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West Nile Virus Bibliography, 2004-2007

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June 2007

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Introduction

West Nile Virus (WVN) was first detected in 1999 in the western hemisphere and has spread throughout the world. West Nile virus is a flavavirus and can infect humans, birds, mosquitoes, horses, and other mammals. The virus has been found in dogs and cats. Transmission of the virus is by mosquitoes that are infected by feeding on infected birds and other animals that are harboring the virus. This disease has caused a reduction in wild bird populations in some areas and has the potential to cause a significant disease problem in humans and other animals. Additional information on West Nile virus can be found in the Web sites listed in the publication.

Multiple sources were searched for citations published between the years 2004 to 2007. The sources of information include peer-reviewed journals, conference proceedings, theses, annual reports, dissertations, books, monographs, letters, Web pages, reviews, and patents.

This bibliographic information is an update to an earlier Animal Welfare Information Center, West Nile Virus Bibliography, 1965-2004.

Information on how to request materials that are included in the collection of the National Agricultural Library (NAL) may be found at: http://www.nal.usda.gov/services/request.shtml.

Readers are cautioned as to the dynamic nature of the internet and the fact that Web addresses and content are subject to change. All sites are current as of April 2007.

Animal Sentinels

Deegan, C.S., J.E. Burns, M. Huguenin, E.Y. Steinhaus, N.A. Panella, S. Beckett, and N. Komar (2005). **Sentinel pigeon surveillance for West Nile virus by using lard-can traps at differing elevations and canopy cover classes.** *Journal of Medical Entomology* 42(6): 1039-1044.

Abstract: Sentinel pigeons, *Columba livia*, were installed in lard-can traps at heights of 1.5 m and 7.6-9.1 m within differing canopy cover classes in New York City. Adult mosquitoes were collected weekly from July to October 2002, as were serum samples from each pigeon. *Culex pipiens L.* and *Culex restuans Theobald* comprised 97% of mosquitoes collected and were most numerous in canopy-level, forested traps. The West Nile virus (family Flaviviridae, genus Flavivirus, WNV) seroconversion rate was significantly greater for pigeons in canopy-level traps, although seroconversions occurred concurrently with human cases in the city and were of little prognostic value to public health agencies. Our results indicate that sentinel pigeons were most effective for monitoring enzootic transmission of WNV when placed in single-sentinel caging 7.6-9.1 m above ground level.

Descriptors: bird diseases, insect vectors, sentinel surveillance, West Nile fever, New York City, polymerase chain reaction methods, species specificity, trees.

Gibbs, S.E., N.L. Marlenee, J. Romines, D. Kavanaugh, J.L. Corn, and D.E. Stallknecht (2006). **Antibodies to West Nile virus in feral swine from Florida, Georgia, and Texas, USA.** *Vector Borne and Zoonotic Diseases* 6(3): 261-265. ISSN: 1530-3667.

Abstract: West Nile virus (WNV) exposure has not yet been reported in feral swine (*Sus scrofa*) despite the broad geographic range and population density of this species. The objectives of this study were to determine the prevalence of antibodies to WNV in feral pigs, and to evaluate serologic diagnostics as applied to this species. Feral pig serum from three states was evaluated for antibodies to WNV. The overall WNV seroprevalence rate for 222 samples collected in 2001-2004 was 22.5%. Seroprevalence rates in Florida, Georgia, and Texas were 17.2%, 26.3%, and 20.5%, respectively. The results of this study demonstrate that feral pigs could represent useful mammalian sentinels of WNV.

Descriptors: West Nile virus, animal sentinals, feral swine, pig serum antibodies, seroprevalence rate, serologic diagnostics.

Gibbs, S., A. Ellis, D. Mead, A. Allison, J. Moulton, E. Howerth, and D. Stallknecht (2005). **West Nile virus detection in the organs of naturally infected blue jays (*Cyanocitta cristata*).** *Journal of Wildlife Diseases.* 41(2): 354-362. ISSN:

0090-3558.
Descriptors: wild birds, sentinel animals, disease detection, immunohistochemistry, reverse transcriptase polymerase chain reaction, Georgia, blue jays, West Nile virus.

Godsey, M.J., M. Blackmore, N. Panella, K. Burkhalter, K. Gottfried, L. Halsey, R. Rutledge, S. Langevin, R. Gates, and K. Lamonte (2005). **West Nile virus epizootiology in the southeastern United States, 2001.** *Vector Borne and Zoonotic Diseases.* 5(1): 82-89. ISSN: 1530-3667.
Descriptors: West Nile virus, virus transmission, insect vectors, Culex, *Culiseta melanura*, mosquitoes, disease reservoirs, birds, disease detection, antibody detection, seroprevalence, chickens, sentinel animals, Southeastern United States, Florida, Georgia.

Lefrancois, T., B.J. Blitvich, J. Pradel, S. Molia, N. Vachiery, and D. Martinez (2006). **West nile virus in guadeloupe: introduction, spread, and decrease in circulation level: 2002-2005.** *Annals of the New York Academy of Sciences* 1081: 206-215. ISSN: 0077-8923.
Abstract: In July 2002, a surveillance system was implemented on Guadeloupe to detect for the potential introduction and monitor the spread of West Nile virus (WNV). From 2002 to 2004, equines and chickens were serologically assayed for antibodies to WNV by IgG and IgM enzyme-linked immunosorbent assay (ELISA), epitope-blocking ELISA, and plaque reduction neutralization tests. After introduction, probably through migratory birds at the end of 2001, many seroconversions occurred between July and October 2002 resulting in a high seroprevalence (19.3%) in equines in 2003. WNV circulation levels decreased dramatically in 2003 and 2004 as assessed by the absence of seroconversion in equine and the very low prevalence in chickens. This decrease coincided with a 7-month drought that presumably caused a decrease in vector populations. In 2005, a sentinel survey was implemented in equines and chickens placed in areas at high risk and the very low rate of seroconversion (1 equine out of 106, no chicken) demonstrated that WNV circulation is now occurring at a very low level.
Descriptors: West Nile virus, equines, chickens, antibodies, serological assay, ELISA, birds, vector populations.

Shaman, J., J. Day, and M. Stieglitz (2005). **Drought-induced amplification and epidemic transmission of West Nile virus in southern Florida.** *Journal of Medical Entomology.* 42(2): 134-141. ISSN: 0022-2585.
Abstract: We show that the spatial-temporal variability of human West Nile (WN) cases and the transmission of West Nile virus (WNV) to sentinel chickens are associated with the spatial-temporal variability of drought and wetting in southern Florida. Land surface wetness conditions at 52 sites in 31 counties in southern Florida for

2001-2003 were simulated and compared with the occurrence of human WN cases and the transmission of WNV to sentinel chickens within these counties. Both WNV transmission to sentinel chickens and the occurrence of human WN cases were associated with drought 2-6 mo prior and land surface wetting 0.5-1.5 mo prior. These dynamics are similar to the amplification and transmission patterns found in southern Florida for the closely related St. Louis encephalitis virus. Drought brings avian hosts and vector mosquitoes into close contact and facilitates the epizootic cycling and amplification of the arboviruses within these populations. Southern Florida has not recorded a severe, widespread drought since the introduction of WNV into the state in 2001. Our results indicate that widespread drought in the spring followed by wetting during summer greatly increase the probability of a WNV epidemic in southern Florida.

Descriptors: West Nile virus, virus transmission, drought, disease outbreaks, sentinel animals, chickens, humans, spatial variation, temporal variation, insect vectors, *Culex nigripalpus*, Culicidae, simulation models, Florida, virus-amplification, land-surface-wetting, hydrology-models.

Animals Susceptible to Disease

Caffrey, C., S.C.R. Smith, and T.J. Weston (2005). **West Nile Virus Devastates an American Crow Population**. *The Condor* P.: 128-32.
Descriptors: West Nile virus, crows, population devastated, birds, sentinals.

Clark, L., J. Hall, R. McLean, M. Dunbar, K. Klenk, R. Bowen, and C.A. Smeraski (2006). **Susceptibility of greater sage-grouse to experimental infection with West Nile virus.** *Journal of Wildlife Diseases* 42(1): 14-22.
Abstract: Populations of greater sage-grouse (*Centrocercus urophasianus*) have declined 45-80% in North America since 1950. Although much of this decline has been attributed to habitat loss, recent field studies have indicated that West Nile virus (WNV) has had a significant negative impact on local populations of grouse. We confirm the susceptibility of greater sage-grouse to WNV infection in laboratory experimental studies. Grouse were challenged by subcutaneous injection of WNV (10(3.2) plaque-forming units [PFUs]). All grouse died within 6 days of infection. The Kaplan-Meier estimate for 50% survival was 4.5 days. Mean peak viremia for nonvaccinated birds was 10(6.4) PFUs/ml (+/-10(0.2) PFUs/ml, standard error of the mean [SEM]). Virus was shed cloacally and orally. Four of the five vaccinated grouse died, but survival time was increased (50% survival=9.5 days), with 1 grouse surviving to the end-point of the experiment (14 days) with no signs of illness. Mean peak viremia for the vaccinated birds was 10(2.3) PFUs/ml (+/-10(0.6) PFUs/ml, SEM). Two birds cleared the virus from their blood before death or euthanasia. These data emphasize the high susceptibility of greater sage-grouse to infection with WNV.
Descriptors: bird diseases immunology, galliformes, viral vaccines immunology, West Nile fever, West Nile virus, animals, wild immunology, wild virology, bird diseases virology, disease susceptibility, enzyme linked immunosorbent assay, injections, random allocation, reverse transcriptase polymerase chain reaction, survival rate, time factors , viremia, West Nile fever immunology, West Nile fever mortality, West Nile fever.

Coumbe, K., T. Mair, J. Samuelson, and D. Sinclair (2006). **West Nile virus in horses.** *Veterinary Record* 159(24): 823.
Descriptors: horses, West Nile virus, West Nile fever, disease, infection.

Dietrich, G., J.A. Montenieri, N.A. Panella, S. Langevin, S.E. Lasater, K. Klenk, J.C. Kile, and N. Komar (2005). **Serologic evidence of West Nile virus infection in free-ranging mammals, Slidell, Louisiana, 2002.** *Vector Borne and Zoonotic Diseases* 5(3): 288-292. ISSN: 1530-3667.

well as future perspectives are discussed. A wide variety of vertebrates, including more than 150 bird species and at least 30 other vertebrate species, are susceptible to WNV infection. The outcome of infection depends on the species, the age of the animal, its immune status and the pathogenicity of the WNV isolate. WNV infection of various birds, especially passeriforms, but also of young chickens and domestic geese, results in high-titred viremia that allows arthropod-borne transmission. For other vertebrate species, only lemurs, lake frogs and hamsters develop suitable viremia levels to support arthropod-borne transmission. The role of vertebrates in direct, non-arthropod-borne transmission, such as via virus-contaminated organs, tissues or excretions is less well characterized. Even though direct transmission can occur among vertebrates of several species, data are lacking on the exact amounts of infectious virus needed. Finally, the increased importance of WNV infections has led to the development of killed, live-attenuated, DNA-recombinant and chimeric veterinary vaccines.
Descriptors: animal diseases, West Nile fever, horse diseases, horses.

Wunschmann, A., J. Shivers, J. Bender, L. Carroll, S. Fuller, M. Saggese, A. Van Wettere, and P. Redig (2005). **Pathologic and immunohistochemical findings in goshawks (*Accipiter gentilis*) and great horned owls (*Bubo virginianus*) naturally infected with West Nile virus.** *Avian Diseases.* 49(2): 252-259. ISSN: 0005-2086.
Abstract: The carcasses of 25 great horned owls and 12 goshawks were investigated for West Nile virus (WNV) infection by immunohistochemistry (IHC) performed on various organs, including brain, spinal cord, heart, kidney, eye, bone marrow, spleen, liver, lungs, pancreas, intestine, and proventriculus, using a WNV-antigen-specific monoclonal antibody and by WNV-specific reverse transcriptase-polymerase chain reaction (RT-PCR), performed on fresh brain tissue only. WNV infection was diagnosed by IHC in all owls and all goshawks. WNV-specific RT-PCR amplified WNV-RNA in the brain of all goshawks but only 12 owls (48%). Cachexia was a common macroscopic finding associated with WNV infection in owls (76%). Myocarditis was occasionally macroscopically evident in goshawks (33%). Microscopically, inflammatory lesions, including lymphoplasmacytic and histiocytic encephalitis, myocarditis, endophthalmitis, and pancreatitis were present in both species but were more common and more severe in goshawks than in owls. The most characteristic brain lesion in owls was the formation of glial nodules, in particular in the molecular layer of the cerebellum, while encephalitis affecting the periventricular parenchyma of the cerebral cortex was common in the goshawks. In owls, WNV-antigen-positive cells were present usually only in very small numbers per organ. Kidney (80%), heart (39%), and cerebellum (37%) were the organs that most commonly contained WNV antigen in owls. WNV antigen was frequently widely distributed in the organs of infected goshawks, with increased amounts of WNV antigen in the heart and the cerebrum. Spleen (75%), cerebellum (66%),

heart (58%), cerebrum (58%), and eye (50%) were often WNV-antigen positive in goshawks. In contrast with the goshawks, WNV antigen was not present in cerebral and retinal neurons of owls. WNV infection appears to be capable of causing fatal disease in great horned owls and goshawks. However, the distribution and severity of histologic lesions, the antigen distribution in the various organs, and the amount of antigen varied among both species. Therefore, the diagnostician may choose organs for histology and immunohistochemistry as well as RT-PCR depending on the investigated species in order to avoid false-negative results.
Descriptors: *Accipiter gentilis*, *Bubo virginianus*, wild birds, birds of prey, West Nile virus, bird diseases , pathogenesis, immunohistochemistry, immunopathology, disease incidence, animal organs, monoclonal antibodies, antigens, reverse transcriptase polymerase chain reaction, cachexia, viral diseases of animals and humans.
Language of Text: Summary in Spanish.

Wunschmann, A. and A. Ziegler (2006). **West Nile virus-associated mortality events in domestic Chukar partridges (*Alectoris chukar*) and domestic Impeyan pheasants (*Lophophorus impeyanus*).** *Avian Diseases* 50(3): 456-459. ISSN: 0005-2086.
Abstract: West Nile virus (WNV) infection was diagnosed in captive juvenile chukars (*Alectoris chukar*), and captive juvenile Impeyan pheasants (*Lophophorus impeyanus*) on the basis of necropsy, histopathology, polymerase chain reaction, and immunohistochemistry. The chukars were kept in a game bird farm that experienced two outbreaks with approximately 25% mortality in hundreds of chukars between September and October 2002 and during the same months in 2003. The submitted pheasants were part of a group of 15 juvenile Impeyan pheasants that all died within approximately 2 wk at the end of August 2002. The macroscopic lesions in the pheasants were dominated by mucosal hemorrhage at the proventricular to ventricular junction and cecal ulcers, whereas the gross lesions in the chukar partridges were nonspecific. The predominant microscopic lesion in the chukar partridges was myocardial necrosis, whereas fibrinous and necrotizing splenitis was prominent in the pheasants. Viral antigen was usually widespread in animals of both species. Spontaneously occurring WNV infection should be considered a differential diagnosis in cases of mortality among select species of galliform birds.
Descriptors: bird diseases, mortality, West Nile fever, necrotizing enterocolitis, heart virology, myocardium pathology, juvenile chukars, juvenile Impeyan pheasants.

Yaremych, S.A., R.E. Warner, P.C. Mankin, J.D. Brawn, A. Raim and R. Novak (2005). **West Nile virus and high death rate in American crows.** S.K. Majumdar, J.E. Huffman, F.J. Brenner and A.I.E. Panah *Wildlife Diseases: Landscape Epidemiology, Spatial Distribution and Utilization of Remote Sensing Technology.,* Pennsylvania Academy of Science, Easton., p. Chapter pagination: 200-204, i-ix. ISBN: 0945809190.

Abstract: After an outbreak of West Nile virus (WNV) infections in Slidell, Louisiana, in 2002, we detected neutralizing antibodies to WNV in 13 of 120 mammals, representing five of six species sampled. Seroprevalence was measured in opossum, *Didelphis virginiana* (75%, n = 8), raccoons, *Procyon lotor* (60%. n = 5), black rats, *Rattus rattus* (6%, n = 36), hispid cotton rats, *Sigmodon hispidus* (4%, n = 24), and eastern gray squirrels, *Sciurus carolinensis* (2%, n = 43).
Descriptors: *Didelphis virginiana*, *Procyon lotor*, *Rattus rattus*, *Sciurus carolinensis*, *Sigmodon hispidus*, viral diseases, West Nile virus, recorded and prevalence, Louisiana, Slidell.

Drummond, R. (2006). **West Nile virus in horses.** *Veterinary Record* 159(26): 895.
Descriptors: West Nile virus, horses, infection, diagnosis.

Durham, A. (2006). **West Nile virus in horses.** *Veterinary Record* 159(21): 723; Discussion 723.
Descriptors: horse diseases, West Nile fever, disease notification, Great Britain, horse diseases transmission, risk factors, travel, virus isolation and purification, West Nile virus, zoonoses.

Farfan Ale, J.A., B.J. Blitvich, N.L. Marlenee, M.A. Lorono Pino, F. Puerto Manzano, J.E. Garcia Rejon, E.P. Rosado Paredes, L.F. Flores Flores, A. Ortega Salazar, J. Chavez Medina, J.C. Cremieux Grimaldi, F. Correa Morales, G. Hernandez Gaona, J.F. Mendez Galvan, and B.J. Beaty (2006). **Antibodies to West Nile virus in asymptomatic mammals, birds, and reptiles in the Yucatan Peninsula of Mexico.** *American Journal of Tropical Medicine and Hygiene* 74(5): 908-914. ISSN: 0002-9637.
Abstract: Surveillance for evidence of West Nile virus (WNV) infection in taxonomically diverse vertebrates was conducted in the Yucatan Peninsula of Mexico in 2003 and 2004. Sera from 144 horses on Cozumel Island, Quintana Roo State, 415 vertebrates (257 birds, 52 mammals, and 106 reptiles) belonging to 61 species from the Merida Zoo, Yucatan State, and 7 farmed crocodiles in Ciudad del Carmen, Campeche State were assayed for antibodies to flaviviruses. Ninety (62%) horses on Cozumel Island had epitope-blocking enzyme-linked immunosorbent assay (ELISA) antibodies to flaviviruses, of which 75 (52%) were seropositive for WNV by plaque reduction neutralization test (PRNT). Blocking ELISA antibodies to flaviviruses also were detected in 13 (3%) animals in the Merida Zoo, including 7 birds and 2 mammals (a jaguar and coyote) seropositive for WNV by PRNT. Six (86%) crocodiles in Campeche State had PRNT-confirmed WNV infections. All animals were healthy at the time of serum collections and none had a history of WNV-like illness.
Descriptors: vertebrata, viral diseases, West Nile virus, prevalence of antibodies in asymptomatic individuals, immune response, antibodies, against viral disease,

prevalence in asymptomatic individuals, Mexico , Yucatan Peninsula, prevalence of antibodies against viral disease in asymptomatic individuals.

Gancz, A.Y., D.A. Smith, I.K. Barker, R. Lindsay, and B. Hunter (2006). **Pathology and tissue distribution of West Nile virus in North American owls (family: Strigidae).** *Avian Pathology* 35(1): 17-29. ISSN: 0307-9457 .

Abstract: This study describes the macroscopic and microscopic lesions and the viral antigen distribution in 82 owls (Family: Strigidae) of 11 North American and one Eurasian species that died following natural West Nile virus infection. The range of lesions seen was greater than that previously reported for owls, and involved more organs. Two patterns of antigen distribution were identified: one that involved the blood and all major organs; and a second where antigen was sparse, localized, and absent from the blood. The first pattern was associated with species of northern natural breeding range, while the second was seen in owls of a more southern distribution and appeared to be associated with a more prolonged course of illness. Further differences in lesion and antigen distribution appeared to be either species related or individual. The findings underline the complexity and variability of West Nile virus pathology within birds of a relatively narrow taxonomic group.

Descriptors: West Nile virus, North American owls, pathology, tissue distribution, natural infection, lesions, antigen distribution.

Gibbs, S.E., A.B. Allison, M.J. Yabsley, D.G. Mead, B.R. Wilcox, and D.E. Stallknecht (2006). **West Nile virus antibodies in avian species of Georgia, USA: 2000-2004.** *Vector Borne and Zoonotic Diseases* 6(1): 57-72. ISSN: 1530-3667.

Abstract: West Nile virus (WNV) was first isolated in the state of Georgia in the summer of 2001. As amplifying hosts of WNV, avian species play an important role in the distribution and epidemiology of the virus. The objective of this study was to identify avian species that are locally involved as potential amplifying hosts of WNV and can serve as indicators of WNV transmission over the physiographic and land use variation present in the southeastem United States. Avian serum samples (n = 14,077) from 83 species of birds captured throughout Georgia during the summers of 2000-2004 were tested by a plaque reduction neutralization test for antibodies to WNV and St. Louis encephalitis virus. Over the 5-year period, WNV-neutralizing antibodies were detected in 869 (6.2%) samples. The WNV seroprevalence increased significantly throughout the study and was species dependent. The highest antibody prevalence rates were detected in rock pigeons (*Columba livia*), northern cardinals (*Cardinalis cardinalis*), common ground doves (*Columbina passerina*), grey catbirds (*Deumetella carolinensis*), and northern mockingbirds (*Mimus polyglottos*). Northem cardinals, in addition to having high geometric mean antibody titers and seroprevalence rates, were commonly found in all land use types and physiographic regions. Rock pigeons, common ground doves, grey catbirds, and northern mockingbirds,

although also having high seroprevalence rates and high antibody titers against WNV, were more restricted in their distribution and therefore may be of more utility when attempting to assess exposure rates in specific habitat types. Of all species tested, northern cardinals represent the best potential avian indicator species for widespread serologic-based studies of WNV throughout Georgia due to their extensive range, ease of capture, and high antibody rates and titers. Due to the large geographic area covered by this species, their utility as a WNV sentinel species may include most of the eastern United States.

Descriptors: aves, viral diseases, West Nile virus, host records and prevalence, Georgia, USA, viral disease.

Hukkanen, R.R., H.D. Liggitt, S.T. Kelley, R. Grant, D. Anderson, B.J. Beaty, N.L. Marlenee, R.A. Hall, and H. Bielefeldt Ohmann (2006). **Comparison of commercially available and novel West Nile virus immunoassays for detection of seroconversion in pig-tailed macaques (*Macaca nemestrina*).** *Comparative Medicine* 56(1): 46-54.

Abstract: We report the assessment and validation of an NS1 epitope-blocking enzyme-linked immunosorbent assay (ELISA) for detection of antibodies to West Nile virus (WNV) in macaques. Sera from naturally infected *Macaca nemestrina* were tested by ELISA and plaque reduction neutralization test (PRNT). Results were correlated with hemagglutination inhibition (HAI) data. Our results demonstrate that the blocking ELISA rapidly and specifically detects WNV infection in *M. nemestrina*. In addition, the diagnostic value of 7 commercially available immunoassays (PanBio immunoglobulin [Ig] M ELISA, PanBio IgG ELISA, PanBio immunofluorescence assay (IFA), InBios IgG ELISA, InBios IgM ELISA, Focus Diagnostics IgG ELISA, and Focus Diagnostics IgM ELISA) in *M. nemestrina* was evaluated and compared with that of the epitope-blocking ELISA. The PanBio IgG ELISA was found to effectively diagnose WNV exposure in *M. nemestrina*. Further, PanBio IFA slides are fast and reliable screening tools for diagnosing flaviviral exposure in *M. nemestrina*.

Descriptors: West Nile virus, immunoassays, detection, pig-tailed macques, NS1 epitope-blocking enzyme-linked immunosorbent assay (ELISA), antibodies, plaque reduction neutralization test (PRNT).

Jacobson, E.R., P.E. Ginn, J.M. Troutman, L. Farina, L. Stark, K. Klenk, K.L. Burkhalter, and N. Komar (2005). **West Nile virus infection in farmed American alligators (*Alligator mississippiensis*) in Florida.** *Journal of Wildlife Diseases* 41(1): 96-106.

Abstract: In September and October 2002, an epizootic of neurologic disease occurred at an alligator farm in Florida (USA). Three affected American alligators (*Alligator mississippiensis*) were euthanatized and necropsied, and results confirmed infection with West Nile virus (WNV). The most significant microscopic lesions were a moderate heterophilic to lymphoplasmacytic meningoencephalomyelitis,

necrotizing hepatitis and splenitis, pancreatic necrosis, myocardial degeneration with necrosis, mild interstitial pneumonia, heterophilic necrotizing stomatitis, and glossitis. Immunohistochemistry identified WNV antigen, with the most intense staining in liver, pancreas, spleen, and brain. Virus isolation and RNA detection by reverse transcription-polymerase chain reaction confirmed WNV infection in plasma and tissue samples. Of the tissues, liver had the highest viral loads (maximum 10(8.9) plaque-forming units [PFU]/0.5 cm3), whereas brain and spinal cord had the lowest viral loads (maximum 10(6.6) PFU/0.5 cm3 each). Virus titers in plasma ranged from 10(3.6) to 10(6.5) PFU/ml, exceeding the threshold needed to infect Culex quinquefasciatus mosquitoes (10(5) PFU/ml). Thus, alligators may serve as a vertebrate amplifying host for WNV.

Descriptors: alligators and crocodiles, neurologic disease, Florida, USA, alligator farms, West Nile fever, West Nile virus isolation and purification, amplifying host for WNV.

Joyner, P.H., S. Kelly, A.A. Shreve, S.E. Snead, J.M. Sleeman, and D.A. Pettit (2006). **West Nile virus in raptors from Virginia during 2003: clinical, diagnostic, and epidemiologic findings.** *Journal of Wildlife Diseases* 42(2): 335-344. ISSN: 0090-3558.

Abstract: Sixty-one birds of prey admitted to The wildlife Center of Virginia (WCV; Waynesboro, Virginia, USA) from June to November 2003 were tested for West Nile 'Arus (WNV) infection. Choanal and/or cloacal swabs were obtained and submitted to Virginia's Division of Consolidated Laboratory Services (Richmond, Virginia, USA) for analysis with real-time reverse transcriptase polymerase chain reaction (RT-PCR). Forty birds of prey were positive for WNV by RT-PCR. Five avian families and nine species of raptors were represented, with great horned owls (*Bubo virginianus*) and red-tailed hawks (*Buteo jamaicensis*) most frequently affected. Presenting clinical signs were consistent with previous reports of WNV infection in raptors; however, these differed between species. Of WNV positive birds, nonspecific signs of illness were the most common clinical findings, particularly in red-tailed hawks; signs included dehydration (n=20), emaciation (n =18), and depression (n =15). Neurologic abnormalities were frequently identified, especially in great horned owls, and included head tremors (n=17), ataxia (n=13), head incoordination (n=7), torticollis (n=3), nystagmus (n=3), and bead tilt (n=3). Great horned owls exhibited aNemia and leukocytosis with heterophilia, eosinophilia, and monocytosis consistent with chronic inflammation. Red-tailed hawks were aNemic with a heterophilic leukocytosis and regenerative left shift. The majority of WNV cases occurred during August and September; there was a marked increase in the number of raptors admitted to WCV during these months followed by a marked decrease during October, November, and December. This pattern differed from mean monthly admissions during the previous 10 years and suggests a negative impact on local raptor popu-

lations. The effects of WNV on avian populations are largely unknown; however, because of their ecological importance, further investigation of the effects of WNV on raptor populations is warranted.
Descriptors: falconiformes, strigiformes, viral diseases, West Nile virus, clinical, diagnostic and epidemiologic findings, transmission of viruses, Virginia.

Miller, D.L., Z.A. Radi, C. Baldwin, and D. Ingram (2005). **Fatal West Nile virus infection in a white-tailed deer (*Odocoileus virginianus*).** *Journal of Wildlife Diseases* 41(1): 246-249.
Abstract: A 3-yr-old male white-tailed deer (*Odocoileus virginianus*) with a history of ataxia and tremors was submitted to the Tifton Veterinary Diagnostic and Investigational Laboratory (The University of Georgia, Tifton, Georgia, USA) for necropsy. Gross findings were unremarkable. Histologically, the brain had multifocal lymphoplasmacytic perivascular inflammation, scattered gliosis, and rare satellitosis. Mild hemorrhage and congestion in the retropharyngeal lymph nodes and mild lymphoid depletion in the tonsil were present. A reverse transcription-polymerase chain reaction test performed on brain yielded a positive result for West Nile virus. This represents the first report of fatal West Nile virus infection in a white-tailed deer.
Descriptors: white-tailed deer, *Odocoileus virginianus*, West Nile fever, West Nile virus isolation and purification, brain stem, fatal outcome, viral RNA analysis, reverse transcriptase polymerase chain reaction methods.

Naugle, D., C. Aldridge, B. Walker, K. Doherty, M. Matchett, J. McIntosh, T. Cornish, and M. Boyce (2005). **West Nile virus and sage-grouse: What more have we learned?** *Wildlife Society Bulletin.* 33(2): 616-623. ISSN: 0091-7648.
Descriptors: *Centrocercus urophasianus*, grouse, West Nile virus, mortality, surveys, Wyoming, Montana, Alberta, Colorado, California.

Nemeth, N., D. Gould, R. Bowen, and N. Komar (2006). **Natural and experimental West Nile virus infection in five raptor species.** *Journal of Wildlife Diseases* 42(1): 1-13.
Abstract: We studied the effects of natural and/or experimental infections of West Nile virus (WNV) in five raptor species from July 2002 to March 2004, including American kestrels (*Falco sparverius*), golden eagles (*Aquila chrysaetos*), red-tailed hawks (*Buteo jamaicensis*), barn owls (*Tyto alba*), and great horned owls (*Bubo virginianus*). Birds were infected per mosquito bite, per os, or percutaneously by needle. Many experimentally infected birds developed mosquito-infectious levels of viremia (>10(5) WNV plaque forming units per ml serum) within 5 days postinoculation (DPI), and/ or shed virus per os or per cloaca. Infection of organs 15-27 days postinoculation was infrequently detected by virus isolation from spleen, kidney, skin, heart, brain, and eye in convalescent birds. Histopathologic findings varied among species and by method of infection. The most common histopathologic lesions

were subacute myocarditis and encephalitis. Several birds had a more acute, severe disease condition represented by arteritis and associated with tissue degeneration and necrosis. This study demonstrates that raptor species vary in their response to WNV infection and that several modes of exposure (e.g., oral) may result in infection. Wildlife managers should recognize that, although many WNV infections are sub-lethal to raptors, subacute lesions could potentially reduce viability of populations. We recommend that raptor handlers consider raptors as a potential source of WNV contamination due to oral and cloacal shedding.

Descriptors: bird diseases, raptors, West Nile fever, West Nile virus, animals, wild virology, species specificity, mosquito vectors, histopathology , species response to WNV infection, mode of exposure, oral and cloacal shedding.

Nemeth, N., D. Hahn, D. Gould, and R. Bowen (2006). **Experimental West Nile Virus Infection in Eastern Screech Owls (*Megascops asio*).** *Avian Diseases.* 50(2): 252-258. ISSN: 0005-2086.

Abstract: Eastern Screech Owls (EASOs) were experimentally infected with the pathogenic New York 1999 strain of West Nile virus (WNV) by subcutaneous injection or per os. Two of nine subcutaneously inoculated birds died or were euthanatized on 8 or 9 days postinfection (DPI) after <24 hr of lethargy and recumbency. All subcutaneously inoculated birds developed levels of viremia that are likely infectious to mosquitoes, with peak viremia levels ranging from 105.0 to 109.6 plaque-forming units/ml. Despite the viremia, the remaining seven birds did not display signs of illness. All birds alive beyond 5 DPI seroconverted, although the morbid birds demonstrated significantly lower antibody titers than the clinically normal birds. Cagemates of infected birds did not become infected. One of five orally exposed EASOs became viremic and seroconverted, whereas WNV infection in the remaining four birds was not evident. All infected birds shed virus via the oral and cloacal route. Early during infection, WNV targeted skin, spleen, esophagus, and skeletal muscle. The two morbid owls had myocardial and skeletal muscle necrosis and mild encephalitis and nephritis, whereas some of the clinically healthy birds that were sacrificed on 14 DPI had myocardial arteritis and renal phlebitis. WNV is a significant pathogen of EASOs, causing pathologic lesions with varying clinical outcomes.

Descriptors: Strigiformes, wild birds, West Nile virus, pathogenicity, vertebrate viruses, subcutaneous injection, drug delivery systems, viremia, disease transmission, insect vectors, symptoms, seroconversion, morbidity, oral administration, disease course, disease severity, hosts, new host records, pathogen-shedding, Internet-resource.

Language of Text: Summary in Spanish.

Nevarez, J.G., M.A. Mitchell, Dae Young Kim, R. Poston, and H.M. Lampinen (2005). **West Nile virus in alligator, *Alligator mississippiensis*, ranches from Louisiana.** *Journal of Herpetological Medicine and Surgery* 15(3): 4-9. ISSN: 1529-9651.

Abstract: West Nile virus (WNV) has been reported to affect various crocodilian species including the American alligator, *Alligator mississippiensis*, the Nile crocodile, *Crocodylus niloticus*, and the Morelet's crocodile, *Crocodylus moreletii*. In the Fall of 2003 an increased number of mortalities were observed at various alligator ranches in Louisiana. Affected animals were reported to have neurologic signs followed by death. At the time West Nile virus (WNV) had already been diagnosed in alligators from Georgia and Florida, but not Louisiana. This report outlines the findings of physical exam, necropsy, histopathology and diagnostic tests of affected animals. Viral isolation, real time RT-PCR and immunohistochemistry have proven to be useful tests for diagnosing WNV in alligators. One interesting aspect of these cases is that only one of the four affected facilities had alligators hatched in Louisiana. The other three had imported hatchlings from Florida and Texas.

Descriptors: *Alligator mississippiensis*, viral diseases, West Nile virus, occurrence at ranches, symptoms and disease origins, transmission of viruses, Louisiana.

Ollis, G., L. Morin, and A. Visser (2005). **Laboratory confirmed positive cases of equine West Nile virus in Alberta in 2003.** *Canadian Veterinary Journal* 46(2): 131-133. ISSN: 0008-5286.

Descriptors: horse diseases, West Nile virus, disease incidence, geographical distribution , symptoms, horses, risk factors, disease surveillance, disease detection, diagnostic techniques, blood serum, immunoglobulin M, enzyme linked immunosorbent assay, disease diagnosis, Alberta.

Read, R.W., D.B. Rodriguez, and B.A. Summers (2005). **West Nile Virus Encephalitis in a Dog.** *Veterinary Pathology.* 42(2): 219-222. ISSN: 0300-9858.

Abstract: In November 2002, a 2-year-old, spayed Maltese Terrier in central Mississippi was presented for an acute illness characterized by uncontrolled hyperactivity that rapidly progressed to generalized tremors, ataxia, and intermittent hyperthermia. Postmortem examination after a 2-week course revealed mild, multifocal, nonsuppurative meningo encephalitis, with focal necrosis in the medulla. Reverse transcriptase-nested-polymerase chain reaction for West Nile virus (WNV) was positive on brain and negative on other tissues. Immunohistochemistry was negative on all tissues. The clinical, postmortem, and laboratory findings are consistent with acute encephalitis due to WNV infection. WNV infection should be considered in dogs showing signs of encephalitis when and where WNV and mosquito vectors occur.

Descriptors: dog diseases, case studies, disease course, Mississippi, West Nile virus, symptoms, diagnosis.

Root, J.J., P.T. Oesterle, N.M. Nemeth, K. Klenk, D.H. Gould, R.G. McLean, L. Clark, and J.S. Hall (2006). **Experimental infection of fox squirrels (*Sciurus niger*) with West Nile virus.** *American Journal of Tropical Medicine and Hygiene* 75(4): 697-701. ISSN: 0002-9637.

Abstract: Tree squirrels (Sciurus spp.) have exhibited high seroprevalence rates, suggesting that they are commonly exposed to West Nile virus (WNV). Many characteristics of WNV infections in tree squirrels, such as the durations and levels of viremia, remain unknown. To better understand WNV infections in fox squirrels (*S. niger*), we subcutaneously inoculated fourteen fox squirrels with WNV. Peak viremias ranged from 10(4.00) plaque-forming units (PFU)/mL of serum on day 2 post-infection (DPI) to 10(4.98) PFU/mL on 3 DPI, although viremias varied between individuals. Oral secretions of some fox squirrels were positive for WNV viral RNA, occasionally to moderate levels (10(3.2) PFU equivalent/swab). WNV PFU equivalents in organs were low or undetectable on 12 DPI; gross and histologic lesions were rare. The viremia profiles of fox squirrels indicate that they could serve as amplifying hosts in nature. In addition, viral RNA in the oral cavity and feces indicate that this species could contribute to alternative WNV transmission in suburban communities.

Descriptors: rodent diseases, squirrels, West Nile fever, West Nile virus, viral biosynthesis, brain pathology, kidney pathology, liver pathology, myocardium pathology, viral RNA analysis, random allocation, reverse transcriptase polymerase chain reaction, virus shedding, mortality, pathology.

Steinman, A., C. Banet Noach, L. Simanov, N. Grinfeld, Z. Aizenberg, O. Levi, D. Lahav, M. Malkinson, S. Perk, and N.Y. Shpigel (2006). **Experimental Infection of Common Garter Snakes (*Thamnophis sirtalis*) with West Nile Virus.** *Vector Borne and Zoonotic Diseases* 6(4): 361-368. ISSN: 1530-3667.

Abstract: The role of various reptilian species in the infectious cycle of several arboviruses is documented, but their role in that of West Nile virus (WNV) is uncertain. Common garter snakes (*Thamnophis sirtalis*) were infected subcutaneously with 10(5) plaque forming units (PFU) WNV-Isr 98, five of nine snakes became viremic, and five exhibited persistent low levels of neutralizing antibodies. Four of the parentally infected snakes died and high titers of virus were found in multiple organ samples. In contrast, orally infected garter snakes did not become viremic, but viral RNA was detected in cloacal swabs. Since oral infection of predator birds by WNV is known, their ingestion of infected snakes may also result in their becoming infected.

Descriptors: reptilian species, West Nile virus, garter snakes, subcutaneous infection, oral infection, viremic, RNA, predator birds.

Stout, W., A. Cassini, J. Meece, J. Papp, R. Rosenfield, and K. Reed (2005). **Serologic evidence of West Nile virus infection in three wild raptor populations.** *Avian Diseases.* 49(3): 371-375. ISSN: 0005-2086.

Abstract: We assayed for West Nile virus (WNV) antibodies to determine the presence and prevalence of WNV infection in three raptor populations in southeast Wisconsin during 2003-04. This study was conducted in the framework of ongoing population studies that started before WNV was introduced to the study area. For 354 samples, 88% of 42 adult Cooper's hawks (*Accipiter cooperii*), 2.1% of 96 nestling Cooper's hawks, 9.2% of 141 nestling red-tailed hawks (*Buteo jamaicensis*), and 12% of 73 nestling great horned owls (*Bubo virginianus*) tested positive for WNV antibodies by the constant virus-serum dilution neutralization test. Samples that tested positive for WNV antibodies were collected across a wide variety of habitat types, including urban habitats (both high and low density), roads, parking areas, recreational areas, croplands, pastures, grasslands, woodlands, and wetlands. Based on the increased prevalence and significantly higher WNV antibody titers in adults compared with nestlings, we suggest that nestlings with detectable antibody levels acquired these antibodies through passive transmission from the mother during egg production. Low levels of WNV antibodies in nestlings could serve as a surrogate marker of exposure in adult raptor populations. Based on breeding population densities and reproductive success over the past 15 yr, we found no apparent adverse effects of WNV infections on these wild raptor populations.
Descriptors: wild birds, hawks, Accipiter, *Buteo jamaicensis*, owls, *Bubo virginianus*, West Nile virus, immunologic techniques, viral diseases of animals and humans, disease detection, disease prevalence, wildlife habitats, biomarkers, maternal immunity, Wisconsin, *Accipiter-cooperii.*
Language of Text: Summary in Spanish.

Sullivan, H., G. Linz, L. Clark, and M. Salman (2006). **West Nile virus antibody prevalence in red-winged blackbirds (*Agelaius phoeniceus*) from North Dakota, USA (2003-2004).** *Vector Borne and Zoonotic Diseases* 6(3): 305-309. ISSN: 1530-3667.
Abstract: This study was designed to explore the role that red-winged blackbirds (*Agelaius phoeniceus*) may have played in disseminating West Nile virus (WNV) across the United States. Using enzyme-linked immunosorbent assays designed to detect WNV antibodies in avian species we were able to determine the WNV antibody prevalence in a cohort of red-winged blackbirds in central North Dakota in 2003 and 2004. The peak WNV antibody prevalence was 22.0% in August of 2003 and 18.3% in July of 2004. The results of this study suggest that red-winged blackbird migratory populations may be an important viral dispersal mechanism with the ability to spread arboviruses such as WNV across the United States.
Descriptors: West Nile virus, red winged black birds, antibody prevalence, migratory populations, disseminating, disperal, spread.

Ternovoi, V.A., E.V. Protopopova, S.G. Surmach, M.V. Gazetdinov, S.I. Zolotykh, A.M. Shestopalov, E.V. Pavlenko, G.N. Leonova, and V.B. Loktev (2006). **[The genotyping of the West Nile virus in birds in the far eastern region of Russia in 2002-2004]**. *Molekuliarnaia Genetika, Mikrobiologiia i Virusologiia*(4): 30-35.

Abstract: Samples from 20 species of trapped and dead birds were collected in the Far Eastern Region in 2002-2004 and were analyzed by the anti-WNV MAb-modified immunoenzyme assay for antigen detection and RT-PCR for viral RNA detection. Five positive samples from cinereous vultures (*Aegypius monachus*) and two positive samples from cattle egret (*Bubulcus ibis*) were found in both tests. The sequencing of the 322 bp fragments of protein E gene showed 99-99.67% homology with the strain WNV/LEIV-VlgOO-27924 of the WNV isolated in Volgograd, Russia, 2000. Additionally, five positive samples from birds (*Pica pica, Corvus macrorhynchos, Larus crossirostris, Parus minor, Emberiza spodocephala*) collected in autumn 2004 were found during screening with anti-WNV MAb-modified ELISA. These results confirm that the WNV is circulating in the Far Eastern Region of Russia and outbreaks of WN fever in humans may be possible. This demonstrates that the genotype 1a of the West Nile virus could spread in the southern regions of the Far East by migrating birds and introduction of the WNV into other southern regions of the Asian part of Russia are probably.

Descriptors: birds, West Nile fever, viral genotype, immunoenzyme techniques, reverse transcriptase polymerase chain reaction, Russia, West Nile virus classification.

Language of Text: Russian.

Tesh, R.B., M. Siirin, H. Guzman, A.P. Travassos da Rosa, X. Wu, T. Duan, H. Lei, M.R. Nunes, and S.Y. Xiao (2005). **Persistent West Nile virus infection in the golden hamster: studies on its mechanism and possible implications for other flavivirus infections.** *Journal of Infectious Diseases* 192(2): 287-295.

Abstract: Golden hamsters (*Mesocricetus auratus*) experimentally infected with West Nile virus (WNV) developed chronic renal infection and persistent shedding of virus in urine for up to 8 months, despite initial rapid clearance of virus from blood and the timely appearance of high levels of specific neutralizing antibodies. Infectious WNV could be recovered by direct culture of their urine and by cocultivation of kidney tissue for up to 247 days after initial infection. Only moderate histopathologic changes were observed in the kidneys or brain of the chronically infected hamsters, although WNV antigen was readily detected by immunohistochemistry within epithelium, interstitial cells, and macrophages in the distal renal tubules. Comparison of WNV isolates from serial urine samples from individual hamsters over several months indicated that the virus underwent both genetic and phenotypic changes during persistent infection. These findings are similar to previous reports of persistent infection with tickborne encephalitis and Modoc viruses.

Descriptors: flavivirus infections, West Nile fever, viral blood antibodies, antibody formation, animal disease models, enzyme linked immunosorbent assay, kidney, *Mesocricetus auratus*, golden hamster, viremia immunology, West Nile virus.

Tiawsirisup, S., K.B. Platt, B.J. Tucker, and W.A. Rowley (2005). **Eastern cottontail rabbits (*Sylvilagus floridanus*) develop West Nile virus viremias sufficient for infecting select mosquito species.** *Vector Borne and Zoonotic Diseases* 5(4): 342-350. ISSN: 1530-3667.

Abstract: The potential of the eastern cottontail rabbit (CTR; *Sylvilagus floridanus*) to contribute to an enzootic West Nile virus (WNV) cycle was demonstrated by characterizing the WNV viremia profile of 15 CTRs and demonstrating that mosquitoes could become infected by feeding on these CTRs. Eight CTRs were infected with a titer of 10(5.0) cell-infectious dose 50% endpoints (CID50s) of WNV (NY99-Crow) by needle and seven CTRs by bite of one or more WNV-infected mosquitoes. There were no marked differences between the WNV viremia profiles of CTRs infected by either method. West Nile virus was detected in serums of all CTRs by 24 h p.i. The daily mean titers of all 15 CTRs on days 1-4 p.i. were 10(4.1+/-0.4), 10(4.7+/-0.3), 10(4.1+/-0.6), and 10(3.7+/-0.6) respectively, declining to 10(1.2+/-0.1) CID50s/ml of serum by day 6 p.i. No virus was detected in the blood of any CTR on day 7 p.i. The average duration of WNV titers of >or=10(4.3) and <10(5.0) CID50s/mL for all CTRs was 2.2 +/- 0.6 and 1.0 +/- 0.1 days, respectively. The minimum estimated infection rates (MEIRs) of *Culex pipiens* (L.) and *Culex salinarius* (Coq.) that fed on CTRs with titers of >or=10(4.3) and >10(5.0) were 11.5 +/- 5.5 and 21 +/- 6.0%, respectively. These rates increased to 20.5 +/- 6.4% and 25.0 +/- 3.0% when CTR serum titers were >10(5.0) CID50s/mL. Neither *Aedes aegypti* (L.) nor *Aedes albopictus* (Skuse) were infected by feeding on CTRs with titers of <10(5.0) CID50s/mL. The MEIRs of these two species were 11.5 +/- 3.5% and 1.5 +/- 0.5% after feeding on CTRs with titers of >10(5.0) CID50s/ml. None of the CTRs infected by mosquito bite or by needle showed any symptoms of WNV disease.

Descriptors: cottontail rabbit, West Nile virus, viremia, Culex mosquitos, infection rates.

van der Meulen, K.M., M.B. Pensaert, and H.J. Nauwynck (2005). **West Nile virus in the vertebrate world.** *Archives of Virology* 150(4): 637-657. ISSN: 0304-8608.

Abstract: West Nile virus (WNV), an arthropod-borne virus belonging to the family Flaviviridae, had been recognized in Africa, Asia and the south of Europe for many decades. Only recently, it has been associated with an increasing number of outbreaks of encephalitis in humans and equines as well as an increasing number of infections in vertebrates of a wide variety of species. In this article, the data available on the incidence of WNV in vertebrates are reviewed. Moreover, the role of vertebrates in the transmission of WNV, the control of WNV infections in veterinary medicine as

Diagnosis, Testing and Detection

Anonymous (2006). **First West Nile Virus screening test approved.** *FDA Consumer* 40(1): 3.
Descriptors: West Nile fever diagnosis, West Nile virus isolation and purification, screening tests.

Anonymous (2005). **Summaries for patients. The cost-effectiveness of screening the U.S. blood supply for West Nile virus.** *Annals of Internal Medicine* 143(7): I44.
Descriptors: blood transfusion, blood borne pathogens, West Nile virus, computer simulation, cost benefit analysis, markov chains, humans, nucleic acid amplification techniques, United States.
Notes: Original Report In: Ann Intern Med. 2005 Oct 4;143(7):486-92.

Avalos Bock, S.A. (2005). **West Nile virus and the U.S. blood supply: New tests substantially reduce the risk of transmission via donated blood products.** *American Journal of Nursing* 105(12): 34, 36-37 .
Descriptors: blood donors, nucleic acid amplification techniques, RNA, viral blood, West Nile fever, United States, West Nile virus.

Balasuriya, U.B., P.Y. Shi, S.J. Wong, V.L. Demarest, I.A. Gardner, P.J. Hullinger, G.L. Ferraro, J.D. Boone, C.L. De Cino, A.L. Glaser, R.W. Renshaw, M. Ledizet, R.A. Koski, and N.J. MacLachlan (2006). **Detection of antibodies to West Nile virus in equine sera using microsphere immunoassay.** *Journal of Veterinary Diagnostic Investigation* 18(4): 392-395.
Abstract: One hundred and ninety-one sera from horses that recently were exposed to West Nile virus (WNV) by either vaccination or natural infection or that were not vaccinated and remained free of infection were used to evaluate fluorescent microsphere immunoassays (MIAs) incorporating recombinant WNV envelope protein (rE) and recombinant nonstructural proteins (rNS1, rNS3, and rNS5) for detection of equine antibodies to WNV. The rE MIA had a diagnostic sensitivity and specificity, respectively, of 99.3% and 97.4% for detection of WNV antibodies in the serum of horses that were recently vaccinated or naturally infected with WNV, as compared to the plaque reduction neutralization test (PRNT). The positive rE MIA results were assumed to be WNV-specific because of the close agreement between this assay and the PRNT and the fact that unvaccinated control horses included in this study were confirmed to be free of exposure to the related St Louis encephalitis virus. The NS protein-based MIA were all less sensitive than either the rE MIA or PRNT (sensitivity 0-48.0), although the rNSI MIA distinguished horses vaccinated with the

recombinant WNV vaccine from those that were immunized with the inactivated WNV vaccine (P < 0.0001) or naturally infected with WNV (P < 0.0001). The rE MIA would appear to provide a rapid, convenient, inexpensive, and accurate test for the screening of equine sera for the presence of antibodies to WNV.
Descriptors: antibodies, horse diseases, West Nile fever, West Nile virus.

Batuello, J.T., J. Youngwerth, and R. Gabel (2005). **Increased serum lipase in West Nile virus infection.** *New England Journal of Medicine* 352(4): 420-421.
Descriptors: lipase blood, West Nile fever enzymology, humans.

Beasley, D.W. (2005). **Recent advances in the molecular biology of west nile virus.** *Current Molecular Medicine* 5(8): 835-850.
Abstract: Since the mid-1990s, West Nile virus (WNV) has emerged as a significant agent of arboviral encephalitis in several regions of the world. In 1999, WNV was introduced into the northeastern United States and was associated with an outbreak of encephalitis affecting humans, birds and horses. Subsequently, the virus has spread across the country, and across southern Canada, and in 2002 and 2003 was associated with the largest outbreaks of arboviral encephalitis recorded in the Western hemisphere. Interestingly, the more recent spread of WNV into Mexico, Central America and the Caribbean has not been associated with the high levels of clinical disease observed in North America. This review addresses the most recent results from studies investigating the molecular biology and evolution of WNV, as well as progress in the development of diagnostic and therapeutic reagents.
Descriptors: West Nile fever, West Nile virus, United States.

Bell, J.A., C.M. Brewer, N.J. Mickelson, G.W. Garman, and J.A. Vaughan (2006). **West Nile virus epizootiology, central Red River Valley, North Dakota and Minnesota, 2002-2005.** *Emerging Infectious Diseases* 12(8): 1245-1247.
Abstract: West Nile virus (WNV) epizootiology was monitored from 2002 through 2005 in the area surrounding Grand Forks, North Dakota. Mosquitoes were tested for infection, and birds were surveyed for antibodies. In 2003, WNV was epidemic; in 2004, cool temperatures precluded WNV amplification; and in 2005, immunity in passerines decreased, but did not preclude, WNV amplification.
Descriptors: bird diseases, West Nile virus, insect vectors, Minnesota, North Dakota, zoonoses.

Brenner, W., G. Storch, R. Buller, R. Vij, S. Devine, and J. Dipersio (2005). **West nile virus encephalopathy in an allogeneic stem cell transplant recipient: Use of quantitative PCR for diagnosis and assessment of viral clearance**. *Bone Marrow Transplantation* 36(4): 369-370. ISSN: 0268-3369.
Descriptors: encephalopathy, quadriplegia, chronic myeloid leukemia, neoplastic

disease, heart disease, magnetic resonance imaging, diagnostic techniques, stem cell transplantation, quantitative polymerase chain reaction, viral clearance.

Buckley, A., A. Dawson, and E.A. Gould (2006). **Detection of seroconversion to West Nile virus, Usutu virus and Sindbis virus in UK sentinel chickens.** *Virology Journal* 3: 71.

Abstract: We previously reported evidence of West Nile virus (WNV) circulation in UK birds, probably introduced by migratory birds from overseas. We now demonstrate WNV-specific seroconversion in sentinel chickens raised on an English farm. Maternal neutralizing antibodies to WNV in hatchlings declined within three weeks. During the following months, healthy chickens developed WNV neutralizing antibodies that were confirmed by immunoblotting and indirect immunofluorescence tests using WNV antigens. The proportion of seropositive chickens was higher for WNV than for Usutu virus or Sindbis virus. Attempts to isolate infectious virus or to detect viral RNA in the sera, failed.

Descriptors: alphavirus infections, viral blood, chickens, encephalitis, flavivirus, sindbis virus, bird diseases, western blotting, Great Britain, fluorescence microscopy, sentinel surveillance, seroepidemiologic studies , West Nile virus isolation and purification.

Bunde, J.M., E.J. Heske, N.E. Mateus Pinilla, J.E. Hofmann, and R.J. Novak (2006). **A survey for West Nile virus in bats from Illinois.** *Journal of Wildlife Diseases* 42(2): 455-458.

Abstract: A blocking enzyme-linked immunosorbent assay was used to test 97 serum samples from big brown bats (*Eptesicus fuscus*) captured in six counties in Illinois between May 2002 and February 2004 for West Nile virus (WNV) antibodies. One female big brown bat tested positive for WNV antibodies. Samples of kidney, liver, and heart tissue were collected from 312 bats of seven species that were submitted to the Illinois (USA) Department of Public Health or the Illinois Department of Agriculture diagnostic laboratories between January 2001 and December 2003. Tissue samples were tested for WNV using TaqMan reverse transcriptase polymerase chain reaction and all were negative. Prevalence of WNV antibodies in the bats (1%) was lower than previously reported for other flaviviruses, but similar to the prevalence (2%) of WNV antibodies reported in bats from New Jersey and New York, USA. Additional research is needed to determine potential impact of WNV infections on bats and to determine whether they play a role in the WNV transmission cycle.

Descriptors: viral blood antibodies, big brown bats, West Nile fever, West Nile virus, enzyme linked immunosorbent assay methods, myocardium, reverse transcriptase polymerase chain reaction methods.

Burkhalter, K.L., R. Lindsay, R. Anderson, A. Dibernardo, W. Fong, and R.S. Nasci (2006). **Evaluation of commercial assays for detecting West Nile virus antigen.** *Journal of the American Mosquito Control Association* 22(1): 64-69.

Abstract: Two commercially available West Nile virus (WNV) detection assays (RAMP WNV test, Response Biomedical Corp., Burnaby, British Columbia, Canada; and VecTest WNV antigen assay, Medical Analysis Systems, Inc., Camarillo, CA) were compared for sensitivity, specificity, and ability to detect WNV in field-collected mosquito pools. Serially diluted stock seed WNV and St. Louis encephalitis virus (SLEV) were used to determine sensitivity and specificity. The RAMP WNV test detected WNV at concentrations as low as 3.17 log10 plaque-forming units per milliliter (PFU/ml), whereas the VecTest assay detected WNV at concentrations as low as 5.17 log10 PFU/ml. Neither test cross-reacted with SLEV. A WNV-specific reverse transcriptase polymerase chain reaction was used to identify positives among field-collected mosquito pools. The RAMP WNV test detected 94% of positive pools and the VecTest assay detected 65% of the positive field-collected pools. Despite these differences, both assays have characteristics that make them useful in WNV surveillance programs.

Descriptors: viral antigens, mosquitoes, diagnostic reagent kits, West Nile virus, immunoenzyme techniques, insect vectors, reverse transcriptase polymerase chain reaction.

Busch, M.P., S. Caglioti, E.F. Robertson, J.D. McAuley, L.H. Tobler, H. Kamel, J.M. Linnen, V. Shyamala, P. Tomasulo, and S.H. Kleinman (2005). **Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing.** *New England Journal of Medicine* 353(5): 460-467.

Abstract: BACKGROUND: The use of nucleic acid amplification tests of "mini-pools" of 16 samples to screen blood donors for West Nile virus RNA began in July 2003. We report the yield and characteristics of positive donations and the incremental yield and safety of nucleic acid amplification tests of individual donations. METHODS: Reactive minipools were analyzed to identify the individual reactive donations. For the regions with the highest yield on minipool testing, retrospective nucleic acid amplification testing was performed on individual donations that were negative on minipool testing. Reactive donations were confirmed by alternative nucleic acid amplification tests and IgM and IgG tests, and donors were followed to document seroconversion. RESULTS: From July 1 through October 31, 2003, 677,603 donations were prospectively screened for West Nile virus by minipool testing, yielding 183 confirmed viremic donations (0.027 percent, or 1 in 3703 donations). Retrospective individual testing of 23,088 donations from high-prevalence regions that were negative on minipool testing yielded 30 additional units with a low level of viremia, with 14 additional viremic units detected by prospective testing of

individual donations late in the 2003 transmission season. Of all the viremic units detected, 5 percent were detected only by individual testing and were negative for IgM antibody, 29 percent were detected by individual testing after IgM seroconversion, and 66 percent were detected by minipool testing. West Nile virus infection was confirmed in both recipients of IgM-negative units that were reactive on individual testing, whereas neither recipient of antibody-positive blood components that were reactive on individual testing was infected. In 2004, prospective testing of individual donations in regions that yielded donations that were reactive on minipool testing resulted in a 32 percent incremental yield of units with a low level of viremia that would have been missed by minipool testing. CONCLUSIONS: Although nucleic acid amplification testing of minipools of blood donations prevented hundreds of cases of West Nile virus infection in 2003, it failed to detect units with a low level of viremia, some of which were antibody-negative and infectious. These data support the use of targeted nucleic acid amplification testing of individual donations in high-prevalence regions, a strategy that was implemented successfully in 2004. Copyright 2005 Massachusetts Medical Society.

Descriptors: blood donors, nucleic acid amplification techniques, viral blood RNA, West Nile fever, West Nile virus isolation and purification, retrospective studies, viremia.

Notes: Comment In: N Engl J Med. 2005 Aug 4;353(5):516-7.

Chen, Y.P., Y.J. Yang, X.D. Wu, and Z.L. Wang (2006). **[Preparation of monoclonal antibodies against West Nile virus envelope protein domain.]**. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi; Zhonghua Shiyan He Linchuang Bingduxue Zazhi; Chinese Journal of Experimental and Clinical Virology* 20(3): 213-5.

Abstract: BACKGROUND: To prepare monoclonal antibodies against West Nile virus (WNV) envelope protein domain. METHODS: BALB/c mice were immunized with recombinant antigen of West Nile virus envelope protein domain, and the spleen cells of the mice were used to prepare the monoclonal antibodies (McAb) by hybridoma technique. RESULTS: Three hybridoma cell strains secreting McAbs against WNV envelope protein domain, designated as 4F7, 6H3 and 8E4, respectively, were obtained and were identified by indirect enzyme linked immunosorbent assay (ELISA), they belonged to IgG1, IgG1 and Ig2a, respectively. Two epitopes of envelope protein domain were determined, among them, 4F7 and 6H3 were against the same epitope and 8E4 to another one. CONCLUSIONS: The results of indirect ELISA, Western blot and indirect immunofluorescence experiment indicated that these three McAbs were specific for West Nile virus envelope protein domain and did not cross-react with Japanese encephalitis virus and other viruses, so they can be used for specific detection of West Nile virus.

Descriptors: West Nile virus, envelope protein, spleen cells, mice, monoclonal anti-

bodies, western blot, enzyme linked immunosorbent assay, Japanese encephalitis.
Language of Text: Chinese.

Chevalier, V., R. Lancelot, A. Diaite, B. Mondet, B. Sall, and X. DE Lamballerie (2006). **Serological assessment of west nile Fever virus activity in the pastoral system of ferlo, senegal.** *Annals of the New York Academy of Sciences* 1081: 216-25.
Abstract: The Ferlo area (north-central Senegal) is characterized by a system of temporary ponds favorable to arboviruses among which West Nile fever (WNF) was already identified. During the rainy season in 2003, a serological study was undertaken on horses to assess the activity of the WNF virus (WNFV) in Barkedji (Ferlo). The observed serological prevalence rate was 78.3% for neutralizing antibodies, with a 95% confidence interval (CI) of [64.0, 92.7]. This prevalence rate significantly increased with age (P = 10(-5)). This study confirmed that WNF was endemic in the Ferlo. The transmission risks depended on the introduction of the WNFV in the ecosystem-probably with migrating birds, on its amplification in hosts and on the vector-population dynamic. Further studies are needed to investigate how the cycle is initiated in Barkedji at the beginning of the rainy season and the impact of climatic variations on the risk of transmission of WNF. A surveillance system should be implemented: (a) to assess the clinical impact of the WNF on human and equine populations, (b) to provide an early detection of virulent strains, and (c) to assess the risk of WNF transmission to disease-free ecosystems via migrating birds.
Descriptors: West Nile virus, serological assessment, virus activity, ponds, horses, antibodies, transmission, migrating birds.

Cunha, B.A. (2006). **West Nile virus encephalitis: clinical diagnostic and prognostic indicators in compromised hosts.** *Clinical Infectious Diseases an Official Publication of the Infectious Diseases Society of America* 43(1): 117.
Descriptors: immunocompromised host, West Nile fever diagnosis, lymphoma, b cell complications, b cell immunology, prognosis, serologic tests, West Nile fever immunology.
Notes: Comment On: Clin Infect Dis. 2006 Mar 1;42(5):680-3.

Deng-Yong-Qiang, Jiang-Tao, Yu-Man, Qin-Cheng-Feng, Chen-Shui-Ping, Zhu-Qing-Yu, and Qin-E-De (2005). **Detection of West Nile virus using real-time PCR assay**. *Zhonghua Weishengwuxue He Mianyixue Zazhi* 25(6): 519-522. ISSN: 0254-5101.
Descriptors: West Nile virus detection, real time PCR assay, reverse transcriptase polymerase chain reaction, genetic techniques.
Language of Text: Chinese.

Ellis, A.E., D.G. Mead, A.B. Allison, S.E.J. Gibbs, N.L. Gottdenker, D.E. Stallknecht, and E.W. Howerth (2005). **Comparison of Immunohistochemistry and Virus Isolation for Diagnosis of West Nile Virus.** *Journal of Clinical Microbiology.* 43(6): 2904-2908. ISSN: 0095-1137.

Abstract: Immunohistochemistry and virus isolation were performed on 1,057 birds. Immunohistochemistry, virus isolation, or both found 325 birds to be West Nile virus positive. Of these, 271 were positive by both methods. These results indicate that virus isolation and immunohistochemistry are approximately equal in their ability to detect West Nile virus.

Descriptors: West Nile virus, diagnosis, birds, virus isolation, immunohistochemistry, comparison.

Ezenwa, V.O., M.S. Godsey, R.J. King, and S.C. Guptill (2006). **Avian diversity and West Nile virus: Testing associations between biodiversity and infectious disease risk.** *Proceedings. Biological Sciences The Royal Society* 273(1582): 109-117.

Abstract: The emergence of several high profile infectious diseases in recent years has focused attention on our need to understand the ecological factors contributing to the spread of infectious diseases. West Nile virus (WNV) is a mosquito-borne zoonotic disease that was first detected in the United States in 1999. The factors accounting for variation in the prevalence of WNV are poorly understood, but recentideas suggesting links between high biodiversity and reduced vector-borne disease risk may help account for distribution patterns of this disease. Since wild birds are the primary reservoir hosts for WNV, we tested associations between passerine (Passeriform) bird diversity, non-passerine (all other orders) bird diversity and virus infection rates in mosquitoes and humans to examine the extent to which bird diversity is associated with WNV infection risk. We found that non-passerine species richness (number of non-passerine species) was significantly negatively correlated with both mosquito and human infection rates, whereas there was no significant association between passerine species richness and any measure of infection risk. Our findings suggest that non-passerine diversity may play a role in dampening WNV amplification rates in mosquitoes, minimizing human disease risk.

Descriptors: biodiversity, birds, West Nile fever transmission, West Nile virus, bird genetics, *Culex sp*., statistical data interpretation, geography, passeriformes, risk factors, West Nile fever epidemiology.

Farajollahi, A., W.J. Crans, D. Nickerson, P. Bryant, B. Wolf, A. Glaser, and T.G. Andreadis (2005). **Detection of West Nile virus RNA from the louse fly *Icosta americana* (Diptera: Hippoboscidae).** *Journal of the American Mosquito Control Association* 21(4): 474-476.

Abstract: West Nile virus (WNV) was detected by Taqman reverse transcription-polymerase chain reaction in 4 of 85 (4.7%) blood-engorged (n = 2) and unengorged

(n = 2) *Icosta americana* (Leach) hippoboscid flies that were collected from wild raptors submitted to a wildlife rehabilitation center in Mercer County, NJ, in 2003. This report represents an additional detection of WNV in a nonculicine arthropod in North America and the first documented detection of the virus in unengorged hippoboscid flies, further suggesting a possible role that this species may play in the transmission of WNV in North America.
Descriptors: West Nile virus, New Jersey, viral RNA analysis, reverse transcriptase polymerase chain reaction, West Nile fever transmission.

Fox, J.L., S.L. Hazell, L.H. Tobler, and M.P. Busch (2006). **Immunoglobulin G avidity in differentiation between early and late antibody responses to West Nile virus.** *Clinical and Vaccine Immunology* 13(1): 33-36. ISSN: 1556-6811.
Abstract: In 1999 West Nile virus (WNV) surfaced in the United States in the city of New York and spread over successive summers to most of the continental United States, Canada, and Mexico. Because WNV immunoglobulin M (IgM) antibodies have been shown to persist for up to 1 year, residents in areas of endemicity can have persistent WNV IgM antibodies that are unrelated to a current illness with which they present. We present data on the use of IgG avidity testing for the resolution of conflicting data arising from the testing of serum or plasma for antibodies to WNV. Thirteen seroconversion panels, each consisting of a minimum of four samples, were used. All samples were tested for the presence of WNV IgM and IgG antibodies, and the avidity index for the WNV IgG-positive samples was calculated. Panels that exhibited a rise in the WNV IgM level followed by a sequential rise in the WNV IgG level were designated "primary." Panels that exhibited a marked rise in the WNV IgG level followed by a sequential weak WNV IgM response and that had serological evidence of a prior flavivirus infection were designated "secondary." All samples from the "primary" panels exhibited low avidity indices (less than 40%) for the first 20 to 30 days after the recovery of the index sample (the sample found to be virus positive). All of the "secondary" samples had elevated WNV IgG levels with avidity indices of > or =55%, regardless of the number of days since the recovery of the index sample. These data demonstrate that it is possible to differentiate between recent and past exposure to WNV or another flavivirus through the measurement of WNV IgG avidity indices.
Descriptors: viral blood antibodies, blood immunoglobulin G, West Nile fever, West Nile virus, biological assay, enzyme linked immunosorbent assay, serologic tests, time factors.

Gallian, P., P. De Micco, X. de Lamballerie, T. Levayer, F. Levacon, P. Guntz, B. Mercier, I. Dupond, C. Cornillot, and G. Andreu (2005). **Prevention of West Nile virus transmission by blood transfusion: a comparison of nucleic acid test screening assays.** *Transfusion* 45(9): 1540-1541.

Descriptors: blood transfusion, mass screening methods, viral RNA, West Nile fever prevention and control, West Nile virus isolation and purification, blood borne pathogens, viral analysis.

Ge, J., B. Chen, M.Y. Zhang, Q.L. Song, Y.X. Zhao, J. Pace, P. Lam, Y. Zhang, C. Obiezu-Forster, H. Goldman, J.H. Keffer, and N. Shaikh (2006). **A novel immunochromatographic assay rapidly detecting West Nile virus-specific IgM in patient's serum and plasma**. *Clinical Chemistry* 52(6, Suppl. S): A41. ISSN: 0009-9147.
Descriptors: West Nile virus detection in serum plasma, immunologic techniques, solid phase sandwich immunoassay, bioassay techniques.
Notes: Meeting Information: 58th Annual Meeting of the American Association of Clinical Chemistry, Chicago, Illinois, USA; July 23 -27, 2006.

Gibbs, S.E., A.E. Ellis, D.G. Mead, A.B. Allison, J.K. Moulton, E.W. Howerth, and D.E. Stallknecht (2005). **West Nile virus detection in the organs of naturally infected blue jays (*Cyanocitta cristata*).** *Journal of Wildlife Diseases* 41(2): 354-362.
Abstract: Blue jays (*Cyanocitta cristata*) are an effective indicator species for West Nile virus (WNV) and may be regionally important in surveillance efforts. The sites of WNV replication and sensitivity of virus detection techniques are undefined for blue jays. The objectives of this study were to describe the gross and microscopic pathology associated with natural WNV infection in blue jays, as well as determine the most appropriate tissues to be used for virus isolation, reverse transcription-nested polymerase chain reaction, and immunohistochemistry (IHC) techniques. Blue jays were collected in Georgia, USA, between May and September 2001. Initial screening by virus isolation indicated that 36 of 59 blue jays chosen for evaluation were WNV positive. From this group, 20 positive and five negative birds were chosen to compare virus detection techniques. Six positive and five negative birds were selected for histopathology examination. Splenomegaly and poor body condition were the most consistent gross findings among positive birds. The most consistent histopathologic findings in the tissues of WNV-positive blue jays were mononuclear leukocytosis and epicarditis/myocarditis. Brain, heart, and lung had the highest viral titers, and WNV antigen was most often detected by IHC in heart, kidney, liver, and lung. Reverse transcription-nested polymerase chain reaction proved to be the most sensitive diagnostic test applied in this study irrespective of the tissue type. Brain tissue could be used effectively for both virus isolation and RT-nPCR, and this tissue is simple to remove and process. The success of IHC is highly dependent on tissue selection, and the use of multiple tissues including heart, kidney, liver, or lung is recommended.
Descriptors: blue jays, *Cyanocitta cristata*, WNV indicator species, disease surveillance, virus detection techniques, immunohistochemistry techniques (IHC), tissue selection, heart, kidney, lung, liver.

Gibbs, S.E., D.M. Hoffman, L.M. Stark, N.L. Marlenee, B.J. Blitvich, B.J. Beaty, and D.E. Stallknecht (2005). **Persistence of antibodies to West Nile virus in naturally infected rock pigeons (*Columba livia*).** *Clinical and Diagnostic Laboratory Immunology* 12(5): 665-667.

Abstract: Wild caught rock pigeons (*Columba livia*) with antibodies to West Nile virus were monitored for 15 months to determine antibody persistence and compare results of three serologic techniques. Antibodies persisted for the entire study as detected by epitope-blocking enzyme-linked immunosorbent assay and plaque reduction neutralization test. Maternal antibodies in squabs derived from seropositive birds persisted for an average of 27 days.

Descriptors: viral blood antibodies, West Nile fever, West Nile virus transmission, wild animals, disease transmission, enzyme-linked immunosorbent assay, plaque assay.

Gibbs, S., A. Ellis, D. Mead, A. Allison, J. Moulton, E. Howerth, and D. Stallknecht (2005). **West Nile virus detection in the organs of naturally infected blue jays (*Cyanocitta cristata*).** *Journal of Wildlife Diseases.* 41(2): 354-362. ISSN: 0090-3558.

Descriptors: wild birds, sentinel animals, disease detection, immunohistochemistry, reverse transcriptase polymerase chain reaction, Georgia, blue jays, West Nile virus.

Godhardt, J.A., K. Beheler, M.J. O'Connor, T.J. Whyte, E.S. Reisdorf, S.J. Ubl, P.N. Bochsler, and K.L. Toohey Kurth (2006). **Evaluation of antigen-capture ELISA and immunohistochemical methods for avian surveillance of West Nile virus.** *Journal of Veterinary Diagnostic Investigation* 18(1): 85-89.

Abstract: Accurate detection of West Nile virus (WNV) in corvids is essential for monitoring the spread of virus during the mosquito season. Viremia in corvids is very high, with titers approaching 10(8) viral particles/ml. In the presence of such marked viremia, the sensitivity of real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis is unnecessary, and more cost-effective methods should be assessed. To this end, antigen-capture ELISA (ACE) and immunohistochemical (IHC) assays were evaluated. Skin, cloacal swab specimens, and feathers from corvids were tested by use of ACE, and results were compared with results obtained from use of real-time RT-PCR analysis. Of the 3 sample types, skin gave the best sensitivity (98%) and specificity (100%). Skin, brain, kidney, and spleen from corvids were analyzed by IHC, and results were compared with real-time RT-PCR results. Kidney and spleen were more often positive by use of IHC than were brain and skin tissue; however, IHC did not perform as well as ACE in the identification of virus-positive birds. Results of this study support the use of a skin sample in an ACE format as an effective surveillance method for corvids.

Descriptors: West Nile virus, ELISA, avian surveillance, antigen capture, evaluation, immunohistochemical methods, accurate detection.

Herrmann, S., B. Leshem, S. Landes, B. Rager-Zisman, and R.S. Marks (2005). **Chemiluminescent optical fiber immunosensor for the detection of anti-West Nile virus IgG**. *Talanta* 66(1): 6-14. ISSN: 0039-9140.
Descriptors: West Nile virus IgG, chemiluminescent optical fiber immunosensor, clinical techniques, diagnostic techniques, ELISA, disease detection.

Hukkanen, R.R., H.D. Liggitt, S.T. Kelley, R. Grant, D. Anderson, B.J. Beaty, N.L. Marlenee, R.A. Hall, and H. Bielefeldt Ohmann (2006). **Comparison of commercially available and novel West Nile virus immunoassays for detection of seroconversion in pig-tailed macaques (*Macaca nemestrina*).** *Comparative Medicine* 56(1): 46-54.
Abstract: We report the assessment and validation of an NS1 epitope-blocking enzyme-linked immunosorbent assay (ELISA) for detection of antibodies to West Nile virus (WNV) in macaques. Sera from naturally infected *Macaca nemestrina* were tested by ELISA and plaque reduction neutralization test (PRNT). Results were correlated with hemagglutination inhibition (HAI) data. Our results demonstrate that the blocking ELISA rapidly and specifically detects WNV infection in *M. nemestrina*. In addition, the diagnostic value of 7 commercially available immunoassays (PanBio immunoglobulin [Ig] M ELISA, PanBio IgG ELISA, PanBio immunofluorescence assay (IFA), InBios IgG ELISA, InBios IgM ELISA, Focus Diagnostics IgG ELISA, and Focus Diagnostics IgM ELISA) in *M. nemestrina* was evaluated and compared with that of the epitope-blocking ELISA. The PanBio IgG ELISA was found to effectively diagnose WNV exposure in *M. nemestrina*. Further, PanBio IFA slides are fast and reliable screening tools for diagnosing flaviviral exposure in *M. nemestrina*.
Descriptors: West Nile virus, immunoassays, detection, pig-tailed macques, NS1 epitope-blocking enzyme-linked immunosorbent assay (ELISA), antibodies, plaque reduction neutralization test (PRNT).

Jacobson, E.R., A.J. Johnson, J.A. Hernandez, S.J. Tucker, A.P.2. Dupuis, R. Stevens, D. Carbonneau, and L. Stark (2005). **Validation and use of an indirect enzyme-linked immunosorbent assay for detection of antibodies to West Nile virus in American Alligators (*Alligator mississippiensis*) in Florida.** *Journal of Wildlife Diseases* 41(1): 107-114.
Abstract: In October 2002, West Nile virus (WNV) was identified in farmed American alligators (*Alligator mississippiensis*) in Florida showing clinical signs and having microscopic lesions indicative of central nervous system disease. To perform seroepidemiologic studies, an indirect enzyme-linked immunosorbent assay (ELISA) was developed to determine exposure of captive and wild alligators to WNV. To validate

the test, a group of WNV-seropositive and -seronegative alligators were identified at the affected farm using hemagglutination inhibition (HAI) and the plaque reduction neutralization test (PRNT). The indirect ELISA utilized a rabbit anti-alligator immunoglobulins polyclonal antibody as the secondary antibody, and inactivated WNV-infected Vero cells were used as the coating antigen. For all samples (n=58), the results of the ELISA were consistent with the HAI and PRNT findings. Plasma was collected from 669 free-ranging alligators from 21 sites across Florida in April and October 2003. Four samples collected in April and six in October were positive for WNV antibodies using HAI, PRNT, and the indirect ELISA. This indicated that wild alligators in Florida have been exposed to WNV. These findings can be used as a baseline for future surveys.
Descriptors: alligators, crocodiles, West Nile fever, West Nile virus, disease reservoirs, enzyme linked immunosorbent assay methods, Florida, seroepidemiologic studies.

Jiang ShuFang, Zhang YingMei, Zhao TongYan, and Dong YanDe (2006). **Establishment and application of plaque assay for the detection of West Nile virus**. *Chinese Journal of Vector Biology and Control* 17(2): 81-82. ISSN: 1003-4692.
Descriptors: West Nile virus, quantitative detection, plaque assay, animal models, vero cells, poultry, agarose overlay, *Culex pipiens*.
Language of Text: Chinese; Summary in English.

Jiang Yan, Zhang ChangYin, and Chen GuoQiang (2005). **Construction of RT-PCR for detection of West Nile virus**. *Chinese Journal of Animal Quarantine* 22(5): 25-27. ISSN: 1005-944x.
Descriptors: West Nile virus, detection, RT-PCR, reverse transcription polymerase chain reaction, E gene.
Language of Text: Chinese; Summary in English.

Johnson, A.J., A.J. Noga, O. Kosoy, R.S. Lanciotti, A.A. Johnson, and B.J. Biggerstaff (2005). **Duplex microsphere-based immunoassay for detection of anti-West Nile virus and anti-St. Louis encephalitis virus immunoglobulin M antibodies**. *Clinical and Diagnostic Laboratory Immunology* 12(5): 566-574. ISSN: 1071-412X.
Descriptors: West Nile virus, St. Louis virus, viral detection, ELISA, immunoglobulin, IgM, duplex microsphere based immunoassay.

Korves, C.T., S.J. Goldie, and M.B. Murray (2006). **Cost-effectiveness of alternative blood-screening strategies for West Nile Virus in the United States.** *PLoS Medicine* 3(2): E21.
Online: http://medicine.plosjournals.org/perlserv/?request=get-document&doi=10.1371/journal.pmed.0030021

Abstract: BACKGROUND: West Nile virus (WNV) is endemic in the US, varying seasonally and by geographic region. WNV can be transmitted by blood transfusion, and mandatory screening of blood for WNV was recently introduced throughout the US. Guidelines for selecting cost-effective strategies for screening blood for WNV do not exist. METHODS AND FINDINGS: We conducted a cost-effectiveness analysis for screening blood for WNV using a computer-based mathematical model, and using data from prospective studies, retrospective studies, and published literature. For three geographic areas with varying WNV-transmission intensity and length of transmission season, the model was used to estimate lifetime costs, quality-adjusted life expectancy, and incremental cost-effectiveness ratios associated with alternative screening strategies in a target population of blood-transfusion recipients. We compared the status quo (baseline screening using a donor questionnaire) to several strategies which differed by nucleic acid testing of either pooled or individual samples, universal versus targeted screening of donations designated for immunocompromised patients, and seasonal versus year-long screening. In low-transmission areas with short WNV seasons, screening by questionnaire alone was the most cost-effective strategy. In areas with high levels of WNV transmission, seasonal screening of individual samples and restricting screening to blood donations designated for immunocompromised recipients was the most cost-effective strategy. Seasonal screening of the entire recipient pool added minimal clinical benefit, with incremental cost-effectiveness ratios exceeding USD 1.7 million per quality-adjusted life-year gained. Year-round screening offered no additional benefit compared to seasonal screening in any of the transmission settings. CONCLUSIONS: In areas with high levels of WNV transmission, seasonal screening of individual samples and restricting screening to blood donations designated for immunocompromised recipients is cost saving. In areas with low levels of infection, a status-quo strategy using a standard questionnaire is cost-effective.
Descriptors: mass screening economics, West Nile fever, blood donors, blood transfusion, cost benefit analysis, immunocompromised host, econometric models, prospective studies, quality adjusted life years, retrospective studies, seasons, United States.
Notes: Comment In: PLoS Med. 2006 Feb;3(2):e99.

Kuehn, B.M. (2006). **Studies propose targeted screening of blood for West Nile virus.** *JAMA the Journal of the American Medical Association* 295(11): 1235-1236.
Descriptors: blood bank standards, blood donors, West Nile fever diagnosis and transmission, West Nile virus isolation and purification, blood transfusion, mass screening, United States.

Lampman, R.L., N.M. Krasavin, M. Szyska, and R.J. Novak (2006). **A comparison of two West Nile virus detection assays (TaqMan reverse transcriptase polymerase chain reaction and VecTest antigen assay) during three consecutive outbreaks in northern Illinois.** *Journal of the American Mosquito Control Association* 22(1): 76-86.

Abstract: Mosquitoes identified as female Culex (Culex) species, primarily mixtures or uniform batches of *Culex pipiens* and *Culex restuans*, were collected daily from gravid traps by 2 mosquito abatement districts (MADs) in Cook County, Illinois. From 2002 through 2004, batches (pools) of mosquitoes were tested by the MADs for West Nile virus (WNV) by using VecTest WNV antigen assays and the same samples were retested, usually within 1-2 wk, for WNV RNA by the TaqMan reverse transcriptase polymerase chain reaction (RT-PCR). There were 952 TaqMan-positive pools out of 3,953 pools over the 3 years, and about one half of that number were VecTest-positive. The difference between the 2 detection assays varied between and within years. The VecTest assays detected about 57% and 69% of the TaqMan RT-PCR-positive pools from Des Plaines Valley MAD and Northwest MAD in 2002, but only about 40% and 46% in 2003, and 36% and 55% in 2004, respectively. Based on a subset of the 2004 data, a linear relationship was found between VecTest detection of WNV and TaqMan cycle threshold between 18 and 28 cycles. A temporal decrease in the difference between the 2 assays was observed in 2003 and 2004, which we conjecture is due, at least partially, to a seasonal decline in the proportion of recently infected mosquitoes. This trend was not observed in 2002 because infection rates indicated a high likelihood of more than 1 infected mosquito per pool at the peak of transmission. Unlike a previous study, the 95% confidence intervals of infection rates based on the 2 detection methods did not always overlap. The highest infection rates occurred in 2002 when mean monthly temperatures were above average.

Descriptors: *Culex sp*., reverse transcriptase polymerase chain reaction, West Nile virus, disease outbreaks, Illinois, insect vectors, seasons, temperature, West Nile fever epidemiology.

Lee, B.Y. and B.J. Biggerstaff (2006). **Screening the United States blood supply for West Nile Virus: A question of blood, dollars, and sense.** *PLoS Medicine* 3(2): E99.

Online: http://medicine.plosjournals.org/perlserv/?request=get-document&doi=10.1371/journal.pmed.0030099

Descriptors: mass screening, West Nile fever, blood bank standards, blood donors, blood transfusion, cost of illness, cost-benefit analysis, health policy, seasons, specimen handling.

Notes: Comment On: PLoS Med. 2006 Feb;3(2):E21.

Lee, D.H., J. Mathew, W. Pfahler, D. Ma, J. Valinsky, A.M. Prince, and L. Andrus (2005). **Individual Donor Nucleic Acid Amplification Testing for Detection of West Nile Virus.** *Journal of Clinical Microbiology.* 43(10): 5111-5116. ISSN: 0095-1137.
Abstract: We have developed an economical, high-throughput nucleic acid amplification test (NAT) for blood-borne viruses, suitable for use in the screening of plasma samples from individual blood donors. This assay system includes a semiautomated procedure, using 96-well glass fiber plates for the extraction of viral nucleic acids from plasma and "universal beacon" technology which permits the detection of all genotypes of highly variable viruses (e.g., human immunodeficiency virus and hepatitis C virus). In this detection system, two fluorescent- detection technologies were employed successfully in a single tube: molecular beacon for West Nile virus (WNV) detection using a 6-carboxyfluorescein fluorophore and TaqMan for internal control detection using a VIC fluorophore. To establish proof of concept, we focused on the development of a robust individual donor NAT for WNV. The assay showed no reactivity to 15 other viruses tested or to 420 blood donor samples from the WNV pre-epidemic season. No cross-contamination was observed on an alternating positive-/negative-well test. The sensitivity (limit of detection, 95%) of the assay for WNV is between 3.79 and 16.3 RNA copies/ml, depending on which material was used as a standard. The assay detected all positive blood donation samples identified by the Roche WNV NAT. The assay can be performed qualitatively for screening and quantitatively for confirmation.
Descriptors: West Nile virus, detection, testing, nucleic acid amplification, blood born viruses, assay, screening, plasma samples.

Levett, P.N., K. Sonnenberg, F. Sidaway, S. Shead, M. Niedrig, K. Steinhagen, G.B. Horsman, and M.A. Drebot (2005). **Use of Immunoglobulin G Avidity Assays for Differentiation of Primary from Previous Infections with West Nile Virus.** *Journal of Clinical Microbiology.* 43(12): 5873-5875. ISSN: 0095-1137.
Abstract: Since its introduction in 1999, West Nile virus (WNV) infections have spread rapidly across the North American continent. Diagnosis of acute WNV infection by detection of WNV-specific immunoglobulin M (IgM) is complicated by the persistence of detectable IgM for more than 1 year in some patients. IgG antibody avidity testing was assessed as a supplemental assay in the diagnosis of current infections. Three groups of serum samples were assayed in parallel by two different IgG avidity test systems (indirect immunofluorescence test [IIFT] and prototype enzyme-linked immunosorbent assay [ELISA]; EUROIMMUN, Luebeck, Germany). Group I (40 sera taken between 2 and 9 days after the onset of influenza-like symptoms) and group II (40 sera taken between 10 and 43 days after onset) were acute and convalescent specimens from patients with a positive anti-WNV IgM test (ELISA; Focus Diagnostics, Cypress, CA). Group III consisted of 43 patient sera collected between

6 and 12 months after infection. IgG antibodies specific for WNV were detected in 38% (ELISA) and 50% (IIFT) of group I sera, in 90% (ELISA and IIFT) of group II sera, and in 100% (ELISA and IIFT) of group III sera. Low-avidity IgG antibodies were demonstrated in 86% (ELISA) and 95% (IIFT) of IgG-positive patient samples taken between 2 and 43 days after the onset of symptoms (groups I and II). High-avidity IgG antibodies were detected in 100% of group III sera obtained 6 months or more after the onset of symptoms (ELISA and IIFT). IgG avidity tests for WNV infections are rapid and simple to perform. The determination of IgG avidity provides additional diagnostic certainty in differentiating between recently acquired and previous infections with WNV.
Descriptors: West Nile virus, diagnosis, detection, specific immunoglobulin, IgM, differentiate, previous infection, recent infection.

Li, L., A. Barrett, and D. Beasley (2005). **Differential expression of domain III neutralizing epitopes on the envelope proteins of West Nile virus strains.** *Virology* 335(1): 99-105. ISSN: 0042-6822.
Descriptors: West Nile virus, viral proteins, viral antigens, epitopes, neutralization, amino acid sequences, antigenic variation, nucleotide sequences, structure activity relationships, viral-envelope-protein, viral-antibodies, molecular-sequence-data, Internet-resource.

Lim, C.K. and I. Kurane (2005). **[Diagnostic tests: West Nile virus]**. *Japanese Journal of Clinical Medicine* 63(Suppl 7): 321-323.
Descriptors: viral blood antibodies, immunoglobulin M blood, RNA, viral isolation and purification, reverse transcriptase polymerase chain reaction, serologic tests, West Nile fever diagnosis, West Nile virus isolation, enzyme linked immunosorbent assay (ELISA) methods, neutralization tests, zoonoses.
Language of Text: Japanese.

Long, M.T., W. Jeter, J. Hernandez, D.C. Sellon, D. Gosche, K. Gillis, E. Bille, and E.P. Gibbs (2006). **Diagnostic performance of the equine IgM capture ELISA for serodiagnosis of West Nile virus infection.** *Journal of Veterinary Internal Medicine* 20(3): 608-613.
Abstract: The objectives of these studies were to assess the diagnostic performance (sensitivity and specificity) of the IgM capture enzyme-linked immunosorbent assay (ELISA; MAC) for diagnosis of West Nile (WN) virus in horses and to examine the performance of this test by using different criteria for seropositivity. A total of 36 horses classified as WN virus infected (group 1) and 383 horses from 4 subpopulations of hoses classified as noninfected (groups 2, 3, 4, and 5) were used in the study. The sensitivity (proportion of infected horses that tested positive for WN virus IgM antibodies) and specificity (proportion of noninfected horses that tested negative)

were calculated at different cutoff points by using receiver operating curve (ROC) analysis. Using a selected cutoff point = 2.0, the sensitivity and specificity of the MAC were 91.7 and 99.2%, respectively. The area under the ROC curve = 0.95 (95% confidence interval [CI], 0.89 to 1.0), suggesting that the MAC is a useful tool for diagnosis of recent WN virus exposure in horses. In fulfillment of the 2nd objective, 2 other indices were developed and these indices approached 1.0 for the AUC with smaller 95% CIs. These indices were then used to test 602 additional diagnostic samples submitted from suspect horses between 2002 and 2004. Using the standard cutoff, 194 (32%) of the horses were interpreted as positive. Utilizing newly predicted cutoff criteria from each index, additional horses were identified as positive. In conclusion, the MAC as used for identification of WN virus-diseased horses undergoing recent exposure performs reliably at the standard cutoff for seropositivity. A negative test might not completely rule out WN virus disease, but horses that test negative were most likely not exposed to WNV. Performance of the test can be further improved by investigation of other indexes of seropositivity.
Descriptors: sensitivity and specificity of the IgM capture enzyme-linked immunosorbent assay (ELISA), diagnosis of West Nile virus, horses, seropositivity, predictive value of tests, serologic tests, West Nile fever.

Mandalakas, A.M., C. Kippes, J. Sedransk, J.R. Kile, A. Garg, J. McLeod, R.L. Berry, and A.A. Marfin (2005). **West Nile virus epidemic, northeast Ohio, 2002.** *Emerging Infectious Diseases* 11(11): 1774-1777. ISSN: 1080-6040.
Abstract: Serum samples and sociodemographic data were obtained from 1,209 Ohio residents. West Nile virus immunoglobulin M (IgM) and IgG antibodies were detected by enzyme-linked immunosorbent assay and confirmed. Children were 4.5 times more likely to become infected yet 110 times less likely to have neuroinvasive disease develop.
Descriptors: viral blood antibodies, disease outbreaks, West Nile fever epidemiology, West Nile virus, humans, enzyme linked immunosorbent assay, blood immunoglobulin G, blood immunoglobulin M, Ohio.

Marciniak, C. and E.L. Rosenfeld (2005). **Serial electrodiagnostic studies in West Nile virus-associated acute flaccid paralysis.** *American Journal of Physical Medicine and Rehabilitation* 84(11): 904-910.
Abstract: A man in his 70s presented for acute rehabilitation with severe acute flaccid asymmetric weakness in both lower limbs. Cerebrospinal fluid and serum immunoglobulin M titers were positive for West Nile virus. Electrodiagnostic studies demonstrated severe diffuse motor axonopathy consistent with an anterior myelitis. Electrodiagnostic and clinical improvements were monitored. Electrodiagnostic testing at 6 and 18 mos demonstrated continuing reinnervation; nascent voluntary motor unit action potentials were first noted proximally and, at 18 mos, distally in

the left lower limb, including muscles in which motor unit potentials were not initially noted. Corresponding clinical improvements, though slow, were demonstrated even at 1(1/2) yrs after onset. Thus, motoric changes after West Nile virus-associated anterior myelitis need to be monitored over a prolonged time period to allow accurate assessment of prognosis for recovery in rehabilitation programs.
Descriptors: myelitis, West Nile fever complications , West Nile virus isolation and purification, acute disease, viral cerebrospinal fluid, muscle weakness, skeletal muscle, paraplegia rehabilitation, time factors.

Mawhorter, S.D., A. Sierk, S.M. Staugaitis, R.K. Avery, R. Sobecks, R.A. Prayson, G.W. Procop, and B. Yen Lieberman (2005). **Fatal West Nile Virus infection after rituximab/fludarabine--induced remission for non-Hodgkin's lymphoma.** *Clinical Lymphoma and Myeloma* 6(3): 248-250.
Abstract: West Nile virus (WNV) infections are potentially life threatening in immunocompromised hosts. Currently, the best diagnostic test is serology. Reverse-transcriptase polymerase chain reaction (RT-PCR) testing has a role, but, because WNV is a cell-associated neurotropic virus, RT-PCR results are frequently negative even in cases of active infection. We present a case in which serology results were persistently negative because the patient was immunocompromised following lymphoma treatment. The role of humoral immunity in resolution of WNV is also discussed.
Descriptors: monoclonal antibodies, administration and dosage, antineoplastic agents, follicular lymphoma, West Nile fever, West Nile virus fatal outcome , lymphoma, follicular drug therapy, remission , vidarabine administration and dosage.

Mazurek, J.M., K. Winpisinger, B.J. Mattson, R. Duffy, and R.L. Moolenaar (2005). **The epidemiology and early clinical features of West Nile virus infection.** *American Journal of Emergency Medicine* 23(4): 536-543.
Abstract: We studied early clinical features of the West Nile virus (WNV) infection. Case patients were Ohio residents who reported to the Ohio Department of Health from August 14 to December 31, 2002, with a positive serum or cerebrospinal fluid for anti-WNV IgM. Of 441 WNV cases, medical records of 224 (85.5%) hospitalized patients were available for review. Most frequent symptoms were fever at a temperature of 38.0 degrees C or higher (n = 155; 69.2%), headache (n = 114; 50.9%), and mental status changes (n = 113; 50.4%). At least one neurological symptom, one gastrointestinal symptom, and one respiratory symptom was present in 186 (83.0%), 119 (53.1%), and 46 (20.5%) patients, respectively. Using multivariate logistic regression and controlling for age, we found that the initial diagnosis of encephalitis (P = .001) or reporting abdominal pain (P < .001) was associated with death. Because initial symptoms of WNV infection are not specific, physicians should maintain a high index of suspicion during the epidemic season, particularly in elderly patients with compatible symptoms.

Descriptors: West Nile fever, hospital statistics, numerical data, encephalitis, guillain barre syndrome, meningitis, Ohio, health care, humans.

Meece, J.K., T.A. Kronenwetter Koepel, M.F. Vandermause, and K.D. Reed (2006). **West Nile virus infection in commercial waterfowl operation, Wisconsin.** *Emerging Infectious Diseases* 12(9): 1451-1453. ISSN: 1080-6040 .

Abstract: A West Nile virus (WNV) outbreak occurred at a commercial waterfowl operation in Wisconsin in 2005. Retrospective analysis of dead and live birds was conducted. WNV was detected by PCR in 84.1% of 88 dead birds; neutralizing antibodies were found in 14 of 30 randomly sampled asymptomatic or recovered birds.

Descriptors: animal husbandry, disease outbreaks, ducks, geese, poultry diseases epidemiology , West Nile fever, Wisconsin.

Murgai, M., M. Mayda, M. Rios, R. Hammamieh, and M. Jett (2006). **Gene expression analysis of the West Nile virus and the Dengue serotypes**. *FASEB Journal* 20(4, Part 1): A62. ISSN: 0892-6638.

Descriptors: West Nile virus, Dengue fever, vector borne diseases, meningitis, encephalitis, microarray gene expression analysis, genetic technique, cDNA microarrays.

Notes: Meeting Information: Experimental Biology 2006 Meeting, San Francisco, California, USA; April 01 -05, 2006.

Niedrig, M., S. Linke, H. Zeller, and C. Drosten (2006). **First international proficiency study on West Nile virus molecular detection.** *Clinical Chemistry* 52(10): 1851-185.

Abstract: BACKGROUND: West Nile virus (WNV) molecular detection is being conducted by a growing number of laboratories, but the degree of proficiency may vary between them. External quality control is needed. METHODS: We have conducted an international quality assurance study on WNV molecular detection. Participating laboratories tested noninfectious samples inactivated by heat and gamma irradiation. Participants received 7 coded lyophilized samples containing WNV of genetic lineages 1a, 1b, and 2 at 2600 to 18 000 000 RNA copies/mL, 3 samples containing heterologous flaviviruses, and 2 negative samples. RESULTS: Thirty laboratories participated. The average laboratory achieved 50% detection probability from 7762 copies/mL onward (probit analysis; 95% CI = 1174-24547 copies/mL). Lineages 1a and 1b were detected with equal efficiencies, but the lineage 2 strain (Ug37) was detected at significantly lower rates. Only 27% of participants were able to detect the 6 samples containing > or =1.8 x 10(4) copies/mL. Three laboratories generated false-positive results in negative samples. Six of 30 laboratories reported correct strain identification in 3 samples containing non-WNV flaviviruses. We observed a significant positive correlation between the capability of detecting

non-WNV flaviviruses and detecting WNV lineage 2. CONCLUSIONS: Most participants showed good performance in detecting lineage 1 WNV, the predominant virus in the Northern Hemisphere. The inability of some laboratories to detect even highly concentrated lineage 2 WNV downgraded the overall outcome. The lineage 2 material received through this study will provide laboratories with the necessary template for improving their assays. Such material is otherwise hard to obtain.
Descriptors: West Nile virus, *Cercopithecus aethiops*, false positive reactions, international cooperation, molecular diagnostic techniques, quality control, vero cells, virology methods.

Niedrig, M., K. Sonnenberg, K. Steinhagen, and J.T. Paweska (2007). **Comparison of ELISA and immunoassays for measurement of IgG and IgM antibody to West Nile virus in human sera against virus neutralisation.** *Journal of Virological Methods* 139(1): 103-105.
Abstract: Two commercial assays for the detection of IgG antibody to West Nile virus (WNV), an indirect enzyme-linked immunosorbent assay (I-ELISA) and indirect fluorescent antibody test (IFAT), were evaluated against the virus neutralisation test. Excellent agreement with the virus neutralisation was obtained with both tests, i.e., 99.5% by I-ELISA and 100% by IFAT. The well-known serological cross-reactivity within the family of the Flaviviridae was analysed using sera with known antibodies against dengue virus, tick borne encephalitis virus and yellow fever virus. IgM and/or IgG positive sera were examined for reactivity by WNV-ELISA and WNV-IFAT. While cross-reactivity between 0 and 18.2% was recorded with IgM positive sera, there was extensive cross-reactivity of 15.7-100% with IgG positive sera.
Descriptors: West Nile virus, antibody, IgG, IgM, immunoassays, ELISA, camparison, indirect fluorescent antibody test.

Nixon, M.L. and H.E. Prince (2006). **West Nile virus immunoglobulin A (WNV IgA) detection in cerebrospinal fluid in relation to WNV IgG and IgM reactivity.** *Journal of Clinical Virology* 37(3): 174-178.
Abstract: BACKGROUND: Diagnostic criteria for neurologic involvement in WNV infection include WNV IgM detection in CSF; however, WNV IgM can persist in CSF >6 months. CSF IgA characterizes other flavivirus infections, but WNV IgA in CSF has not been evaluated. WNV IgM in CSF correlates with IgM in serum but the presence of WNV IgA in CSF compared to serum is unknown. OBJECTIVES: Evaluate WNV IgA detection in CSF as a marker of WNV neuroinvasive infection, initially with samples pre-selected based on WNV IgG and IgM reactivity and subsequently with all available CSF samples submitted for WNV antibody testing over an entire WNV season. STUDY DESIGN: Selected CSF samples and CSF/serum pairs previously tested for WNV IgG and IgM were assayed for WNV IgA. Subsequently, all available CSF samples tested for WNV antibod-

ies during the 2005 season were tested for WNV IgA, including those where paired sera were available and tested for IgA, IgG and IgM. RESULTS: For most samples, including paired CSF and serum, the IgA result qualitatively agreed with the IgM result, regardless of the IgG result. CONCLUSION: IgA detection is equivalent to IgM detection as a marker of WNV infection in CSF.
Descriptors: West Nile virus, detection, cerebrolspinal fluid, IgM, IgA, diagnostic criteria.

Ohajuruka, O.A., R.L. Berry, S. Grimes, and S. Farkas (2005). **West Nile Virus detection in kidney, cloacal, and nasopharyngeal specimens.** *Emerging Infectious Diseases* 11(9): 1437-1439. ISSN: 1080-6040 .
Abstract: We compared kidney tissue samples and cloacal and nasopharyngeal swab samples from field-collected dead crows and blue jays for West Nile virus surveillance. Compared to tissue samples, 35% more swab samples were false negative. Swab samples were usually positive only when the corresponding tissue sample was strongly positive.
Descriptors: cloaca, crow, kidney, nasopharynx, West Nile virus isolation and purification, false negative reactions, linear models, Ohio , population surveillance methods, reverse transcriptase polymerase chain reaction.

Ollis, G., L. Morin, and A. Visser (2005). **Laboratory confirmed positive cases of equine West Nile virus in Alberta in 2003.** *Canadian Veterinary Journal* 46(2): 131-133. ISSN: 0008-5286.
Descriptors: horse diseases, West Nile virus, disease incidence, geographical distribution , symptoms, horses, risk factors, disease surveillance, disease detection, diagnostic techniques, blood serum, immunoglobulin M, enzyme linked immunosorbent assay, disease diagnosis, Alberta.

Padgett, K.A., B. Cahoon Young, R. Carney, L. Woods, D. Read, S. Husted, and V. Kramer (2006). **Field and laboratory evaluation of diagnostic assays for detecting West Nile virus in oropharyngeal swabs from California wild birds.** *Vector Borne and Zoonotic Diseases* 6(2): 183-191. ISSN: 1530-3667.
Abstract: Three diagnostic assays for detecting West Nile virus (WNV) in avian oral swabs were evaluated in California in 2004 and 2005: two commercial antigen-capture assays, VecTest and Rapid Analyte Measurement Platform (RAMP), and reverse transcriptase-polymerase chain reaction (RT-PCR) of oral swabs in a specialized viral transport medium (VTM). Results from this study demonstrated that VTM was excellent for transportation and maintenance of WNV in avian oral swab samples and allowed for detection by RT-PCR and subsequent confirmation by virus isolation. Oral swabs and kidney tissue in VTM tested by RT-PCR were found to have similar accuracy in detecting WNV in corvids. The two antigen-capture assays,

VecTest and RAMP, provided few false positives for corvids, with over 95% specificity. When performed by multiple local agencies throughout the state, VecTest and RAMP were similarly sensitive for oral swabs of American Crows (*Corvus brachyrhynchos*) (70% and 64%, respectively). Data from known WNV positive corvid oral swabs in VTM tested by antigen-capture assays at a diagnostic laboratory suggested that RAMP was more sensitive than VecTest. Due to high probability of false negatives, neither test is recommended for use on non-corvids. While WNV antigen-capture assays were effective screening tools for corvids, they were markedly less sensitive for Western Scrub Jays (*Aphelocoma californica*).
Descriptors: bird diseases, oropharynx, West Nile fever, West Nile virus, false negative reactions, false positive reactions, reproducibility of results, reverse transcriptase polymerase chain reaction methods, species specificity.

Panella, N.A., K.L. Burkhalter, S.A. Langevin, A.C. Brault, L.M. Schooley, B.J. Biggerstaff, R.S. Nasci, and N. Komar (2005). **Rapid West Nile virus antigen detection.** *Emerging Infectious Diseases* 11(10): 1633-1635. ISSN: 1080-6040.
Abstract: We compared the VecTest WNV antigen assay with standard methods of West Nile virus (WNV) detection in swabs from American Crows (*Corvus brachyrhynchos*) and House Sparrows (*Passer domesticus*). The VecTest detected WNV more frequently than the plaque assay and was comparable to a TaqMan reverse transcription-polymerase chain reaction.
Descriptors: viral analysis, bird diseases, diagnostic reagent kits, West Nile fever, West Nile virus isolation and purification, crows, plaque assay, reverse transcriptase polymerase chain reaction, sparrows, specimen handling methods, time factors.

Pierson, T.C., M.D. Sanchez, B.A. Puffer, A.A. Ahmed, B.J. Geiss, L.E. Valentine, L.A. Altamura, M.S. Diamond, and R.W. Doms (2006). **A rapid and quantitative assay for measuring antibody-mediated neutralization of West Nile virus infection.** *Virology* 346(1): 53-65. ISSN: 0042-6822.
Abstract: West Nile virus (WNV) is a neurotropic flavivirus within the Japanese encephalitis antigenic complex that is responsible for causing West Nile encephalitis in humans. The surface of WNV virions is covered by a highly ordered icosahedral array of envelope proteins that is responsible for mediating attachment and fusion with target cells. These envelope proteins are also primary targets for the generation of neutralizing antibodies in vivo. In this study, we describe a novel approach for measuring antibody-mediated neutralization of WNV infection using virus-like particles that measure infection as a function of reporter gene expression. These reporter virus particles (RVPs) are produced by complementation of a sub-genomic replicon with WNV structural proteins provided in trans using conventional DNA expression vectors. The precision and accuracy of this approach stem from an ability to measure the outcome of the interaction between antibody and viral antigens under conditions

that satisfy the assumptions of the law of mass action as applied to virus neutralization. In addition to its quantitative strengths, this approach allows the production of WNV RVPs bearing the prM-E proteins of different WNV strains and mutants, offering considerable flexibility for the study of the humoral immune response to WNV in vitro. WNV RVPs are capable of only a single round of infection, can be used under BSL-2 conditions, and offer a rapid and quantitative approach for detecting virus entry and its inhibition by neutralizing antibody.
Descriptors: viral antibodies immunology, West Nile virus, cell line, *Cercopithecus aethiops*, cricetinae, reporter genes, neutralization tests, time factors, viral envelope proteins, West Nile fever immunology.

Prince, H.E. and M. Lape Nixon (2005). **Evaluation of a West Nile virus immunoglobulin A capture enzyme-linked immunosorbent assay.** *Clinical and Diagnostic Laboratory Immunology* 12(1): 231-233.
Abstract: An in-house-developed enzyme-linked immunosorbent assay detected West Nile virus (WNV) immunoglobulin A (IgA) in 65 of 68 sera from WNV-infected patients; 40 of 63 WNV IgM-positive, IgG-negative serum or plasma specimens; 65 of 67 WNV IgM-positive, IgG-positive specimens; 0 of 70 WNV IgM-negative, IgG-negative specimens; and 0 of 64 archived blood donation sera. WNV IgA is thus highly prevalent among WNV-infected patients and typically appears after WNV IgM but before WNV IgG.
Descriptors: enzyme-linked immunosorbent assay, immunoglobulin A immunology, West Nile fever, West Nile virus, immunoglobulin G, immunoglobulin M, reproducibility of results.

Prince, H.E., M. Lape Nixon, M.P. Busch, L.H. Tobler, G.A. Foster, and S.L. Stramer (2005). **Utilization of follow-up specimens from viremic blood donors to assess the value of west nile virus immunoglobulin G avidity as an indicator of recent infection.** *Clinical and Diagnostic Laboratory Immunology* 12(9): 1123-1126.
Abstract: The value of West Nile virus immunoglobulin G avidity for distinguishing recent from past infection was investigated using 348 follow-up specimens from 170 viremic blood donors. Low avidity accurately indicated infection within the previous 4 months. However, due to rapid avidity maturation in some individuals, high avidity did not accurately indicate past infection.
Descriptors: blood donors, West Nile fever, West Nile virus antibodies, antibody affinity, follow-up studies, blood immunoglobulin G.

Reds-Study-Grp (2005). **Detection of West Nile virus RNA and antibody in frozen plasma components from a voluntary market withdrawal during the 2002 peak epidemic**. *Transfusion (Malden)* 45(4): 480-486. ISSN: 0041-1132.

Descriptors: West Nile virus, frozen blood components, viremia, serologic markers, RNA, IgM, risk factors.

Reisen, W.K., S.S. Wheeler, S. Yamamoto, Y. Fang, and S. Garcia (2005). **Nesting Ardeid colonies are not a focus of elevated West Nile virus activity in southern California.** *Vector Borne and Zoonotic Diseases* 5(3): 258-266. ISSN: 1530-3667.

Abstract: A large nesting colony of Ardeid birds at the Finney-Ramer Wildlife Refuge in Imperial County, California, did not appear to be a focus of West Nile virus (WNV) amplification during the summer of 2004. Blood samples taken during June and July from 155 nestlings of four species of Ardeid birds (cattle egrets, black-crowned night herons, great egrets, and snowy egrets) and five nestling double-crested cormorants yielded a single WNV isolation from a 3-week-old cattle egret. Antibody was detected by enzyme immunoassay from 20 nestlings (13%), 14 (70%) of which were confirmed as positive by plaque reduction neutralization test (PRNT). However, titration end points against WNV and St. Louis encephalitis virus (SLEV) were similar precluding viral identification. The grouping of positives within few nests, highest PRNT titers in youngest birds (<1 weeks of age), the decline of titer with nestling age, and the lack of antibody specificity indicated that antibody may have been acquired maternally and did not represent new infections. Infection rates in Culex tarsalis mosquitoes collected near the Ardeid colony at Ramer Lake (3.1 per 1,000) were statistically similar to rates estimated at the nearby Wister Unit wetlands (5.3 per 1,000) that lacked an Ardeid nesting colony. Black-crowned night heron nestlings experimentally infected with the NY99 strain of WNV produced viremias >5 log10 plaque forming units (PFU)/mL and were considered moderately competent hosts, whereas cattle egret nestlings had viremias that remained <5 log10 PFU/mL and were incompetent hosts.

Descriptors: viral blood antibodies, bird diseases, culex mosquitoes, insect vectors, West Nile fever, West Nile virus, wild animals, birds, California, disease reservoirs, St. Louis encephalitis virus, neutralization tests, species specificity, zoonoses.

Reisen, W., S. Wheeler, S. Yamamoto, Y. Fang, and S. Garcia (2005). **Nesting ardeid colonies are not a focus of elevated West Nile virus activity in southern California.** *Vector Borne and Zoonotic Diseases* 5(3): 258-266. ISSN: 1530-3667.

Abstract: A large nesting colony of Ardeid birds at the Finney-Ramer Wildlife Refuge in Imperial County, California, did not appear to be a focus of West Nile virus (WNV) amplification during the summer of 2004. Blood samples taken during June and July from 155 nestlings of four species of Ardeid birds (cattle egrets, black-crowned night herons, great egrets, and snowy egrets) and five nestling double-crested cormorants yielded a single WNV isolation from a 3-week-old cattle egret. Antibody was detected by enzyme immunoassay from 20 nestlings (13%), 14 (70%) of which were confirmed as positive by plaque reduction neutralization test (PRNT).

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Descriptors: *Ardea alba*, *Bubulcus ibis*, *Egretta thula*, *Nycticorax nycticorax*, *Phalacrocorax auritus*, viral diseases, West Nile virus, nestling infection rate and host competence survey, California, , Imperial County, Finney Ramer Wildlife Refuge.

Saldanha, J., S. Shead, A. Heath, and M. Drebot (2005). **Collaborative study to evaluate a working reagent for West Nile virus RNA detection by nucleic acid testing**. *Transfusion* 45(1): 97-102. ISSN: 0041-1132.
Online: http://www.blackwellpublishing.com/journal.asp?ref=0041-1132
Descriptors: West Nile virus, RNA detection, nucleic acid testing, assays.

Sanchez Guerrero, S.A., S. Romero Estrella, A. Rodriguez Ruiz, L. Infante Ramirez, A. Gomez, E. Villanueva Vidales, M. Garcia Torres , A.M. Dominguez, J.A. Vazquez, E.D. Calderon, L. Valiente Banuet, J.M. Linnen, A. Broulik, W. Harel, and R.A. Marin Y Lopez (2006). **Detection of West Nile virus in the Mexican blood supply.** *Transfusion* 46(1): 111-117.
Abstract: BACKGROUND: West Nile virus (WNV) is the etiologic agent of an emerging disease in the Western Hemisphere that can be transmitted to humans by blood transfusion. WNV first appeared in the United States in 1999, in Canada in 2001, and in Mexico in 2002. The aim of this nationwide study was to determine the prevalence of WNV in blood donors in Mexico as a first step in preventing its transfusion-associated transmission. STUDY DESIGN AND METHODS: In July and August 2004, a total of 3856 fresh plasma specimens collected from each state's center for blood transfusion in 29 of 31 Mexican states were screened with an investigational WNV assay (Procleix,(R) Gen-Probe Inc. and Chiron Corp.), a nucleic acid test based on transcription-mediated amplification (TMA). Reactive specimens were confirmed with a second TMA-based test, the alternative WNV assay (Gen-Probe), and with WNV capture enzyme-linked immunosorbent assays (ELISAs) for detection of immunoglobulin M (IgM) and IgG antibodies. In addition, 3714 frozen plasma samples collected in 2002 and 2003 were similarly tested. RESULTS: One of

3856 fresh samples from an asymptomatic donor from Chihuahua was reactive by both TMA-based tests and IgM ELISA, suggesting a recently acquired infection. The observed percentage of viremic donors blood donors was 0.03 percent. Results from frozen samples were not included in the prevalence calculation and none were TMA-reactive for WNV. CONCLUSIONS: WNV is present in the Mexican blood supply and measures should be taken to reduce the risk of transfusion transmission.
Descriptors: viral antibodies in blood, blood banks, blood donors, communicable diseases, West Nile fever, West Nile virus, blood transfusion, emerging diseases prevention and control, enzyme linked immunosorbent assay, Mexico, reverse transcriptase polymerase chain reaction.

Shaikh, N., H. Goldman, J. Ge, M. Zhang, B. Chen, J. Paice, P. Lam, and J. Keffer (2006). **Efficacy of spectral's WNV IgM status test to rapidly detect the presence of WNV IgM antibodies in patients suspected of West Nile virus infection.** *Clinical Chemistry* 52(6, Suppl. S): A48. ISSN: 0009-9147.
Descriptors: West Nile virus, IgM antibodies, serology, nervous system disease, ELISA, immunologic techniques.
Notes: Meeting Information: 58th Annual Meeting of the American Association of Clinical Chemistry, Chicago, Illinois, USA; July 23 -27, 2006.

Shirato, K., H. Miyoshi, H. Kariwa, and I. Takashima (2005). **Detection of West Nile virus and Japanese encephalitis virus using real-time PCR with a probe common to both viruses.** *Journal of Virological Methods* 126(1-2): 119-125.
Abstract: A diagnostic method to distinguish between West Nile virus (WNV) and Japanese encephalitis virus (JEV) based on fluorogenic real-time polymerase chain reaction (TaqMan) assays was developed. To detect WNV and JEV with a single probe, a probe was designed to correspond to sequences in the core protein region that are shared by both viruses. The specificity of this assay depended on the primer sets used, which were specific to the target virus sequences: the primer set for WNV could detect only WNV strains and the primer set for JEV could detect only JEV strains. The assays were tested by detection of viruses from experimentally infected animal tissues. The method described in this study will be useful for the simultaneous discrimination of WNV and JEV in areas where JEV is endemic, such as East Asia.
Descriptors: Japanese encephalitis virus (JEV), diagnosis, polymerase chain reaction methods, West Nile virus, DNA primers, specificity of assays, core protein regions.

Sirigireddy, K.R., G.A. Kennedy, A. Broce, L. Zurek, and R.R. Ganta (2006). **High prevalence of west nile virus: a continuing risk in acquiring infection from a mosquito bite.** *Vector Borne and Zoonotic Diseases* 6(4): 351-360. ISSN: 1530-3667.
Abstract: The prevalence of West Nile Virus (WNV) was evaluated by diplex real-time RT-PCR assay for the years 2001-2005 in Culex species of mosquitoes, several

species of dead birds, and clinically suspected mammals collected in Kansas. The analysis was performed using a TaqMan-based diplex real-time RT-PCR assay targeted against two regions of the WNV genome, envelope glycoprotein gene and 3' untranslated region. The assay aided in the accurate detection of WNV in mosquitoes at high prevalence for the years 2002-2005. Similarly, high incidence of birds that tested positive for WNV was detected in 2002-2004. WNV positives in mammals by the diplex real rime RT-PCR assay included horses, squirrels, mules, sheep and a mountain goat. Majority of the equine WNV positives were detected only in the year 2002. Sequence analysis of a segment of the envelope glycoprotein gene from 31 randomly selected WNV positive samples revealed variations in six samples at one or two nucleotide positions. The identity of high levels of WNV positives in Kansas parallels the recent reports on the widespread distribution of the virus in the United States. The continued detection of WNV in the mosquitoes is of significant public health concern and calls for continued surveillance and public health activities.
Descriptors: West Nile virus, prevalnce, risk, evaluated, dead birds, mosquitoes, surveillance, public health, distribution.

Stone, W.B., J.E. Therrien, R. Benson, L. Kramer, E.B. Kauffman, M. Eidson, and S. Campbell (2005). **Assays to detect west Nile virus in dead birds.** *Emerging Infectious Diseases* 11(11): 1770-1773. ISSN: 1080-6040.
Abstract: Using oral swab samples to detect West Nile virus in dead birds, we compared the Rapid Analyte Measurement Platform (RAMP) assay with VecTest and real-time reverse-transcriptase-polymerase chain reaction. The sensitivities of RAMP and VecTest for testing corvid species were 91.0% and 82.1%, respectively.
Descriptors: aves, diagnostic techniques, oral swab sampling and molecular assays to detect viral pathogen in dead specimens, comparative study, viral diseases, West Nile virus, comparative evaluation.

Stramer, S.L., B. Custer, M.P. Busch, and R.Y. Dodd (2006). **Strategies for testing blood donors for West Nile virus.** *Transfusion* 46(12): 2036-2037.
Descriptors: blood donors, West Nile virus, surveillance, strategies, detection, testing.

Tang, Y., C. Anne Hapip, B. Liu, and C.T. Fang (2006). **Highly sensitive TaqMan RT-PCR assay for detection and quantification of both lineages of West Nile virus RNA.** *Journal of Clinical Virology* 36(3): 177-182.
Abstract: BACKGROUND: Starting in 1999, the West Nile virus (WNV) epidemic represents the largest outbreak of arboviral encephalitis ever recorded in the U.S. The effective means to determine an infection are detection of viral nucleic acid and/or viral specific immunoglobulin, IgM and/or IgG. OBJECTIVE: To develop a highly sensitive and specific TaqMan RT-PCR assay for the detection

and quantification of WNV RNA of lineage 1 and lineage 2. STUDY DESIGN: A TaqMan RT-PCR primer-probe was designed to perfectly match target sequences of all sequenced WNV strains and isolates, which added a layer of protection against false-negative results due to strain variability. In addition, the inclusion of a low level RNA internal control (IC) in the assay increased the precision and accuracy of the assay. RESULTS: By optimizing the RNA preparation procedure for increased WNV RNA recovery, together with optimizing the primer-probe and TaqMan conditions for improved amplification efficiency, we developed a highly sensitive assay with the detection limit of 10 copies/mL. To evaluate the assay, we tested plasma samples from 12 transfusion-transmitted implicated cases in 2002 and from 68 positive blood donors in 2003. All tested specimens were WNV positive. The viral load of 68 positive blood donor samples collected in 2003 ranged from 10 copies/mL to 67,000 copies/mL with a mean of 8100 copies/mL. Furthermore, high sensitivity of the assay was achieved without compromising specificity. All 100 routine donor samples tested negative. CONCLUSIONS: The assay results demonstrate that our in-house TaqMan RT-PCR procedure can detect and quantify WNV RNA of lineage1 and lineage 2 in human plasma with high sensitivity and specificity.
Descriptors: viral blood RNA, reverse transcriptase polymerase chain reaction, West Nile fever, West Nile virus isolation and purification, blood donors, blood transfusion, DNA primers.

Tanner, J., J. Traub Dargatz, A. Hill, H. Van Campen, A. Knight, W. Cunningham, and M. Salman (2006). **Evaluation of factors associated with positive IgM capture ELISA results in equids with clinical signs compatible with West Nile virus infection: 1,017 cases (2003).** *Journal of the American Veterinary Medical Association.* 228(3): 414-421. ISSN: 0003-1488.
Descriptors: horses, horse diseases, West Nile virus, viral diseases of animals and humans, immunoglobulin M, enzyme linked immunosorbent assay, disease diagnosis, disease prevalence, vaccination, symptoms, gender differences, disease severity, risk factors, Colorado.

Tilley, P.A., J.D. Fox, G.C. Jayaraman, and J.K. Preiksaitis (2006). **Nucleic acid testing for west nile virus RNA in plasma enhances rapid diagnosis of acute infection in symptomatic patients.** *Journal of Infectious Diseases* 193(10): 1361-1364.
Abstract: Although nucleic acid amplification testing (NAAT) for West Nile virus (WNV) is useful in screening blood donors, such methods have not been studied in symptomatic patients. For diagnosis of WNV infection, 1.0 mL of plasma was tested by NAAT, and WNV-specific immunoglobulin M was assayed. Of 276 WNV cases, 191 were tested by both serology and NAAT. Of these, 86 (45.0%), 111 (58.1%), and 180 (94.2%) were detected by NAAT, serology, and combined NAAT and serology, respectively. NAAT-based screening was most useful within 8 days of the onset

of symptoms. Viremia is common in early symptomatic WNV infection, and NAAT enhances diagnostic yield.
Descriptors: nucleic acid amplification techniques, viral RNA analysis, West Nile fever, West Nile virus isolation and purification, Alberta, routine diagnostic tests, immunoglobulin M, predictive value of tests, viral blood RNA, reverse transcriptase polymerase chain reaction, specimen handling, viremia diagnosis.

Tilley, P.A., G.A. Zachary, R. Walle, and P.F. Schnee (2005). **West Nile virus detection and commercial assays.** *Emerging Infectious Diseases* 11(7): 1154-1155. ISSN: 1080-6040.
Descriptors: viral blood antibodies, blood immunoglobulin M, diagnostic reagent kits, West Nile fever diagnosis, West Nile virus isolation and purification, blood immunoglobulin G, time factors.

Tilley, P.A.G., R. Walle, A. Chow, G.C. Jayaraman, K. Fonseca, M.A. Drebot, J. Preiksaitis, and J. Fox (2005). **Clinical Utility of Commercial Enzyme Immunoassays during the Inaugural Season of West Nile Virus Activity, Alberta, Canada.** *Journal of Clinical Microbiology.* 43(9): 4691-4695. ISSN: 0095-1137.
Abstract: West Nile virus (WNV) has spread rapidly across North America, creating a need for rapid and accurate laboratory diagnosis on a large scale. Immunoglobulin M (IgM) capture enzyme immunoassays (EIA) became commercially available in the summer of 2003, but limited data are available on their clinical performance. Consolidated human WNV diagnostic testing for the province of Alberta, Canada, at the public health laboratory permitted a large-scale evaluation of the assays, covering a wide clinical spectrum. Two thousand nine hundred sixty-nine sera were tested, from 2,553 Alberta residents, and 266 cases were identified. Sensitivities of the Focus assay and first-generation Panbio IgM capture EIA were 79 and 80%, respectively. During the first week of illness only 53 to 58% of cases were positive, but sensitivity was 96 to 97% after day 8. Sensitivity for neurological cases was 92% overall. Specificity was high for the Focus kit at 98.9%, but only 82.9% for the first Panbio kit. A positive Focus WNV IgG result with a twofold rise in IgG index was a reliable indicator of acute flavivirus infection (67/67 WNV). Agreement between the IgG test and hemagglutinin inhibition titers in paired sera was at least 82%. Commercial IgM and IgG EIA proved useful for WNV diagnosis, provided follow-up sera were collected after 8 days of illness.
Descriptors: West Nile virus, detection, surveillance, diagnosis, immunoglobulin M, enzyme assays, EIA, Canada.

Tobler, L.H., C. Bianco, S.A. Glynn, G.B. Schreiber, B.J. Dille, H.E. Prince, R.S. Lanciotti, J.M. Linnen, J. Gallarda, V. Shyamala, D. Smith, S.H. Kleinman, and M.P. Busch (2005). **Detection of West Nile virus RNA and antibody in frozen plasma com-**

ponents from a voluntary market withdrawal during the 2002 peak epidemic. *Transfusion* 45(4): 480-486.

Abstract: BACKGROUND: The US West Nile virus (WNV) epidemic in the summer and fall of 2002 included the first documented cases of transfusion-transmitted WNV infection. In December 2002, the FDA supported a voluntary market withdrawal by the blood banking community of frozen blood components collected in WNV high-activity areas. At the time, the prevalence of viremia and serologic markers for WNV in the blood supply was undefined. STUDY DESIGN AND METHODS: In collaboration with America's Blood Centers, 1468 frozen plasma components (of approx. 60,000 frozen units voluntarily withdrawn from the market) were selectively retrieved from the peak epidemic regions and season (June 23, 2002-September 28, 2002). These units were unlinked, subaliquoted, and tested by WNV enzyme immunoassays (EIAs; Focus Technologies and Abbott Laboratories) and nucleic acid amplification tests (NATs; Gen-Probe Inc. and Roche Molecular Systems). RESULTS: Of the 1468 EIA results from Abbott and Focus, 7 were anti-immunoglobulin M (IgM)- and anti-immunoglobulin G (IgG)-reactive by both assays, 8 and 1 were IgM-only-reactive, and 8 and 23 were IgG-only-reactive, respectively. NAT by Gen-Probe and Roche Molecular Systems yielded one RNA-positive, antibody-negative unit containing approximately 440 RNA copies per mL. An additional 10-fold replicate NAT testing by Gen-Probe on 14 of 15 IgM-reactive specimens yielded 2 additional IgM- and IgG-reactive units with low-level viremia (i.e., 7/10 and 2/10 replicates tested reactive). CONCLUSION: The prevalence of acute (RNA-positive) and recent (IgM-seroreactive) WNV infections indicates that transfusion risk in high-risk areas could have been considerable and that voluntary market withdrawal of frozen components likely averted some WNV transfusion transmissions. The existence of very-low-level viremic units raises concerns, because WNV minipool NAT screening will miss such units and individual NAT may not completely correct this situation.

Descriptors: blood banks, plasma virology, West Nile fever epidemiology, West Nile virus isolation and purification, viral blood antibodies, consumer product safety, disease outbreaks, viral analysis, risk factors, seroepidemiologic studies.

Notes: Comment In: Transfusion. 2005 Apr;45(4):460-2.

Tong, Y., X.p. Zeng, T. Liu, X. Xiao, J.w. Zhu, and B. Tucker (2006). **Studies on West Nile virus detection using two rapid methods.** *Zhongguo Meijieshengwuxue Ji Kongzhi Zazhi* 17(4): 307-310. ISSN: 1003-8280.

Descriptors: mosquitoes, diagnostic techniques, quick detection methods, evaluation for viral disease, viral diseases, West Nile virus(WNV), Ramp system, VecTest, China.

Language of Text: Chinese; Summary in Chinese, English.

Tonry, J.H., C.B. Brown, C.B. Cropp, J.K. Co, S.N. Bennett, V.R. Nerurkar, T. Kuberski, and D.J. Gubler (2005). **West Nile virus detection in urine.** *Emerging Infectious Diseases* 11(8): 1294-1296. ISSN: 1080-6040.

Abstract: We report West Nile virus (WNV) RNA in urine collected from a patient with encephalitis 8 days after symptom onset. Viral RNA was detected by reverse transcriptase-polymerase chain reaction (RT-PCR). Sequence and phylogenetic analysis confirmed the PCR product to have > or = 99% similarity to the WNV strain NY 2000-crow3356.

Descriptors: West Nile fever, West Nile virus isolation and purification, viral blood antibodies, viral genetics, reverse transcriptase polymerase chain reaction, sequence alignment, DNA sequence analysis.

Tyler, K.L., J. Pape, R.J. Goody, M. Corkill, and B.K. Kleinschmidt DeMasters (2006). **CSF findings in 250 patients with serologically confirmed West Nile virus meningitis and encephalitis.** *Neurology* 66(3): 361-365.

Abstract: OBJECTIVE: To provide a large, comprehensive evaluation of the CSF findings in patients with serologically confirmed West Nile virus (WNV), CNS disease, and their correlation with outcome. METHODS: CSF samples from 334 WNV-infected hospitalized patients were analyzed. Information was available and extracted from the medical records of 250 of these patients, and CSF parameters correlated with clinical and epidemiologic features of disease (e.g., patient age, sex, outcome). RESULTS: Patients with meningitis had a mean of 226 cells/mm3, and those with encephalitis had a mean of 227 cells/mm3. Three percent of meningitis patients and 5% of encephalitis patients had fewer than 5 cells/mm3, and approximately 8% of both groups had more than 500 cells/mm3. Patients with meningitis had a mean of 41% neutrophils, and those with encephalitis had 45%. Forty-five percent of meningitis patients and 37% of encephalitis patients had at least 50% neutrophils in their initial CSF specimen. Neither the mean percent neutrophils nor their distribution differed significantly between groups. Forty-seven percent of encephalitis patients and 16% of meningitis patients had CSF protein of 100 mg/dL or greater ($p < 0.01$). Although specific CSF parameters, including nucleated cell count and protein concentration, correlated significantly with outcome, multivariate analysis suggested that their total predictive value was modest. Age was an additional predictor of outcome independent of CSF variables in all patients. CONCLUSIONS: Serologically confirmed West Nile virus meningitis and encephalitis produce similar degrees of CSF pleocytosis and are frequently associated with substantial CSF neutrophilia. Patients with encephalitis have higher CSF protein concentrations and are more likely to have adverse outcomes, including admission to long-term care facilities or even death after their acute illness. CSF findings were only a modest predictor of disease outcome, with patient age adding important independent prognostic

information.

Descriptors: encephalitis, cerebrospinal fluid, viral meningitis, West Nile fever, age factors, cell count, leukocytosis, multivariate analysis, osmolar concentration, predictive value of tests, serologic tests, treatment outcome.

Vinayagamoorthy, T., K. Mulatz, M. Drebot, and R. Hodkinson (2005). **Molecular typing of West Nile Virus, Dengue, and St. Louis encephalitis using multiplex sequencing.** *Journal of Molecular Diagnostics* 7(2): 152-159.

Abstract: We report the development of an assay to simultaneously identify three of the clinically important flaviviruses (West Nile Virus, Dengue, and St. Louis encephalitis). This assay is based on the nucleotide sequence variations within a 266-bp region of the non-structural protein 5. Further, based on the nucleotide variations in the same region of the non-structural protein 5, four of the present Dengue serotypes were identified. To identify some of the subtypes of WNV we have developed a second assay using multiplex sequencing technology. The format of the result of this assay is an electropherogram of two genomic segments of the WNV genome: a 48-nucleotide sequence from the anchored core protein C and a 45-nucleotide sequence coding for the non-structural proteins (proteinase and putative helicase genes).

Descriptors: dengue virus, St. Louis encephalitis virus, viral genome, DNA sequence analysis, West Nile virus, DNA base sequence, viral genetics, molecular sequence data, variation genetics, West Nile virus classification.

Wunschmann, A., J. Shivers, J. Bender, L. Carroll, S. Fuller, M. Saggese, A. Van Wettere, and P. Redig (2005). **Pathologic and immunohistochemical findings in goshawks (*Accipiter gentilis*) and great horned owls (*Bubo virginianus*) naturally infected with West Nile virus.** *Avian Diseases.* 49(2): 252-259. ISSN: 0005-2086.

Abstract: The carcasses of 25 great horned owls and 12 goshawks were investigated for West Nile virus (WNV) infection by immunohistochemistry (IHC) performed on various organs, including brain, spinal cord, heart, kidney, eye, bone marrow, spleen, liver, lungs, pancreas, intestine, and proventriculus, using a WNV-antigen-specific monoclonal antibody and by WNV-specific reverse transcriptase-polymerase chain reaction (RT-PCR), performed on fresh brain tissue only. WNV infection was diagnosed by IHC in all owls and all goshawks. WNV-specific RT-PCR amplified WNV-RNA in the brain of all goshawks but only 12 owls (48%). Cachexia was a common macroscopic finding associated with WNV infection in owls (76%). Myocarditis was occasionally macroscopically evident in goshawks (33%). Microscopically, inflammatory lesions, including lymphoplasmacytic and histiocytic encephalitis, myocarditis, endophthalmitis, and pancreatitis were present in both species but were more common and more severe in goshawks than in owls. The most characteristic brain lesion in owls was the formation of glial nodules, in particular

in the molecular layer of the cerebellum, while encephalitis affecting the periventricular parenchyma of the cerebral cortex was common in the goshawks. In owls, WNV-antigen-positive cells were present usually only in very small numbers per organ. Kidney (80%), heart (39%), and cerebellum (37%) were the organs that most commonly contained WNV antigen in owls. WNV antigen was frequently widely distributed in the organs of infected goshawks, with increased amounts of WNV antigen in the heart and the cerebrum. Spleen (75%), cerebellum (66%), heart (58%), cerebrum (58%), and eye (50%) were often WNV-antigen positive in goshawks. In contrast with the goshawks, WNV antigen was not present in cerebral and retinal neurons of owls. WNV infection appears to be capable of causing fatal disease in great horned owls and goshawks. However, the distribution and severity of histologic lesions, the antigen distribution in the various organs, and the amount of antigen varied among both species. Therefore, the diagnostician may choose organs for histology and immunohistochemistry as well as RT-PCR depending on the investigated species in order to avoid false-negative results.

Descriptors: *Accipiter gentilis*, *Bubo virginianus*, wild birds, birds of prey, West Nile virus, bird diseases , pathogenesis, immunohistochemistry, immunopathology, disease incidence, animal organs, monoclonal antibodies, antigens, reverse transcriptase polymerase chain reaction, cachexia, viral diseases of animals and humans.

Language of Text: Summary in Spanish.

Zhang, J.S., P.H. Zhang, B.Y. Si, H. Yang, and W.C. Cao (2005). **[Comparison and discrimination of the biological characteristics between West Nile virus and Japanese encephalitis virus]**. *Chinese Journal of Experimental and Clinical Virology* 19(4): 340-343.

Abstract: BACKGROUND: To compare the biological characteristics of West Nile virus (WNV) and Japanese encephalitis virus (JEV), including cells sensitivity, pathogenicity, viral morphology, as well as the results of immunological and molecular biological detection. METHODS: Cytopathic effect (CPE) and pathogenicity were observed in C6/36 cells and in suckling mice inoculated intracerebrally with the WNV or JEV, respectively. The sliced tissue samples for electron microscopic examination were prepared for the morphologic observation of the viruses. Serum antibody to WNV or JEV was detected using indirect immunofluorescence assay (IFA), and the viral RNA was analyzed by RT-PCR method. RESULTS: WNV or JEV-caused CPE was characterized by cell fusion and cell shedding, respectively. There was no significant difference in the pathogenicity to suckling mice between WNV and JEV. The morphologic observation showed that the shape and size of the two virions were similar. WNV and JEV were found to have antigenic cross-reactivity. The viral RNA could be detected from both WNV and JEV samples with universal primer set, but only nucleoside fragments of corresponding virus could be amplified when specific

primers were used. CONCLUSION: CPE in C6/36 cell and detection of the viral RNA should be useful in discrimination of WNV and JEV, and simultaneously examining the titers of serum antibodies against WNV and JEV may be helpful to diagnosis of infection with these agents.
Descriptors: West Nile virus, Japanese encephalitis virus, biological characteristics, pathogenicity, morphology, viral RNA, diagnosis.
Language of Text: Chinese.

Zhang, Z., F. Wilson, R. Read, L. Pace, and S. Zhang (2006). **Detection and characterization of naturally acquired West Nile virus infection in a female wild turkey.** *Journal of Veterinary Diagnostic Investigation* 18(2): 204-208.
Abstract: An adult female wild turkey exhibiting disorientation and failure to flee when approached was submitted to the Mississippi Veterinary Research and Diagnostic Laboratory. Gross pathologic examination revealed evidence of dehydration and the presence of modest numbers of adult nematodes in the small intestine. Histologic examination revealed extensive multifocal perivascular lymphocytic infiltration in brain, marked heterophilic hyperplasia in bone marrow, and multifocal interstitial lymphocytic infiltration in heart, pancreas, ventriculus, and skeletal muscles. West Nile virus (WNV) was isolated from the brain, lung, and kidney tissues using cultured Vero cells. Higher copies of viral RNA were detected from brain, lung, and kidney than from heart, liver, or spleen by quantitative real-time reverse transcription-polymerase chain reaction (RRT-PCR) analysis. Immunohistochemical (IHC) analysis detected WNV antigen in various tissues including neurons, kidney, respiratory tract epithelium, heart, and bone marrow. On the basis of the data from this investigation, it is concluded that WNV caused encephalitis along with many other pathologic changes in the affected wild turkey.
Descriptors: poultry diseases, turkeys, West Nile fever, West Nile virus isolation and purification, wild animals, *Cercopithecus aethiops*, histocytochemistry, kidney, lung pathology, pathology of adult female wild turkey.

Disease Reservoirs

Allison, A., N. Gottdenker, and D. Stallknecht (2005). **Wintering of neurotropic velogenic Newcastle disease virus and West Nile virus in double-crested cormorants (*Phalacrocorax auritus*) from the Florida keys.** *Avian Diseases.* 49(2): 292-297. ISSN: 0005-2086.

Abstract: During November 2002, six double-crested cormorants (DCCs; *Phalacrocorax auritus*) were found moribund in Big Pine Key, FL, exhibiting clinical signs indicative of neurologic disease. Postmortem diagnostic evaluations were performed on two adult birds. Virulent Newcastle disease virus (NDV) was isolated from a cloacal swab from cormorant 1. West Nile virus (WNV) was isolated from the brain and lung of cormorant 2. Nucleotide sequence analysis of a portion of the fusion (F) protein gene of the NDV cormorant isolate revealed it shared a 100% deduced amino acid identity with only two viruses: the 1992 epizootic cormorant isolate from Minnesota and the 1992 turkey isolate from North Dakota. The epidemiologic significance of the recognition of virulent NDV on cormorant wintering grounds during a nonepizootic period, in addition to the potential implications of the concurrent isolation of NDV and WNV from cormorants, is discussed.

Descriptors: Phalacrocorax, wild birds, bird diseases, alternative hosts, Newcastle disease virus, Newcastle disease, West Nile virus, viral diseases, animals, humans, overwintering, nucleotide sequences, amino acid sequences, pathogen identification, epidemiology, geographical distribution, disease outbreaks, disease reservoirs, Florida.

Language of Text: Summary in Spanish.

Gingrich, J.B., R.D. Anderson, G.M. Williams, L. O'Connor, and K. Harkins (2006). **Stormwater ponds, constructed wetlands, and other best management practices as potential breeding sites for West Nile virus vectors in Delaware during 2004.** *Journal of the American Mosquito Control Association* 22(2): 282-291.

Abstract: We performed longitudinal surveys of mosquito larval abundance (mean mosquito larvae per dip) in 87 stormwater ponds and constructed wetland in Delaware from June to September 2004. We analyzed selected water quality factors, water depth, types of vegetation, degree of shade, and level of insect predation in relation to mosquito abundance. The 2004 season was atypical, with most ponds remaining wet for the entire summer. In terms of West Nile virus (WNV) vectors, wetlands predominantly produce *Aedes vexans, Culex pipiens pipiens*, and *Culex restuans*. Retention ponds generally produced the same species as wetlands, except that *Cx. p. pipiens* was more abundant than *Cx. restuans* in retention ponds. *Aedes vexans* and *Culex salinarius* were the most abundant species to Conservation Restoration Enhancement

Program ponds. Sand filters uniquely produced high numbers of *Cx. restuans, Cx. p. pipiens*, and *Aedes japonicus japonicus*, a newly invasive vector species. Site that alternately dried and flooded, mostly detention ponds, forebays of retention ponds, and some wetlands often produced Ae. vexans, an occasional WNV bridge vector species. Overall, seasonal distribution of vectors was bimodal, with peaks occurring during early and late summer. Ponds with shallow sides and heavy shade generally produced an abundance of mosquitoes, unless insect predators were abundant. Bright, sunny ponds with steep sides and little vegetation generally produced the fewest mosquitoes. The associations among mosquito species and selected vegetation types are discussed.
Descriptors: Culicidae virology, insect vectors, West Nile virus, Aedes, Anopheles, Culex virology, Delaware, fresh water ecology, population density, seasons , waste disposal.

Godsey, M.J., M. Blackmore, N. Panella, K. Burkhalter, K. Gottfried, L. Halsey, R. Rutledge, S. Langevin, R. Gates, and K. Lamonte (2005). **West Nile virus epizootiology in the southeastern United States, 2001.** *Vector Borne and Zoonotic Diseases.* 5(1): 82-89. ISSN: 1530-3667.
Descriptors: West Nile virus, virus transmission, insect vectors, Culex, *Culiseta melanura*, mosquitoes, disease reservoirs, birds, disease detection, antibody detection, seroprevalence, chickens, sentinel animals, Southeastern United States, Florida , Georgia.

Hull, J., A. Hull, W. Reisen, Y. Fang, and H. Ernest (2006). **Variation of West Nile virus antibody prevalence in migrating and wintering hawks in central California.** *Condor* 108(2): 435-439. ISSN: 0010-5422.
Abstract: To assess the extent of West Nile virus (WNV) exposure of migrating (Marin Headlands) and wintering (Central Valley) hawks in California, plasma from 271 Red-tailed Hawks (*Buteo jamaicensis*), 19 Red-shouldered Hawks (*B. lineatus*), and 30 Cooper's Hawks (*Accipiter cooperii*) was tested for WNV antibodies during the winter of 2004-2005. WNV antibodies were found in 5% of migrating and 15% of wintering Red-tailed Hawks, 20% of migrating and 58% of wintering Red-shouldered Hawks, and 13% of migrating Cooper's Hawks. No individuals demonstrated visible signs of WNV illness. Red-tailed Hawks that tested positive for WNV antibodies displayed no difference from Red-tailed Hawks without WNV antibodies in weight to wing chord ratio or white blood cell counts. In the Central Valley, WNV antibodies were significantly more prevalent in Red-shouldered Hawks than in Red-tailed Hawks. Significantly more Red-tailed Hawks sampled on wintering grounds tested positive for WNV antibodies than Red-tailed Hawks sampled during migration.
Descriptors: *Accipiter cooperii*, *Buteo jamaicensis*, *Buteo lineatus*, migration, viral diseases, West Nile virus, antibody prevalence, temporal variation, migrating and

wintering individuals, immune response, viral disease antibody prevalence, California, central.
Language of Text: English, Spanish.

Joyner, P.H., S. Kelly, A.A. Shreve, S.E. Snead, J.M. Sleeman, and D.A. Pettit (2006). **West Nile virus in raptors from Virginia during 2003: clinical, diagnostic, and epidemiologic findings.** *Journal of Wildlife Diseases* 42(2): 335-344. ISSN: 0090-3558.
Abstract: Sixty-one birds of prey admitted to The wildlife Center of Virginia (WCV; Waynesboro, Virginia, USA) from June to November 2003 were tested for West Nile 'Arus (WNV) infection. Choanal and/or cloacal swabs were obtained and submitted to Virginia's Division of Consolidated Laboratory Services (Richmond, Virginia, USA) for analysis with real-time reverse transcriptase polymerase chain reaction (RT-PCR). Forty birds of prey were positive for WNV by RT-PCR. Five avian families and nine species of raptors were represented, with great horned owls (*Bubo virginianus*) and red-tailed hawks (*Buteo jamaicensis*) most frequently affected. Presenting clinical signs were consistent with previous reports of WNV infection in raptors; however, these differed between species. Of WNV positive birds, nonspecific signs of illness were the most common clinical findings, particularly in red-tailed hawks; signs included dehydration (n=20), emaciation (n =18), and depression (n =15). Neurologic abnormalities were frequently identified, especially in great horned owls, and included head tremors (n=17), ataxia (n=13), head incoordination (n=7), torticollis (n=3), nystagmus (n=3), and bead tilt (n=3). Great horned owls exhibited aNemia and leukocytosis with heterophilia, eosinophilia, and monocytosis consistent with chronic inflammation. Red-tailed hawks were aNemic with a heterophilic leukocytosis and regenerative left shift. The majority of WNV cases occurred during August and September; there was a marked increase in the number of raptors admitted to WCV during these months followed by a marked decrease during October, November, and December. This pattern differed from mean monthly admissions during the previous 10 years and suggests a negative impact on local raptor populations. The effects of WNV on avian populations are largely unknown; however, because of their ecological importance, further investigation of the effects of WNV on raptor populations is warranted.
Descriptors: falconiformes, strigiformes, viral diseases, West Nile virus, clinical, diagnostic and epidemiologic findings, transmission of viruses, Virginia.

Owen, J., F. Moore, N. Panella, E. Edwards, R. Bru, M. Hughes, and N. Komar (2006). **Migrating birds as dispersal vehicles for West Nile virus**. *EcoHealth* 3(2): 79-85. ISSN: 1612-92021612-9210.
Descriptors: West Nile virus, migrating birds, dispersal vehicles, locomotor activity, virus spread.

Reisen, W.K., Y. Fang, H.D. Lothrop, V.M. Martinez, J. Wilson, P. O'Connor, R. Carney, B. Cahoon Young, M. Shafii, and A.C. Brault (2006). **Overwintering of West Nile virus in southern California.** *Journal of Medical Entomology* 43(2): 344-355. ISSN: 0022-2585.

Abstract: West Nile virus (family Flaviviridae, genus Flavivirus, WNV) invaded southern California dining 2003, successfully overwintered, amplified to epidemic levels, and then dispersed to every county in the state. Although surveillance programs successfully tracked and measured these events, mechanisms that allowed the efficient overwintering and subsequent amplification of WNV have not been elucidated. Our current research provided evidence for three mechanisms whereby WNV may have persisted in southern California during the winters of 2003-2004 and 2004-2005: 1) continued enzootic transmission, 2) vertical transmission by Culex mosquitoes, and 3) chronic infection in birds. WNV was detected in 140 dead birds comprising 32 species, including 60 dead American crows, thereby verifying transmission during the November-March winter period. Dead American crows provide evidence of recent transmission because this species always succumbs rapidly after infection. However, WNV RNA was not detected concurrently in 43,043 reproductively active female mosquitoes comprising 11 species and tested in 1,258 pools or antibody in sera from 190 sentinel chickens maintained in 19 flocks. Although efficient vertical transmission by WNV was demonstrated experimentally for Culex tarsalis Coquillett infected per os, 369 females collected diapausing in Kern County and tested in 32 pools were negative for WNV. Vertical transmission was detected in Culex pipiens quinquefinciatus Say adults reared from field-collected immatures collected from Kern County and Los Angeles during the summer transmission period. Chronic infection was detected by finding WNV RNA in 34 of 82 birds that were inoculated with WNV experimentally, held for >6 wk after infection, and then necropsied. Frequent detection of WNV RNA in kidney tissue in experimentally infected birds >6 wk postinfection may explain, in part, the repeated detection of WNV RNA in dead birds recovered during winter, especially in species such as mourning doves that typically do not die after experimental infection. In summary, our study provides limited evidence to support multiple modes of WNV persistence in southern California. Continued transmission and vertical transmission by *Culex p. quinquefasciatus* Say seem likely candidates for further study.

Descriptors: aves, viral diseases, West Nile virus, overwintering, transmission of viruses, California, south, viral disease overwintering.

Sabio, I.J., A.J. Mackay, A. Roy, and L.D. Foil (2006). **Detection of West Nile virus RNA in pools of three species of ceratopogonids (Diptera: Ceratopogonidae) collected in Louisiana.** *Journal of Medical Entomology* 43(5): 1020-1022. ISSN: 0022-2585.

Abstract: Light traps were used to collect ceratopogonids in East Baton Rouge

parish, Louisiana. In total, 46,496 ceratopogonids were sorted from 4,968 light trap collections from 20 November 2002 through 25 November 2004. Two hundred and nine pools containing specimens of 18 species of *Culicoides Latreille*, seven pools containing specimens of *Atrichopogon Kieffer*, and five pools containing specimens of *Forcipomyia Meigen* were tested for West Nile virus (family Flaviviridae, genus Flavivirus, WNV) RNA using real-time reverse transcriptase polymerase chain reaction. Five out of the 209 pools of Culicoides specimens were positive for WNV RNA.
Descriptors: West Nile virus, detection, trap collection, Culicoides, RNA, reverse transcriptase polymerase chain reaction, Louisiana.

General

Amy, B.W. (2005). **West Nile virus.** *Journal of the Mississippi State Medical Association* 46(5): 138-140.
Descriptors: West Nile fever epidemiology, West Nile virus, hotlines, Mississippi, prevention and control.

Anon (2006). **West nile virus a looming deadly threat for liver transplantation.** *Transplantation* 82(1 Suppl 2): 150.
Descriptors: West Nile virus, liver transplantation, deadly threat, infection, abstract.

Anon (2006). **West nile virus activity--United States, January 1-November 7, 2006.** *MMWR. Morbidity and Mortality Weekly Report* 55(44): 1204-1205.
Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Standard Time, November 7, 2006. A total of 41 states and the District of Columbia had reported 3,830 cases of human WNV illness to CDC.
Descriptors: West Nile fever, bird diseases, culicidae, horse diseases, rodent diseases, sciuridae, United States, West Nile virus isolation and purification.

Anon (2006). **West Nile virus activity--United States, January 1-October 10, 2006.** *MMWR. Morbidity and Mortality Weekly Report* 55(40): 1097-1098.
Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Daylight Time, October 10, 2006. A total of 41 states and the District of Columbia had reported 3,135 cases of human WNV illness to CDC. A total of 1,717 (55%) cases for which such data were available occurred in males; median age of patients was 50 years (range: 3 months-99 years). Dates of illness onset ranged from January 6 to September 25; a total of 97 cases were fatal.
Descriptors: West Nile fever epidemiology, West Nile virus isolation and purification, bird diseases, culicidae, horse diseases, rodent diseases, sciuridae, United States.

Anon (2006). **West Nile virus activity--United States, January 1-September 12, 2006.** *MMWR. Morbidity and Mortality Weekly Report* 55(36): 996.
Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Daylight Time, September 12, 2006. A total of 36 states and the District of Columbia had reported 1,634 cases of human WNV illness to CDC. A total of 921 (57%) cases for which such data were

available occurred in males; median age of patients was 51 years (range: 3 months-95 years). Dates of illness onset ranged from January 6 to September 10; a total of 52 cases were fatal.
Descriptors: West Nile fever, humans, birds, humans, United States, fatality reports.

Anon (2006). **West Nile virus activity--United States, January 1-August 15, 2006.** *Morbidity and Mortality Weekly Report* 55(32): 879-880.
Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Daylight Time, August 15, 2006. A total of 26 states had reported 388 cases of human WNV illness to CDC. A total of 214 (56%) cases for which such data were available occurred in males; median age of patients was 49 years (range: 2-91 years). Dates of illness onset ranged from January 6 to August 10; a total of 13 cases were fatal. A total of 68 presumptive West Nile viremic blood donors (PVDs) have been reported to ArboNET during 2006. Of these, 20 were reported from Nebraska; 18 were reported from Texas; five were reported from California; four were reported from Utah; three each were reported from Oklahoma and South Dakota; two each were reported from Idaho, Iowa, Kentucky, and Mississippi; and one each was reported from Arizona, Colorado, Minnesota, Nevada, North Dakota, Wisconsin, and Wyoming. Of the 68 PVDs, 10 persons (median age: 43 years [range: 18-59 years]) subsequently had West Nile fever.
Descriptors: West Nile fever epidemiology, humans, bird diseases, culicidae, horse diseases, rodent diseases, United StatesWest Nile virus isolation and purification.

Anon (2006). **Assessing capacity for surveillance, prevention, and control of West Nile virus infection--United States, 1999 and 2004.** *Morbidity and Mortality Weekly Report* 55(6): 150-153.
Abstract: Indigenous human disease caused by West Nile virus (WNV) was first identified in the United States in August 1999 in the greater New York City area. By the end of 2004, human WNV disease had been reported in all states except Washington, Hawaii, and Alaska, and WNV transmission to humans had been documented by five routes: mosquito bites (principally from Culex spp.), blood transfusions, organ transplantation, transplacental transfer, and breastfeeding. During 1999-2005, a total of 19,525 cases of WNV disease in humans and 771 deaths were reported in the United States. In 2000, CDC first published guidelines for WNV surveillance, prevention, and control and created ArboNET, an electronic surveillance and reporting system. Beginning in 1999, WNV surveillance and prevention activities had been initiated in selected states and large cities through the CDC Epidemiology and Laboratory Capacity (ELC) cooperative agreements for emerging infectious diseases and subsequently expanded to all 50 states, six large cities/counties, and Puerto Rico. In 2005, to assess the capacity of state and large-city/county health departments to conduct WNV surveillance, prevention, and control activities,

the Council of State and Territorial Epidemiologists (CSTE), with assistance from the Association of Public Health Laboratories (APHL) and CDC, surveyed WNV programs in the 50 states and six large-city/county health departments. This report describes the results of that assessment, which indicated that all participating states and cities had well-developed surveillance and control programs for human, avian, equine, or mosquito WNV.
Descriptors: public health practice, West Nile fever epidemiology, prevention and control, population surveillance, United States, West Nile virus.

Anon (2005). **West Nile virus activity--United States, January 1-December 1, 2005.** *Morbidity and Mortality Weekly Report* 54(49): 1253-1256.
Abstract: West Nile virus (WNV) is the leading cause of arboviral encephalitis in the United States. Originally discovered in Africa in 1937, WNV was first detected in the western hemisphere in 1999 in New York City. Since then it has caused seasonal epidemics of febrile illness and severe neurologic disease. During January 1-December 1, 2005, a total of 2,744 cases of WNV disease in humans were reported in the United States, an increase from 2,359 during the same period in 2004. A total of 1,165 cases were WNV neuroinvasive disease (WNND). WNV infections in humans, birds, mosquitoes, and nonhuman mammals are reported to CDC through ArboNET, an Internet-based arbovirus surveillance system managed by state health departments and CDC. During 2005, WNV transmission to humans or animals expanded into 21 counties that had not previously reported transmission and recurred in 1,196 counties where transmission had been reported in previous years. This report summarizes provisional WNV surveillance data through December 1, 2005, and highlights the need for ongoing surveillance, mosquito control, promotion of personal protection from mosquito bites, and research into additional prevention strategies.
Descriptors: West Nile fever epidemiology, birds, culicidae, dogs, horses, population surveillance, sciuridae, United States, West Nile virus isolation and purification.

Anon (2005). **Update: West Nile virus activity--United States, 2005.** *Morbidity and Mortality Weekly Report* 54(43): 1105-1106.
Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Standard Time, November 1, 2005.
Descriptors: West Nile fever epidemiology, birds, culicidae, dogs, horses, sciuridae, United States, West Nile virus isolation and purification.

Anonymous (2006). **First West Nile Virus screening test approved.** *FDA Consumer* 40(1): 3.
Descriptors: West Nile fever diagnosis, West Nile virus isolation and purification, screening tests.

Anonymous (2005). **Summaries for patients. The cost-effectiveness of screening the U.S. blood supply for West Nile virus.** *Annals of Internal Medicine* 143(7): I44.
Descriptors: blood transfusion, blood borne pathogens, West Nile virus, computer simulation, cost benefit analysis, markov chains, humans, nucleic acid amplification techniques, United States.
Notes: Original Report In: Ann Intern Med. 2005 Oct 4;143(7):486-92.

Anonymous (2005). **West Nile Virus.** *The Nurse Practitioner* 30(8): 51-52.
Descriptors: West Nile fever diagnosis, West Nile fever prevention and control, West Nile fever transmission.

Anonymous (2005). **All you need to know about West Nile virus.** *Nursing Times* 101(26): 28.
Descriptors: communicable diseases, West Nile fever, birds, insect vectors, mosquito control, seasonal changes.

Avalos Bock, S.A. (2005). **West Nile virus and the U.S. blood supply: New tests substantially reduce the risk of transmission via donated blood products.** *American Journal of Nursing* 105(12): 34, 36-37 .
Descriptors: blood donors, nucleic acid amplification techniques, RNA, viral blood, West Nile fever, United States, West Nile virus.

Averett, E., J.S. Neuberger, G. Hansen, and M.H. Fox (2005). **Evaluation of West Nile virus education campaign.** *Emerging Infectious Diseases* 11(11): 1751-1753.
Abstract: We evaluated the 2003 Kansas West Nile virus public education campaign. Awareness was widespread but compliance was low. Spanish-speaking persons were poorly informed. Relevant factors included population segment variability, campaign content, media choice, and materials delivery methods.
Descriptors: health education, program evaluation, West Nile fever, West Nile virus, health surveys , hispanic Americans, Kansas, mass media.

Batalis, N.I., L. Galup, S.R. Zaki, and J.A. Prahlow (2005). **West Nile virus encephalitis.** *American Journal of Forensic Medicine and Pathology* 26(2): 192-196.
Abstract: West Nile virus (WNV) is a mosquito-borne virus that has caused a large number of deaths in the United States since the first outbreak in New York City in 1998. The outbreak initially was limited to the northeast but has since spread across the entire continental United States. WNV causes a variety of clinical symptoms, but the most severe consequences result from central nervous system infection, resulting in meningitis, encephalitis, or meningoencephalitis. We present a case of a 62-year-old male with metastatic cancer, who died as a result of WNV encephalitis. This is followed by a discussion on the epidemiology of WNV and a detailed summary of

the methods and resources available to make a diagnosis of WNV infection postmortem. The material presented in the discussion should provide the forensic pathologist with all the information necessary to make a diagnosis of WNV infection postmortem. If nothing else, the routine collection and storage of serum, cerebrospinal fluid, and tissue for every case can enable the forensic pathologist to make this diagnosis even in cases in which WNV is not suspected until after autopsy.
Descriptors: mosquito vectors, West Nile fever, brain pathology, carcinoma, small cell therapy, chills, confusion, fever, forensic pathology, lung neoplasms, psychomotor agitation.

Beasley, D.W. (2005). **Recent advances in the molecular biology of west nile virus.** *Current Molecular Medicine* 5(8): 835-850.
Abstract: Since the mid-1990s, West Nile virus (WNV) has emerged as a significant agent of arboviral encephalitis in several regions of the world. In 1999, WNV was introduced into the northeastern United States and was associated with an outbreak of encephalitis affecting humans, birds and horses. Subsequently, the virus has spread across the country, and across southern Canada, and in 2002 and 2003 was associated with the largest outbreaks of arboviral encephalitis recorded in the Western hemisphere. Interestingly, the more recent spread of WNV into Mexico, Central America and the Caribbean has not been associated with the high levels of clinical disease observed in North America. This review addresses the most recent results from studies investigating the molecular biology and evolution of WNV, as well as progress in the development of diagnostic and therapeutic reagents.
Descriptors: West Nile fever, West Nile virus, United States.

Beveroth, T.A., M.P. Ward, R.L. Lampman, A.M. Ringia, and R.J. Novak (2006). **Changes in seroprevalence of West Nile virus across Illinois in free-ranging birds from 2001 through 2004.** *American Journal of Tropical Medicine and Hygiene* 74(1): 174-179. ISSN: 0002-9637.
Abstract: Of the 5,236 birds sampled for antibodies to West Nile virus (WNV) in Illinois from 2001 through 2004, 348 (6.6%) birds were seropositive. Our multiple year surveillance identified several avian species that had particularly high percentages of seropositive individuals. The importance of these species in the enzootic and/or epizootic transmission of WNV is discussed relative to their regional abundance and literature on host competency. The species with the highest exposure rates to WNV differed both temporally and regionally. In general, birds that bred or were born in Illinois were more likely to have antibodies than transient birds. There was also a significant difference in the seroprevalence between adults (12.1%) and juveniles (5.5%), indicating that the acquired antibody response from previous years is a critical concern when interpreting seroprevalence rates in wild-caught birds. The most common hosts for St. Louis encephalitis virus were also the most common hosts for

WNV, which strongly supports the role of similar vectors for both flaviviruses. Avian species with high WNV seroprevalence rates tended to be those that bred throughout the year, have open cup nests, and live in close proximity to humans.
Descriptors: aves, age, viral disease prevalence relations, viral diseases, West Nile virus, host records, prevalence, annual changes and age relations, Illinois, viral disease prevalence and host records.

Bowman, C., A.B. Gumel, P. van den Driessche, J. Wu, and H. Zhu (2005). **A mathematical model for assessing control strategies against West Nile virus.** *Bulletin of Mathematical Biology* 67(5): 1107-33.
Abstract: Since its incursion into North America in 1999, West Nile virus (WNV) has spread rapidly across the continent resulting in numerous human infections and deaths. Owing to the absence of an effective diagnostic test and therapeutic treatment against WNV, public health officials have focussed on the use of preventive measures in an attempt to halt the spread of WNV in humans. The aim of this paper is to use mathematical modelling and analysis to assess two main anti-WNV preventive strategies, namely: mosquito reduction strategies and personal protection. We propose a single-season ordinary differential equation model for the transmission dynamics of WNV in a mosquito-bird-human community, with birds as reservoir hosts and culicine mosquitoes as vectors. The model exhibits two equilibria; namely the disease-free equilibrium and a unique endemic equilibrium. Stability analysis of the model shows that the disease-free equilibrium is globally asymptotically stable if a certain threshold quantity (R0), which depends solely on parameters associated with the mosquito-bird cycle, is less than unity. The public health implication of this is that WNV can be eradicated from the mosquito-bird cycle (and, consequently, from the human population) if the adopted mosquito reduction strategy (or strategies) can make R0<1. On the other hand, it is shown, using a novel and robust technique that is based on the theory of monotone dynamical systems coupled with a regular perturbation argument and a Liapunov function, that if R0>1, then the unique endemic equilibrium is globally stable for small WNV-induced avian mortality. Thus, in this case, WNV persists in the mosquito-bird population.
Descriptors: theoretical models, West Nile fever, birds, communicable disease control methods, culicidae, insect control methods, insect repellents, protective clothing, West Nile virus.

Bradbury, J. (2005). **Toll gate for West Nile virus brain entry.** *Lancet Infectious Diseases* 5(1): 9.
Descriptors: blood brain barrier, membrane glycoproteins, West Nile fever, West Nile virus, culicidae, mice, toll-like receptors.

Briese, T. and K.A. Bernard (2005). **West Nile virus--An old virus learning new tricks?** *Journal of Neurovirology* 11(5): 469-475.
Abstract: West Nile virus (WNV) has spread across the United States causing annual outbreaks since its emergence in 1999. Although severe disease develops only in about 1% of infections, WNV has claimed a total of 564 lives in the 5 years from 1999 to 2003. Observation of flaccid paralysis due to WNV infection at a higher incidence than previously documented and the devastating mortality recorded in infected American bird species triggered concerns about a potentially enhanced virulence of this virus. Here we summarize recent observations made during the American outbreaks regarding host range and transmission modes of WNV, and discuss epidemiological aspects of the emergence of this pathogen in the new habitat.
Descriptors: disease outbreaks, West Nile fever, West Nile virus, transmission of animal diseases, arthropod vectors, birds, disease reservoirs, mortality, pregnancy complications, United States.

Busch, M.P., S. Caglioti, E.F. Robertson, J.D. McAuley, L.H. Tobler, H. Kamel, J.M. Linnen, V. Shyamala, P. Tomasulo, and S.H. Kleinman (2005). **Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing.** *New England Journal of Medicine* 353(5): 460-467.
Abstract: BACKGROUND: The use of nucleic acid amplification tests of "mini-pools" of 16 samples to screen blood donors for West Nile virus RNA began in July 2003. We report the yield and characteristics of positive donations and the incremental yield and safety of nucleic acid amplification tests of individual donations. METHODS: Reactive minipools were analyzed to identify the individual reactive donations. For the regions with the highest yield on minipool testing, retrospective nucleic acid amplification testing was performed on individual donations that were negative on minipool testing. Reactive donations were confirmed by alternative nucleic acid amplification tests and IgM and IgG tests, and donors were followed to document seroconversion. RESULTS: From July 1 through October 31, 2003, 677,603 donations were prospectively screened for West Nile virus by minipool testing, yielding 183 confirmed viremic donations (0.027 percent, or 1 in 3703 donations). Retrospective individual testing of 23,088 donations from high-prevalence regions that were negative on minipool testing yielded 30 additional units with a low level of viremia, with 14 additional viremic units detected by prospective testing of individual donations late in the 2003 transmission season. Of all the viremic units detected, 5 percent were detected only by individual testing and were negative for IgM antibody, 29 percent were detected by individual testing after IgM seroconversion, and 66 percent were detected by minipool testing. West Nile virus infection was confirmed in both recipients of IgM-negative units that were reactive on individual testing, whereas neither recipient of antibody-positive blood components that were

reactive on individual testing was infected. In 2004, prospective testing of individual donations in regions that yielded donations that were reactive on minipool testing resulted in a 32 percent incremental yield of units with a low level of viremia that would have been missed by minipool testing. CONCLUSIONS: Although nucleic acid amplification testing of minipools of blood donations prevented hundreds of cases of West Nile virus infection in 2003, it failed to detect units with a low level of viremia, some of which were antibody-negative and infectious. These data support the use of targeted nucleic acid amplification testing of individual donations in high-prevalence regions, a strategy that was implemented successfully in 2004. Copyright 2005 Massachusetts Medical Society.
Descriptors: blood donors, nucleic acid amplification techniques, viral blood RNA, West Nile fever, West Nile virus isolation and purification, retrospective studies, viremia.
Notes: Comment In: N Engl J Med. 2005 Aug 4;353(5):516-7.

Busch, M.P., D.J. Wright, B. Custer, L.H. Tobler, S.L. Stramer, S.H. Kleinman, H.E. Prince, C. Bianco, G. Foster, L.R. Petersen, G. Nemo, and S.A. Glynn (2006). **West Nile virus infections projected from blood donor screening data, United States, 2003.** *Emerging Infectious Diseases* 12(3): 395-402.
Abstract: National blood donor screening for West Nile virus (WNV) RNA using minipool nucleic acid amplification testing (MP-NAT) was implemented in the United States in July 2003. We compiled national NAT yield data and performed WNV immunoglobulin M (IgM) testing in 1 WNV-epidemic region (North Dakota). State-specific MP-NAT yield, antibody seroprevalence, and the average time RNA is detectable by MP-NAT were used to estimate incident infections in 2003. WNV donor screening yielded 944 confirmed viremic donors. MP-NAT yield peaked in August with >0.5% of donations positive for WNV RNA in 4 states. Peak IgM seroprevalence for North Dakota was 5.2% in late September. The average time viremia is detectable by MP-NAT was 6.9 days (95% confidence interval [CI] 3.0-10.7). An estimated 735,000 (95% CI 322,000-1,147,000) infections occurred in 2003, with 256 (95% CI 112-401) infections per neuroinvasive case. In addition to preventing transfusion-transmitted WNV infection, donor screening can serve as a tool to monitor seasonal incidence in the general population.
Descriptors: blood donors, West Nile fever, RNA, viral blood, seasons, sensitivity and specificity, time factors, United State, West Nile virus.

Chang Hua, Hua QunYi, Xiang Xun, Zeng ZhaoWen, and Duan Gang (2005). **Recent advances in the study of West Nile virus**. *Journal of Yunnan Agricultural University* 21(1): 77-80. ISSN: 1004-390X.
Descriptors: West Nile virus, diagnosis and prevention, vaccination, epidemiology,

pathogenicity, literature review.
Language of Text: Chinese; Summary in English.

Corrigan, R.L., C. Waldner, T. Epp, J. Wright, S.M. Whitehead, H. Bangura, E. Young, and H.G. Townsend (2006). **Prediction of human cases of West Nile virus by equine cases, Saskatchewan, Canada, 2003.** *Preventive Veterinary Medicine* 76(3-4): 263-72.
Abstract: In 2003, an outbreak of West Nile virus (WNV) occurred in Saskatchewan, Canada from July to September. One-hundred thirty-three horse cases and 947 human cases were recorded and data were analyzed retrospectively for evidence of clustering to determine if clinical infection in the horse population could be used to estimate human risk of infection with WNV. Kulldorff's scan statistic was used to identify spatial-temporal clusters in both the human and horse cases. In most areas, human clusters were not preceded by horse clusters. In one area, a significant cluster of horse cases preceded human cases by 1 week; however, 1 week does not provide sufficient time for human-health authorities to act and provide advance warning for the public.
Descriptors: disease outbreaks, horse diseases epidemiology, diseases transmission, West Nile fever epidemiology, zoonoses, cluster analysis, horse diseases prevention and control, predictive value of tests, retrospective studies, Saskatchewan epidemiology, space time clustering, time factors, West Nile fever, prevention and control, West Nile fever transmission, West Nile virus isolation and purification.

Crawford, C., D. Hoch, M. Long, and J. Levy (2005). **Prevalence of West Nile virus in feral cats.** *Journal of Veterinary Internal Medicine* 19(3): 471. ISSN: 0891-6640.
Descriptors: feral cats, population studies, West Nile virus , ELISA, virus neutralization assay, diagnostic techniques.
Notes: Meeting Information: 23rd Annual Forum of the American College of Veterinary Internal Medicine, Baltimore, Maryland, USA; June 1-4, 2005.

Davis, L.B., E. Hayes, D. O'leary, T. Smith, A. Marfin, A. Hinckley, P. Collins, K. Kniss, and G. Campbell (2005). **Age and gender as risk factors for West Nile virus neuroinvasive disease in children in the United States, 1999-2004.** *American Journal of Epidemiology* 161(11, Suppl. S): S113. ISSN: 0002-9262.
Descriptors: children, neuroinvasive disease, meningitis, encephalitis, West Nile virus, risk factors, age, gender, USA.
Notes: Meeting Information: Joint Meeting of the Society for Epidemiologic Research/Canadian Society for Epidemiology and Biostatistics, Toronto, Canada; June 27 -30, 2005.

Davis, L.E., R. DeBiasi, D.E. Goade, K.Y. Haaland, J.A. Harrington, J.B. Harnar, S.A. Pergam, M.K. King, B.K. DeMasters, and K.L. Tyler (2006). **West Nile virus neuroinvasive disease.** *Annals of Neurology* 60(3): 286-300.

Abstract: Since 1999, there have been nearly 20,000 cases of confirmed symptomatic West Nile virus (WNV) infection in the United States, and it is likely that more than 1 million people have been infected by the virus. WNV is now the most common cause of epidemic viral encephalitis in the United States, and it will likely remain an important cause of neurological disease for the foreseeable future. Clinical syndromes produced by WNV infection include asymptomatic infection, West Nile Fever, and West Nile neuroinvasive disease (WNND). WNND includes syndromes of meningitis, encephalitis, and acute flaccid paralysis/poliomyelitis. The clinical, laboratory, and diagnostic features of these syndromes are reviewed here. Many patients with WNND have normal neuroimaging studies, but abnormalities may be present in areas including the basal ganglia, thalamus, cerebellum, and brainstem. Cerebrospinal fluid invariably shows a pleocytosis, with a predominance of neutrophils in up to half the patients. Diagnosis of WNND depends predominantly on demonstration of WNV-specific IgM antibodies in cerebrospinal fluid. Recent studies suggest that some WNV-infected patients have persistent WNV IgM serum and/or cerebrospinal fluid antibody responses, and this may require revision of current serodiagnostic criteria. Although there is no proven therapy for WNND, several vaccines and antiviral therapy with antibodies, antisense oligonucleotides, and interferon preparations are currently undergoing human clinical trials. Recovery from neurological sequelae of WNV infection including cognitive deficits and weakness may be prolonged and incomplete.

Descriptors: nervous system diseases, West Nile fever , West Nile virus.

Dean, J.L. and B.J. Palermo (2005). **West Nile virus encephalitis**. *Current Infectious Disease Reports* 7(4): 292-296. ISSN: 1523-3847.

Abstract: West Nile virus (WNV) is a small RNA virus. It was first isolated in the blood of a febrile woman in the West Nile district of Uganda in 1937. Although WNV has caused human disease in Africa and Europe since its identification, the first documented human infections occurred in the United States in 1999. Wild birds are the reservoir for WNV, and most transmission to humans occurs after the bite of an infected mosquito. In humans, 80% of infections are asymptomatic and nearly 20% cause a mild self-limiting illness called WNV fever. Less than 1% will develop central nervous system (CNS) infection, which manifests as meningitis, encephalitis, or acute flaccid paralysis. The case fatality rate for CNS infection is approximately 15%. Human vaccine is not available. Personal mosquito protection remains the best prevention, and treatment is supportive.

Descriptors: West Nile virus, RNA virus, encephalitis, brain diseases, wild birds as disease reservoirs, transmission, disease prevention, meningitis.

Deardorff, E., J. Estrada Franco, A.C. Brault, R. Navarro Lopez, A. Campomanes Cortes, P. Paz Ramirez, M. Solis Hernandez, W.N. Ramey, C.T. Davis, D.W. Beasley, R.B. Tesh, A.D. Barrett, and S.C. Weaver (2006). **Introductions of West Nile virus strains to Mexico.** *Emerging Infectious Diseases* 12(2): 314-318.
Abstract: Complete genome sequencing of 22 West Nile virus isolates suggested 2 independent introductions into Mexico. A previously identified mouse-attenuated glycosylation variant was introduced into southern Mexico through the southeastern United States, while a common US genotype appears to have been introduced incrementally into northern Mexico through the southwestern United States.
Descriptors: West Nile virus, bird diseases, horse diseases, Mexico, mice, molecular sequence data, United States.

Debiasi, R.L. and K.L. Tyler (2006). **West Nile virus meningoencephalitis.** *Nature: Clinical Practice Neurology* 2(5): 264-275. ISSN: 1745-834X.
Abstract: Since its first appearance in the US in 1999, West Nile virus (WNV) has emerged as the most common cause of epidemic meningoencephalitis in North America. In the 6 years following the 1999 outbreak, the geographic range and burden of the disease in birds, mosquitoes and humans has greatly expanded to include the 48 contiguous US and 7 Canadian provinces, as well as Mexico, the Caribbean islands and Colombia. WNV has shown an increasing propensity for neuroinvasive disease over the past decade, with varied presentations including meningitis, encephalitis and acute flaccid paralysis. Although neuroinvasive disease occurs in less than 1% of infected individuals, it is associated with high mortality. From 1999-2005, more than 8,000 cases of neuroinvasive WNV disease were reported in the US, resulting in over 780 deaths. In this review, we discuss epidemiology, risk factors, clinical features, diagnosis and prognosis of WNV meningoencephalitis, along with potential treatments.
Descriptors: West Nile fever epidemiology, United States.

Diamond, M.S. (2005). **Development of effective therapies against West Nile virus infection.** *Expert Review of Anti Infective Therapy* 3(6): 931-944.
Abstract: Since its entry into North America in 1999, West Nile virus has spread throughout the USA and Canada, and now annually causes a clinical spectrum of human disease ranging from a self-limiting acute febrile illness to potentially lethal encephalitis. Although no therapy is currently approved for use in humans, several strategies are being pursued to develop effective prophylaxis and treatments. This review describes the epidemiology, clinical presentation and pathogenesis of West Nile virus infection, and highlights recent progress towards an effective therapy.

Descriptors: antiviral agents, pharmaceutical trends, West Nile fever, West Nile virus drug effects, literature review.

Diamond, M.S. and R.S. Klein (2006). **A genetic basis for human susceptibility to West Nile virus.** *Trends in Microbiology* 14(7): 287-289.

Abstract: West Nile virus (WNV) infects thousands of humans annually and causes a spectrum of disease ranging from an acute febrile illness to lethal encephalitis. A new study suggests a link between CCR5Delta32 (a common mutant allele of the chemokine and HIV receptor CCR5) and fatal WNV infection. The study highlights a possible risk in targeting this receptor for the prevention and/or treatment of infectious diseases.

Descriptors: genetic predisposition to disease, CCR5 receptors genetics, West Nile fever, mice, West Nile virus, risk factors.

DiMenna, M.A., R.J. Bueno, R.R. Parmenter, D.E. Norris, J.M. Sheyka, J.L. Molina, E.M. LaBeau, E.S. Hatton, and G.E. Glass (2006). **Emergence of West Nile virus in mosquito (Diptera: Culicidae) communities of the New Mexico Rio Grande Valley.** *Journal of Medical Entomology* 43(3): 594-599.

Abstract: The first appearances of West Nile virus (family Flaviviridae, genus Flavivirus, WNV) in New Mexico were reported in late summer to early fall 2002. Several dead birds tested positive for WNV, and 78 equine cases were confirmed. All mosquito pools tested (n = 268) were negative. A statewide surveillance program was launched in May 2003 to study the emergence and spread of this new arbovirus in mosquitoes from the Rio Grande valley. Mosquitoes were trapped at 32 sites along a 750-km stretch of the Rio Grande valley. Sites were trapped for one night either weekly or biweekly, by using CO2-baited CDC light traps and gravid traps. Pools of captured mosquitoes were tested for WNV by reverse transcription-polymerase chain reaction. By mid-July 2003, WNV levels in the mosquito population had reached levels that were detectable by the surveillance program. Positive pools of mosquitoes were found in the Rio Grande valley from mid-July through late September. In total, 75 positive pools were found, from sites throughout the study area. The predominant species infected with WNV in this region were *Culex tarsalis* (Coquillett) in rural areas, and *Culex salinarius* (Coquillett) and *Culex pipiens quinquefasciatus* (Say) in urban areas. There were 202 human cases and 438 equine cases of WNV in New Mexico in 2003, which corresponded well in time with the positive mosquitoes. Our results seemed to be consistent with introduction of WNV in late summer 2002, followed by a period of transmission and amplification cycles between local avian hosts and mosquito vectors.

Descriptors: West Nile virus, horse diseases, insect vectors, New Mexico, seasons, West Nile fever transmission.

Docherty, D.E., M.D. Samuel, C.A. Nolden, K.F. Egstad, and K.M. Griffin (2006). **West Nile virus antibody prevalence in wild mammals, southern Wisconsin**. *Emerging Infectious Diseases* 12(12): 1982-1984. ISSN: 1080-6040.
Descriptors: wild mammals, southern Wisconsin, West Nile antibody, population studies, disease transmission.

Drebot, M.A. and H. Artsob (2005). **West Nile virus. Update for family physicians.** *Canadian Family Physician Medecin De Famille Canadien* 51: 1094-1099.
Abstract: OBJECTIVE: To review the epidemiology and disease manifestations of West Nile virus (WNV) in North America and to describe the current status of therapeutic approaches and vaccines for treating or preventing viral illness. QUALITY OF EVIDENCE: Since 1999, research initiatives investigating the ecology, epidemiology, and biology of WNV have increased substantially. These studies provide a foundation for understanding current activity and predicting future activity and for describing the effect of WNV on human health. MAIN MESSAGE: West Nile virus is transmitted to humans primarily through bites from infected mosquitoes. Most people infected have no symptoms; a few have clinical manifestations ranging from febrile illness to neurologic syndromes and possibly death. Risk of serious disease increases with age, and substantial long-term morbidity has been observed in patients who develop severe neurologic illness. No specific antiviral therapy or vaccine currently exists. CONCLUSION: West Nile virus has established itself in North America and has become an important public health concern. Decreasing risk of virus-associated illness requires seasonal preventive and control measures.
Descriptors: West Nile fever diagnosis, West Nile fever epidemiology, Canada, family practice.

Esteves, A., A. Almeida, R. Galao, R. Parreira, J. Piedade, J. Rodrigues, C. Sousa, and M. Novo (2005). **West Nile virus in Southern Portugal, 2004.** *Vector Borne and Zoonotic Diseases.* 5(4): 410-413. ISSN: 1530-3667.
Descriptors: West Nile virus, disease detection, insect vectors, Culicidae, *Culex pipiens*, *Culex univittatus*, nucleotide sequences, sequence homology, phylogeny, epidemiological studies , Portugal, infection-rate.

Everson, K. (2006). **West Nile virus up in 2005.** *Canadian Medical Association Journal* 174(1): 21.
Descriptors: birds virology, climate, West Nile fever mortality, Canada, disease reservoirs , mortality trends, movement, population dynamics, seasons, zoonoses.

Fonseca, K., G.D. Prince, J. Bratvold, J.D. Fox, M. Pybus, J.K. Preksaitis, and P. Tilley (2005). **West Nile virus infection and conjunctival exposure.** *Emerging Infectious Diseases* 11(10): 1648-1649. ISSN: 1080-6040.

Descriptors: bird diseases, conjunctiva, crows, occupational exposure, West Nile fever transmission, West Nile virus isolation and purification.

Gerhardt, R. (2006). **West nile virus in the United States (1999-2005).** *Journal of the American Animal Hospital Association* 42(3): 170-177 .

Abstract: The accidental introduction of West Nile Virus into New York City from the Old World in 1999 resulted in an epidemic in humans, horses, and birds that swept to the west coast in just 3 years. The virus is transmitted by infective mosquitoes among susceptible native birds, which serve as amplifying hosts. Clinical disease occurs in humans and horses, but not enough virus is produced in their blood to infect other mosquitoes; therefore, humans and horses are considered dead-end hosts. Humans can best protect themselves by remaining indoors during periods of high mosquito activity and/or by using recommended repellents. Effective vaccines are available for horses.

Descriptors: West Nile virus, United States, virus transmission, mosquitoes, humans, birds, horses, dead end hosts.

Green, M.S., M. Weinberger, J. Ben Ezer, H. Bin, E. Mendelson, D. Gandacu, Z. Kaufman, R. Dichtiar, A. Sobel, D. Cohen, and M.Y. Chowers (2005). **Long-term Death Rates, West Nile virus epidemic, Israel, 2000.** *Emerging Infectious Diseases* 11(11): 1754-1757. ISSN: 1080-6040.

Abstract: We studied the 2-year death rate of 246 adults discharged from hospital after experiencing acute West Nile Virus infection in Israel during 2000. The age- and sex-adjusted death rates were significantly higher than in the general population. This excess was greater for men. Significant adverse prognostic factors were age, male sex, diabetes mellitus, and dementia.

Descriptors: communicable diseases, emerging mortality, disease outbreaks, West Nile fever mortality, aged humans, Israel, risk factors, survival rate, West Nile virus.

Hayes, E.B. and D.J. Gubler (2006). **West Nile virus: Epidemiology and clinical features of an emerging epidemic in the United States.** *Annual Review of Medicine* 57: 181-194. ISSN: 0066-4219.

Abstract: West Nile virus (WNV) was first detected in North America in 1999 during an outbreak of encephalitis in New York City. Since then the virus has spread across North America and into Canada, Latin America, and the Caribbean. The largest epidemics of neuroinvasive WNV disease ever reported occurred in the United States in 2002 and 2003. This paper reviews new information on the epidemiology and clinical aspects of WNV disease derived from greatly expanded surveillance and research on WNV during the past six years.

Descriptors: disease outbreaks, West Nile fever epidemiology, West Nile virus , United States.

Hayes, E.B., N. Komar, R.S. Nasci, S.P. Montgomery, D.R. O'Leary, and G.L. Campbell (2005). **Epidemiology and transmission dynamics of West Nile virus disease.** *Emerging Infectious Diseases* 11(8): 1167-1173. ISSN: 1080-6040.

Abstract: From 1937 until 1999, West Nile virus (WNV) garnered scant medical attention as the cause of febrile illness and sporadic encephalitis in parts of Africa, Asia, and Europe. After the surprising detection of WNV in New York City in 1999, the virus has spread dramatically westward across the United States, southward into Central America and the Caribbean, and northward into Canada, resulting in the largest epidemics of neuroinvasive WNV disease ever reported. From 1999 to 2004, >7,000 neuroinvasive WNV disease cases were reported in the United States. In 2002, WNV transmission through blood transfusion and organ transplantation was described for the first time, intrauterine transmission was first documented, and possible transmission through breastfeeding was reported. This review highlights new information regarding the epidemiology and dynamics of WNV transmission, providing a new platform for further research into preventing and controlling WNV disease.

Descriptors: disease outbreaks, insect vectors, West Nile fever epidemiology, West Nile virus growth and development, humans, United States.

Hayes, E.B., J.J. Sejvar, S.R. Zaki, R.S. Lanciotti, A.V. Bode, and G.L. Campbell (2005). **Virology, pathology, and clinical manifestations of West Nile virus disease.** *Emerging Infectious Diseases* 11(8): 1174-1179. ISSN: 1080-6040.

Abstract: West Nile virus (WNV) causes epidemics of febrile illness, meningitis, encephalitis, and flaccid paralysis. Since it was first detected in New York City in 1999, and through 2004, >16,000 WNV disease cases have been reported in the United States. Over the past 5 years, research on WNV disease has expanded rapidly. This review highlights new information regarding the virology, clinical manifestations, and pathology of WNV disease, which will provide a new platform for further research into diagnosis, treatment, and possible prevention of WNV through vaccination.

Descriptors: West Nile fever pathology, West Nile virus growth and development, antiviral agents, viral vaccines.

Hodge, J.G.J. and J.P. O'Connell (2005). **West Nile virus: Legal responses that further environmental health.** *Journal of Environmental Health* 68(1): 44-47.

Descriptors: environmental health, mosquito control, West Nile fever prevention and control, birds, consumer participation, health education, insecticides, New York City, risk assessment, United States.

Holcomb, S.S. (2005). **Guidelines for West Nile virus.** *Nurse Practitioner* 30(9): 7 , 11, 14.

Descriptors: communicable diseases, emerging diseases diagnosis, practice guidelines, West Nile fever diagnosis, West Nile fever prevention and control, health education, mosquito control, population surveillance methods, primary prevention methods, risk factors, United States, West Nile virus.

Jamieson, D.J., D.B. Jernigan, J.E. Ellis, and T.A. Treadwell (2005). **Emerging infections and pregnancy: West Nile virus, monkeypox, severe acute respiratory syndrome, and bioterrorism.** *Clinics in Perinatology* 32(3): 765-776.

Abstract: As new infectious diseases, such as West Nile virus, monkeypox, and severe acute respiratory syndrome (SARS) are recognized in the United States, there are critical questions about how these infectious diseases will affect pregnant women and their infants. In addition, the implications of bioterrorist attacks for exposed pregnant women need to be considered. In this article, the authors address the following questions for a number of infectious disease threats: (1) does pregnancy affect the clinical course of these novel infectious diseases?, (2) what are the implications for prophylaxis and treatment of exposed or infected pregnant women, and (3) are these novel infectious diseases transmitted during pregnancy, labor and delivery, or breastfeeding?

Descriptors: bioterrorism, emerging communicable diseases, monkeypox, pregnancy complications, infectious diseases, severe acute respiratory syndrome (SARS), West Nile virus, smallpox, West Nile fever epidemiology.

Jester, P.M., S.J. Tilden, Y. Li, R.J. Whitley, and W.M. Sullender (2006). **Regulatory challenges: Lessons from recent West Nile virus trials in the United States.** *Contemporary Clinical Trials* 27(3): 254-259.

Abstract: Delays in research on emerging infections could deprive the public of appropriate therapies. This report describes challenges encountered in implementing two multicenter protocols of West Nile virus (WNV) infections in the United States during 2003. Protocol development times, federal regulatory approvals, and local Institutional Review Boards (IRB) approvals were compiled. Twenty eight institutions participated in a natural history study and 27 in a therapeutic trial of WNV developed through the National Institute of Allergy and Infectious Disease Collaborative Antiviral Study Group (CASG). The CASG compiled protocol development times, federal regulatory approvals, and local IRB approvals. Additional information on the local IRB process was obtained by survey of the investigators. Because of the lengthy development and approval process, protocols were distributed after the start of the epidemic season, most sites were unable to enroll subjects at the peak of the season, and a number of sites lacked IRB approval at the end of the season.

Descriptors: clinical trials standards, West Nile fever prevention and control, clinical

trials methods, disease progression, ethics committees, multicenter studies, seasons, time factors, United States, epidemiology.

Johnson, G.D., M. Eidson, K. Schmit, A. Ellis, and M. Kulldorff (2006). **Geographic prediction of human onset of West Nile virus using dead crow clusters: An evaluation of year 2002 data in New York State.** *American Journal of Epidemiology* 163(2): 171-180.

Abstract: The risk of becoming a West Nile virus case in New York State, excluding New York City, was evaluated for persons whose town of residence was proximal to spatial clusters of dead American crows (*Corvus brachyrhynchos*). Weekly clusters were delineated for June-October 2002 by using both the binomial spatial scan statistic and kernel density smoothing. The relative risk of a human case was estimated for different spatial-temporal exposure definitions after adjusting for population density and age distribution using Poisson regression, adjusting for week and geographic region, and conducting Cox proportional hazards modeling, where the week that a human case was identified was treated as the failure time and baseline hazard was stratified by region. The risk of becoming a West Nile virus case was positively associated with living in towns proximal to dead crow clusters. The highest risk was consistently for towns associated with a cluster in the current or prior 1-2 weeks. Weaker, but positive associations were found for towns associated with a cluster in just the 1-2 prior weeks, indicating an ability to predict onset in a timely fashion.

Descriptors: bird diseases, epidemiology, crows, West Nile fever, cluster analysis, disease reservoirs, New York, population surveillance, proportional hazards models, West Nile virus isolation and purification.

Kilpatrick, A.M., L.D. Kramer, M.J. Jones, P.P. Marra, and P. Daszak (2006). **West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior.** *PLoS Biology* 4(4): E82.

Online: http://biology.plosjournals.org/perlserv/?request=get-document&doi=10.1371/journal.pbio.0040082

Abstract: West Nile virus (WNV) has caused repeated large-scale human epidemics in North America since it was first detected in 1999 and is now the dominant vector-borne disease in this continent. Understanding the factors that determine the intensity of the spillover of this zoonotic pathogen from birds to humans (via mosquitoes) is a prerequisite for predicting and preventing human epidemics. We integrated mosquito feeding behavior with data on the population dynamics and WNV epidemiology of mosquitoes, birds, and humans. We show that *Culex pipiens*, the dominant enzootic (bird-to-bird) and bridge (bird-to-human) vector of WNV in urbanized areas in the northeast and north-central United States, shifted its feeding preferences from birds to humans by 7-fold during late summer and early fall, coinciding with the dispersal of its preferred host (American robins, *Turdus migratorius*)

and the rise in human WNV infections. We also show that feeding shifts in *Cx. tarsalis* amplify human WNV epidemics in Colorado and California and occur during periods of robin dispersal and migration. Our results provide a direct explanation for the timing and intensity of human WNV epidemics. Shifts in feeding from competent avian hosts early in an epidemic to incompetent humans after mosquito infection prevalences are high result in synergistic effects that greatly amplify the number of human infections of this and other pathogens. Our results underscore the dramatic effects of vector behavior in driving the transmission of zoonotic pathogens to humans.
Descriptors: West Nile virus, vector-borne diseases, mosquito hosts, *Culex pipens*, feeding preferences of mosquitos, birds to human shift in preference, seasonal shift, WNV epidemics.

Kipp, A.M., J.A. Lehman, R.A. Bowen, P.E. Fox, M.R. Stephens, K. Klenk, N. Komar, and M.L. Bunning (2006). **West Nile virus quantification in feces of experimentally infected American and fish crows.** *American Journal of Tropical Medicine and Hygiene* 75(4): 688-690. ISSN: 0002-9637.
Abstract: To better understand the potential environmental health risk presented by West Nile virus (WNV)-contaminated feces, we quantified the amount of WNV present in the feces of experimentally infected American crows (*Corvus brachyrhynchos*) and fish crows (*Corvus ossifragus*). Peak fecal titers ranged from 10(3.5) to 10(8.8) plaque-forming units (PFU)/g for 10 American crows and from 10(2.3) to 10(6.4) PFU/g for 10 fish crows. The presence of infectious WNV in bird feces indicates a potential for direct transmission of WNV. Thus, handlers of sick or dead birds should take appropriate precautions to avoid exposure to fecal material.
Descriptors: bird diseases, crows, feces, virus shedding, West Nile fever, West Nile virus isolation and purification.

Kleinman, S., S.A. Glynn, M. Busch, D. Todd, L. Powell, L. Pietrelli, G. Nemo, G. Schreiber, C. Bianco, and L. Katz (2005). **The 2003 West Nile virus United States epidemic: The America's Blood Centers experience**. *Transfusion* 45(4): 469-479. ISSN: 0041-1132.
Online: http://www.blackwell-synergy.com/servlet/useragent?func=showIssues&code=trf
Descriptors: West Nile virus, disease epidemics, blood centers, viraemia, nucleic acid amplification technology, RNA, polymerase chain reaction, viral disease testing, detection, surveillance.

Komar, N. and G.G. Clark (2006). **West Nile virus activity in Latin America and the Caribbean**. *Pan American Journal of Public Health* 19(2): 112-117. ISSN: 1020-4989.

Descriptors: West Nile virus, Caribbean, Latin America, disease detection and transmission, birds, humans, horses, chickens.
Language of Text: Spanish.

Kondo, T. (2005). **West Nile virus infection in horses**. *Journal of Veterinary Medicine* 58(2): 135-138. ISSN: 0447-0192.
Descriptors: West Nile virus, horses, viral infection, epidemiology, zoonoses.
Language of Text: Japanese.

Kondro, W. (2006). **West Nile virus still a threat.** *Canadian Medical Association Journal* 175(6): 570.
Descriptors: West Nile fever, Canada, incidence, West Nile fever transmission.

Korves, C.T., S.J. Goldie, and M.B. Murray (2006). **Blood screening for west nile virus: the cost-effectiveness of a real-time, trigger-based strategy.** *Clinical Infectious Diseases* 43(4): 490-493.
Abstract: Previous studies have demonstrated that universal blood screening for West Nile virus is not cost-effective. A newly proposed, real-time, trigger-based screening strategy was analyzed and was also shown to be not cost-effective. These results were highly sensitive to pricing of screening assays.
Descriptors: mass screening economics, West Nile fever diagnosis, West Nile virus isolation and purification, cost-benefit analysis.

Lefrancois, T., B.J. Blitvich, J. Pradel, S. Molia, N. Vachiery, G. Pallavicini, N.L. Marlenee, S. Zientara, M. Petitclerc, and D. Martinez (2005). **West Nile virus surveillance, Guadeloupe, 2003-2004**. *Emerging Infectious Diseases* 11(7): 1100-1103. ISSN: 1080-6040.
Descriptors: West Nile virus surveillance, antibodies, Guadeloupe, epidemiology, poultry, seroprevalence, donkeys, fowls, horses.

Lim ChangKweng and I. Kurane (2005). **The spread and resurgence of West Nile virus disease: Recent developments in the virology, epidemiology, and clinical characteristics**. *Yamaguchi Journal of Veterinary Medicine*(32): 1-12. ISSN: 0388-9335.
Descriptors: West Nile virus, epidemiology, clinical characteristics, birds, literature review, virology, pathology, prevention.
Language of Text: Japanese; Summary in English.

Loeb, M., S.J. Elliott, B. Gibson, M. Fearon, R. Nosal, M. Drebot, C. D'Cuhna, D. Harrington, S. Smith, P. George, and J. Eyles (2005). **Protective behavior and West Nile virus risk**. *Emerging Infectious Diseases* 11(9): 1433-1436. ISSN: 1080-6040.

Descriptors: West Nile virus, protective behavior traits, survey , human disease outbreaks, reduced infection risk .

MacDonald, R.D. and V.F. Krym (2005). **West Nile virus. Primer for family physicians.** *Canadian Family Physician Medecin De Famille Canadien* 51: 833-837. ISSN: 0008-350X .

Abstract: OBJECTIVE: To provide primary care physicians with an understanding of West Nile virus in North America. This article focuses on epidemiology, clinical features, diagnosis, and prevention of infection. QUALITY OF EVIDENCE: MEDLINE and EMBASE searches revealed epidemiologic, surveillance, cohort, and outcome studies providing level II evidence. There were no randomized controlled trials of treatment. Recommended prevention and treatment strategies are based on level II and III evidence. MAIN MESSAGE: The mosquito-borne virus that first appeared on this continent in 1999 is now prevalent throughout North America. Most infections are asymptomatic. Fewer than 1% of those infected develop severe illness; 3% to 15% of those with severe illness die. While methods for controlling the mosquito population are available, we lack evidence that they reduce infection in the general human population. Family physicians have an important role in advising their patients on ways to prevent infection and in identifying patients who might be infected with West Nile virus. CONCLUSION: The general population is at low risk of West Nile virus infection. Prevention of infection rests on controlling the mosquito population and educating people on how to protect themselves against mosquito bites.

Descriptors: West Nile fever, prevention and control, family practice, disease diagnosis.

Mattar, S., E. Edwards, J. Laguado, M. Gonzalez, J. Alvarez, and N. Komar (2005). **West Nile virus antibodies in Colombian horses.** *Emerging Infectious Diseases* 11(9): 1497-1498. ISSN: 1080-6040 .

Descriptors: viral blood antibodies, emerging communicable diseases, flavivirus, West Nile virus, Colombia, hemolytic plaque technique, horses.

Mazurek, J.M., K. Winpisinger, B.J. Mattson, R. Duffy, and R.L. Moolenaar (2005). **The epidemiology and early clinical features of West Nile virus infection.** *American Journal of Emergency Medicine* 23(4): 536-543.

Abstract: We studied early clinical features of the West Nile virus (WNV) infection. Case patients were Ohio residents who reported to the Ohio Department of Health from August 14 to December 31, 2002, with a positive serum or cerebrospinal fluid for anti-WNV IgM. Of 441 WNV cases, medical records of 224 (85.5%) hospitalized patients were available for review. Most frequent symptoms were fever at a temperature of 38.0 degrees C or higher (n = 155; 69.2%), headache (n = 114;

50.9%), and mental status changes (n = 113; 50.4%). At least one neurological symptom, one gastrointestinal symptom, and one respiratory symptom was present in 186 (83.0%), 119 (53.1%), and 46 (20.5%) patients, respectively. Using multivariate logistic regression and controlling for age, we found that the initial diagnosis of encephalitis (P = .001) or reporting abdominal pain (P < .001) was associated with death. Because initial symptoms of WNV infection are not specific, physicians should maintain a high index of suspicion during the epidemic season, particularly in elderly patients with compatible symptoms.
Descriptors: West Nile fever, hospital statistics, numerical data, encephalitis, guillain barre syndrome, meningitis, Ohio, health care, humans.

Michaels, S.R., G.A. Balsamo, M. Kukreja, C. Anderson, S. Straif Bourgeois, G. Talati, and R.C. Ratard (2005). **Surveillance for West Nile virus cases in Louisiana 2001-2004.** *Journal of the Louisiana State Medical Society Official Organ of the Louisiana State Medical Society* 157(5): 269-272.
Abstract: West Nile virus (WNV) was first detected in Louisiana during August of 2001. An outbreak of 204 human cases of neuro-invasive disease (NID) and 25 deaths occurred in 2002. In the 2 years following, lower numbers of human cases were identified (101 NID cases in 2003 and 84 in 2004) but intense localized foci were observed. The incidence of NID has been particularly high in the elderly (65 years and older). The distribution of West Nile cases has consisted of sporadic cases with a few very intense foci. Annually, human cases have occurred from June through December, with a peak number of new cases in August. As compared with other WNV serosurveys conducted in the United States, it appears that the WNV seroprevalence in Louisiana is not elevated.
Descriptors: West Nile fever, humans, Louisiana, population surveillance, seroepidemiologic studies , survival rate.

Michaelson, P.G. and E.A. Mair (2005). **West Nile virus: A primer for the otolaryngologist.** *Otolaryngology - Head and Neck Surgery* 132(3): 347-352.
Abstract: BACKGROUND: Since recognition in the United States with a 1999 New York City epidemic, West Nile virus has enduringly migrated westward, leaving few states unaffected. Infection rates are rising at an alarming rate, doubling every year since introduction, with more than 9800 cases in 2003 alone and more than 260 deaths. Patients may present with myriad symptoms including a maculopapular rash that affects the face and trunk and diffuse lymphadenopathy, both of which may result in the initial consultation of the otolaryngologist. We review the clinical history of West Nile virus and its epidemiology, laboratory findings, and variable clinical presentation, with an emphasis on otolaryngologic manifestations. STUDY DESIGN AND SETTING COMPREHENSIVE: review of the literature over the past 50 years with an emphasis on what the present-day otolaryngologist needs to

know concerning West Nile virus. Clinical manifestations of the head and neck such as encephalitis, meningitis, maculopapular rash, lymphadenopathy and dysphagia are discussed. RESULTS: To date, there are no articles in the otolaryngology literature discussing West Nile virus. These patients may present initially to multiple providers in diverse specialties because of multifarious initial signs and symptoms. The otolaryngologist must be educated on this quickly growing affliction and practice with a high index of suspicion. CONCLUSIONS: In this article we describe the clinical manifestations of West Nile virus, with an emphasis on the otolaryngologic manifestations. The otolaryngologist must become educated about this entity to facilitate preventative measures, adequately treat, and assist other providers in hopeful control and potential eradication of this infectious threat.

Descriptors: West Nile fever diagnosis, West Nile fever epidemiology, otolaryngology.

Montgomery, S.P., J.A. Brown, M. Kuehnert, T.L. Smith, N. Crall, R.S. Lanciotti, A. Macedo de Oliveira, T. Boo, and A.A. Marfin (2006). **Transfusion-associated transmission of West Nile virus, United States 2003 through 2005.** *Transfusion* 46(12): 2038-2046.

Abstract: BACKGROUND: National blood donation screening for West Nile virus (WNV) started in June 2003, after the documentation of WNV transfusion-associated transmission (TAT) in 2002. STUDY DESIGN AND METHODS: Blood donations were screened with investigational nucleic acid amplification assays in minipool formats. Blood collection agencies (BCAs) reported screening results to state and local public health authorities. Donor test results and demographic information were forwarded to CDC via ArboNET, the national electronic arbovirus surveillance system. State health departments and BCAs also reported suspect WNV TATs to CDC, which investigated these reports to confirm WNV infection in blood transfusion recipients in the absence of likely mosquito exposure. RESULTS: During 2003 to 2005, a total of 1,425 presumptive viremic donors were reported to CDC from 41 states. Of 36 investigations of suspected WNV TAT in 2003, 6 cases were documented. Estimated viremia levels were available for donations implicated in four TAT cases; the median estimated viremia was 0.1 plaque-forming units (PFUs) per mL (range, 0.06-0.50 PFU/mL; 1 PFU equals approximately 400 copies/mL). CONCLUSIONS: National blood screening for WNV identified and removed more than 1,400 potentially infectious blood donations in 2003 through 2005. Despite the success of screening in 2003, some residual WNV TAT risk remained due to donations containing very low levels of virus. Screening algorithms employing selected individual-donation testing were designed to address this residual risk and were fully implemented in 2004 and 2005. Continued vigilance for TAT will evaluate the effectiveness of these strategies.

Descriptors: blood donation, West Nile virus, transfusion transmission, blood collection, viremic donors, surveillance system , screening.

Morales, M.A., M. Barrandeguy, C. Fabbri, J.B. Garcia, A. Vissani, K. Trono, G. Gutierrez, S. Pigretti, H. Menchaca, N. Garrido, N. Taylor, F. Fernandez, S. Levis, and D. Enria (2006). **West Nile virus isolation from equines in Argentina, 2006.** *Emerging Infectious Diseases* 12(10): 1559-1561. ISSN: 1080-6040 .

Abstract: West Nile virus (WNV) was isolated from the brains of 3 horses that died from encephalitis in February 2006. The horses were from different farms in central Argentina and had not traveled outside the country. This is the first isolation of WNV in South America.

Descriptors: West Nile virus, horses, encephalitis, South America, Argentina, virus isolation.

Murray, K., S. Baraniuk, M. Resnick, R. Arafat, C. Kilborn, K. Cain, R. Shallenberger, T.L. York, D. Martinez, J.S. Hellums, D. Hellums, M. Malkoff, N. Elgawley, W. McNeely, S.A. Khuwaja, and R.B. Tesh (2006). **Risk factors for encephalitis and death from West Nile virus infection**. *Epidemiology and Infection* 134(6): 1325-1332.

Abstract: We conducted a nested case-control study to determine potential risk factors for developing encephalitis from West Nile virus (WNV) infection. Retrospective medical chart reviews were completed for 172 confirmed WNV cases hospitalized in Houston between 2002 and 2004. Of these cases, 113 had encephalitis, including 17 deaths, 47 had meningitis, and 12 were fever cases; 67% were male. Homeless patients were more likely to be hospitalized from WNV compared to the general population. A multiple logistic regression model identified age [odds ratio (OR) 1.1, P<0.001], history of hypertension, including those cases taking hypertension-inducing drugs (OR 2.9, P=0.012), and history of cardiovascular disease (OR 3.5, P=0.061) as independent risk factors for developing encephalitis from WNV infection. After adjusting for age, race/ethnicity (being black) (OR 12.0, P<0.001), chronic renal disease (OR 10.6, P<0.001), hepatitis C virus (OR 23.1, P=0.0013), and immunosuppression (OR 3.9, P=0.033) were identified as risk factors for death from WNV infection.

Descriptors: West Nile virus, risk factors, encephalitis control study, fever, meningitis, age, immunosuppression.

Murray, S. and E. Weir (2005). **West Nile virus.** *Canadian Medical Association Journal* 173(5): 484.

Descriptors: West Nile fever diagnosis, West Nile virus, diagnosis, insect bites and stings, insecticides, mosquito control, risk factors.

Orme Zavaleta, J., J. Jorgensen, B. D'Ambrosio, E. Altendorf, and P.A. Rossignol (2006). **Discovering spatio-temporal models of the spread of West Nile virus.** *Risk Analysis* 26(2): 413-422.

Abstract: Emerging infectious diseases are characterized by complex interactions among disease agents, vectors, wildlife, humans, and the environment. Since the appearance of West Nile virus (WNV) in New York City in 1999, it has infected over 8,000 people in the United States, resulting in several hundred deaths in 46 contiguous states. The virus is transmitted by mosquitoes and maintained in various bird reservoir hosts. Its unexpected introduction, high morbidity, and rapid spread have left public health agencies facing severe time constraints in a theory-poor environment, dependent largely on observational data collected by independent survey efforts and much uncertainty. Current knowledge may be expressed as a priori constraints on models learned from data. Accordingly, we applied a Bayesian probabilistic relational approach to generate spatially and temporally linked models from heterogeneous data sources. Using data collected from multiple independent sources in Maryland, we discovered the integrated context in which infected birds are plausible indicators for positive mosquito pools and human cases for 2001 and 2002.

Descriptors: biological models, West Nile fever transmission, Bayesian theorem, birds, disease outbreaks, disease reservoirs, Maryland epidemiology, risk, West Nile virus isolation and purification.

Overstreet, M. (2005). **Patient education series. West Nile virus.** *Nursing* 35(8): 64.

Descriptors: West Nile fever diagnosis, West Nile fever therapy, West Nile fever transmission, West Nile virus.

Ozer, N. (2006). **Bati Nil virusu ve vektorleri. [West Nile virus and its vectors]**. *Mikrobiyoloji Bulteni* 40(1-2): 121-128.

Abstract: There are more than five hundred known arthropod-borne viruses (arboviruses) all around the world and approximately hundred of them may cause disease in humans. During the past 20 years there has been a dramatic resurgence or emergence of epidemic arboviral diseases affecting both humans and domestic animals. Many factors play important roles in the emergence of arboviral diseases like Yellow Fever, Dengue, West Nile encephalitis, and of other diseases such as malaria and leishmaniasis in countries where they have not been previously encountered and in the increase in incidences where they have been under control. Some of these are demographic factors such as global population increase and uncontrolled urbanization; social changes such as modern transportation, human encroachment on natural disease hotspots; changes in agricultural activities such as the use of new irrigation techniques; deforestation; genetic changes in the pathogens; preventive measures and probably global climate changes. Mosquitoes are among the most important vectors carrying viruses belonging to Alphavirus, Flavivirus, Bunyavirus and Phlebo-

virus genera. All of the above factors have contributed to the increase in mosquito populations and closer contact between humans and mosquito vectors. West Nile virus notable after the epidemic of 1996 in Romania in Europe is one of the latest examples indicating that viruses can jump continents and produce epidemics. In this review article, the distribution of West Nile virus and its principal vectors and also its importance by means of public health, have been discussed.
Descriptors: West Nile virus, arthropod-borne arboviruses, mosquito populations, literature review.
Language of Text: Turkish.

Paddock, C.D., W.L. Nicholson, J. Bhatnagar, C.S. Goldsmith, P.W. Greer, E.B. Hayes, J.A. Risko, C. Henderson, C.G. Blackmore, R.S. Lanciotti, G.L. Campbell, and S.R. Zaki (2006). **Fatal hemorrhagic fever caused by West Nile virus in the United States.** *Clinical Infectious Diseases an Official Publication of the Infectious Diseases Society of America* 42(11): 1527-1535.
Abstract: BACKGROUND: Most West Nile virus (WNV) infections in humans are asymptomatic; severe disease occurs in relatively few patients and typically manifests as encephalitis, meningitis, or acute flaccid paralysis. A few cases of life-threatening disease with diffuse hemorrhagic manifestations have been reported in Africa; however, this clinical presentation has not been documented for any of the >16,700 cases of WNV disease reported in the United States during 1999-2004. We describe a case of fulminant WNV infection in a 59-year-old Florida man who died following a brief illness that resembled hemorrhagic disease caused by Rickettsia reckettsii, dengue virus or yellow fever virus. METHODS: Traditional and contemporary diagnostic assays, including culture isolation, electron microscopic examination, reverse-transcriptase polymerase chain reaction amplification, and immunohistochemical stains, were used to confirm systemic WNV infection in the patient. RESULTS: WNV was isolated in a cell culture from a skin biopsy specimen obtained from the patient shortly prior to death. Electron microscopic examination identified the isolate as a flavivirus, and reverse-transcriptase polymerase chain reaction amplified specific WNV sequences from the isolate and patient tissue. Quantitative polymerase chain reaction identified approximately 1x10(7) viral copies/mL in the patient's serum. WNV antigens were detected by immunohistochemical stains in intravascular mononuclear cells and endothelium in skin, lung, liver, kidney, spleen, bone marrow, and central nervous system; no viral antigens were identified in neurons or glial cells of the central nervous system. CONCLUSIONS: Although hemorrhagic disease is a rare manifestation of WNV infection, the findings provided by this report may offer new insights regarding the clinical spectrum and pathogenesis of WNV disease in humans.

Descriptors: hemorrhagic fevers, viral virology, West Nile Virus fever, fatal outcome, hemorrhagic fevers, viral epidemiology, skin pathology, United States.

Patnaik, J.L., H. Harmon, and R.L. Vogt (2006). **Follow-up of 2003 human West Nile virus infections, Denver, Colorado.** *Emerging Infectious Diseases* 12(7): 1129-1131. ISSN: 1080-6040 .

Abstract: Tri-County Health Department and Boulder County Public Health conducted a follow-up study of all nonfatal West Nile virus (WNV) cases reported during 2003 in 4 metropolitan Denver, Colorado, counties. Self-reported patient information was obtained approximately 6 months after onset. A total of 656 (81.2%) eligible WNV patients are included in this study.

Descriptors: West Nile fever, epidemiology, Colorado, middle aged humans, odds ratio, risk factors.

Paz, S. (2006). **The West Nile Virus outbreak in Israel (2000) from a new perspective: The regional impact of climate change.** *International Journal of Environmental Health Research* 16(1): 1-13.

Abstract: The West Nile Virus (WNV) outbreak in Israel in 2000 appeared after medical and climatic warning signs. Re-analysis of the epidemic from a new viewpoint, the regional impact of global warming, especially the worsening in the summers' heat conditions, is presented. The disease appeared averagely at a lag of 3-9 weeks (strongest correlation = lag of 7 weeks). The minimum temperature was found as the most important climatic factor that encourages the disease earlier appearance. Extreme heat is more significant than high air humidity for increasing WNV cases. An early extreme rise in the summer temperature could be a good indicator of increased vector populations. While 93.5% of cases were in the metropolitan areas, the disease was not reported in the sub-arid regions. The outbreak development was comparable to the cases from Romania (1996) and NYC (1999). Each of those epidemics appeared after a long heatwave.

Descriptors: disease outbreaks, disease vectors, West Nile fever epidemiology, West Nile virus, climate, humidity, Israel epidemiology, seasons, temperature, time factors, urban population.

Petropoulou, K.A., S.M. Gordon, R.A. Prayson, and P.M. Ruggierri (2005). **West Nile virus meningoencephalitis: MR imaging findings.** *American Journal of Neuroradiology* 26(8): 1986-1995.

Abstract: BACKGROUND AND PURPOSE: Reports of MR imaging in West Nile virus (WNV) meningoencephalomyelitis are few and the described findings limited. The purpose of this study was to review the spectrum of MR imaging findings for WNV meningoencephalomyelitis and investigate whether any of the findings correlates with clinical presentation of flaccid paralysis. METHODS: We reviewed

the MR imaging findings of 17 patients with confirmed WNV encephalitis and/or myelitis. MR imaging brain studies were evaluated for location of signal intensity abnormalities, edema, hydrocephalus, or abnormal enhancement. MR imaging spine studies were evaluated for signal intensity abnormalities in cord and/or enhancement. RESULTS: Retrospective review of the MR imaging studies of 17 patients was performed by 2 neuroradiologists. Eleven of 16 brain MR images demonstrated abnormalities. Eight (50%) patients had abnormal studies related to meningoencephalitis. All 8 patients had abnormal findings in the deep gray matter and/or brain stem; 2 had additional white matter abnormalities. Three patients with abnormal MR studies of the spine had extremity weakness on examination. The imaging findings included abnormal signal intensity more pronounced in the ventral horns and/or enhancement around the conus medullaris and cauda equina. One patient had additional abnormalities in the pons. CONCLUSION: Abnormal MR imaging findings in patients with WNV meningoencephalomyelitis are nonspecific but not uncommon. Anatomic areas commonly affected are basal ganglia, thalami, mesial temporal structures, brain stem, and cerebellum. Extremity weakness or flaccid paralysis corresponds to spinal cord/cauda equina abnormalities.
Descriptors: magnetic resonance imaging, meningoencephalitis, West Nile fever, brain pathology, extremities, muscle weakness, paraplegia, retrospective studies, spinal cord, humans.

Pfleiderer, C., C. Koenig, M. Chudy, M. Schmidt, W.K. Roth, E. Seifried, and C.M. Nuebling (2005). **Prevalence of West Nile virus in central Europe**. *Vox Sanguinis* 89(Suppl. 1): 103. ISSN: 0042-9007.
Descriptors: West Nile virus prevalence, central Europe, epidemiology.
Notes: Meeting Information: 15th Regional Congress of the International Society of Blood Transfusion, Europe, Athens, Greece; July 2-6, 2005.

Pfleiderer, C., C. Konig, M. Chudy, M. Schmidt, W.K. Roth, and C.M. Nubling (2006). **Molecular epidemiology of West Nile Virus in humans.** *Developments in Biologicals* 126: 197-201; Discussion 326-327.
Abstract: After the introduction of West Nile Virus into the United States of America in 1999 followed by annual WNV epidemics during the mosquito seasons and spreading of the virus from the East (New York; 1999) to the West of the U.S. (California; 2003/2004) there appeared the question of whether a similar scenario could happen in Europe, too. To be able to answer this question the German Ministry of Health decided to investigate the prevalence and incidence of WNV infections in German blood donors. First a test algorithm was established taking into account the high level of cross-reactivity between different flavivirus infections in serological test systems. AntiWNV-suspicious specimens were further investigated for their neutralisation capacity and by an antiWNV confirmation assay developed in-house. As a

preliminary result of our studies a very low prevalence of WNV infections in healthy German blood donors was measured. Development of highly specific test systems is necessary for accurate and reliable differential diagnosis of flavivirus infections.
Descriptors: West Nile fever, West Nile virus isolation and purification, viral blood antibodies, Austria, epidemiology, blood donors, Germany.

Prince, H.E., L.H. Tobler, M. Lape Nixon, G.A. Foster, S.L. Stramer, and M.P. Busch (2005). **Development and Persistence of West Nile Virus-Specific Immunoglobulin M (IgM), IgA, and IgG in Viremic Blood Donors.** *Journal of Clinical Microbiology.* 43(9): 4316-20. ISSN: 0095-1137.
Abstract: West Nile Virus (WNV) antibody development and persistence were investigated in blood donors who made WNV RNA-positive (viremic) donations in 2003. Plasma samples from the index donations and follow-up serum or plasma samples were tested for WNV immunoglobulin M (IgM), IgA, and IgG by using enzyme-linked immunosorbent assays. Antibody development was investigated with 154 samples collected from 84 donors 1 to 21 days after their RNA-positive, antibody-negative, index donation. WNV IgM and IgA were first detected on day 3, and all samples collected after day 9 were WNV IgM and IgA positive; WNV IgG was first detected on day 4, and all samples collected after day 16 were positive. Antibody persistence in this donor group (index donations antibody negative) was evaluated by using 128 samples collected from 89 donors on days 22 to 440 of follow-up; 88% of samples were WNV IgM positive, 86% were WNV IgA positive, and 100% were WNV IgG positive. In linear regression analysis, trendlines for WNV IgM and IgA reached the value discriminating positive from negative results at 218 days and 232 days of follow-up, respectively. Similar WNV IgM and IgA persistence trends characterized 27 donors whose index samples were positive for WNV IgM and IgA, as well as 14 donors whose index samples were positive for WNV IgG but negative for WNV IgM. These findings show that WNV IgG emerges after WNV IgM and IgA and that both WNV IgM and IgA typically persist for at least 6 months after infection. Thus, unlike some other flavivirus infections, WNV infection is not characterized by a relatively rapid disappearance of virus-specific IgA.
Descriptors: West Nile virus, viremic blood donors, specific immunoglobulin M, IgM, persistence, plasma samples, enzyme linked immunosorbent assays.

Rao, N., D. Char, and S. Gnatz (2005). **Rehabilitation outcomes of 5 patients with severe West Nile virus infection: A case series.** *Archives of Physical Medicine and Rehabilitation* 86(3): 449-452. ISSN: 0003-9993.
Abstract: OBJECTIVE: To report inpatient rehabilitation outcome in severe cases of West Nile virus (WNV) infection. DESIGN: Retrospective case series. SETTING: Freestanding rehabilitation hospital. PARTICIPANTS: Five consecutive patients admitted to an inpatient rehabilitation hospital with proven WNV infection.

PATIENTS: had severe neurologic manifestations and functional deficits. INTERVENTIONS: Not applicable. MAIN OUTCOME MEASURE: FIM instrument scores. RESULTS: On admission, overall FIM scores ranged from 31 to 68, with a mean of 44.40+/-15.85; overall FIM scores ranged from 52 to 90, with a mean of 76.00+/-15.03 at discharge. CONCLUSIONS: All patients demonstrated significant functional improvement (P <.001). Length of stay ranged from 10 to 71 days, with a mean of 32 days. Lower functional outcome and higher cost of care were noted in patients and was attributable to severe muscle weakness and axonal neuropathy.
Descriptors: West Nile fever rehabilitation, comorbidity, hospitalization economics, rehabilitation centers, retrospective studies, treatment outcome.

Rappole, J.H., B.W. Compton, P. Leimgruber, J. Robertson, D.I. King, and S.C. Renner (2006). **Modeling movement of West Nile virus in the Western hemisphere.** *Vector Borne and Zoonotic Diseases* 6(2): 128-139. ISSN: 1530-3667.
Abstract: We modeled West Nile virus (WNV) movement rates and patterns based on a migratory bird agent (the Swainson's Thrush) and a resident bird agent (the House Sparrow), and compared the results of these models with actual movement data to investigate the likelihood that the pattern of WNV outbreaks observed in the New World was consistent with migrant bird-mediated spread, as reported from the Old World. We found that, contrary to Old World patterns, WNV activity in the Western Hemisphere does not seem consistent with movement by infected migrant birds. Instead WNV spread appears best explained by a non-directional movement, perhaps that of dispersing resident birds.
Descriptors: bird disease transmission, West Nile fever, disease outbreaks, biological models , North America, West Nile virus.

Rawal, A., P.J. Gavin, and C.D. Sturgis (2006). **Cerebrospinal fluid cytology in seasonal epidemic West Nile virus meningo-encephalitis.** *Diagnostic Cytopathology* 34(2): 127-129.
Abstract: The incidence of West Nile Virus (WNV) infection has progressively increased in North America since the first epidemic in 1999. Formal scholarly documentation of cerebrospinal fluid (CSF) cytology changes in patients with WNV infection is limited. We report our experience with CSF cytospins from a population of consecutive patients with documented CSF WNV-specific IgM. Thirty-two patients (12 male, 20 female) with a median age of 52 yr (range, 19-88) diagnosed with WNV meningo-encephalitis were studied. Symptoms were present for a mean of 5 days (range, 1-14) prior to lumbar puncture. CSF proteins were elevated in 94% of patients (30/32) with a mean value of 79 mg/dl (range, 36-185). CSF glucose was normal to elevated in all cases. All cytomorphologically adequate samples demonstrated a pleocytosis with a mean of 156 cells/mm3 (range, 13-683). Nearly, all (26/28) patients showed increased CSF neutrophils--mean 43% (range, 1-83).

Mean lymphocyte and monocyte fractions were 44% (range, 8-85) and 14% (range, 2-27), respectively. Three cases showed 1-4% plasma cells. Mean total leukocyte counts (TLC) (197 cells/mm3) and mean neutrophil fractions (50%) were greater in patients sampled within the first 3 days of symptoms than in those sampled beyond day 3 (mean TLC, 126 cells/mm3; mean neutrophil fraction, 37%). Relative lymphocyte proportions increased from a mean of 39 to 48% after 3 days of illness. WNV should be considered as a potential etiology of infectious CSF pleocytosis in the North American late summer and early fall seasons. 2006 Wiley-Liss, Inc.
Descriptors: cerebrospinal fluid, meningoencephalitis, West Nile virus, immunoglobulin M metabolism, seasons, West Nile virus.

Redig, P., M. Saggese, T. Tully, A. Roy, B. Ritchie, and A. Allison (2005). **Update on West Nile virus disease and vaccine**. In: *Small animal and exotics: Proceedings of the North American Veterinary Conference, January 8, 2005-January 12, 2005, Orlando, Florida, USA,* Eastern States Veterinary Association: Gainesville, USA, Vol. 19, p. 1197-1198.
Online: http://www.navc.org
Descriptors: West Nile virus, virus disease, vaccines, updates.

Reds-Study-Grp (2005). **Detection of West Nile virus RNA and antibody in frozen plasma components from a voluntary market withdrawal during the 2002 peak epidemic**. *Transfusion (Malden)* 45(4): 480-486. ISSN: 0041-1132.
Descriptors: West Nile virus, frozen blood components, viremia, serologic markers, RNA, IgM, risk factors.

Rios, M., M.J. Zhang, A. Grinev, K. Srinivasan, S. Daniel, O. Wood, I.K. Hewlett, and A.I. Dayton (2006). **Monocytes-macrophages are a potential target in human infection with West Nile virus through blood transfusion.** *Transfusion* 46(4): 659-667.
Abstract: BACKGROUND: West Nile virus (WNV) transmission by transfusion was documented in 2002. Approximately 80 percent of WNV infections are asymptomatic and 1 percent develop severe neurological illness. In animals, Langerhans-dendritic cells support initial viral replication, followed by replication in lymphoid tissues and dissemination to organs and possibly to the CNS. The cellular tropism of WNV infection after transfusion and the particular human blood cells that sustain viral replication remain largely unknown. Whether primary monocyte-derived macrophages (MDMs) support WNV infection-replication and produce infectious virions, with an in vitro system, was investigated. STUDY DESIGN AND METHODS: Elutriated monocytes (CD33+/CD14+) from suitable blood donors were cultured in the presence of macrophage-colony-stimulating factor, infected with WNV-NY99 at different time points, washed, and cultivated for up to 47 days. Supernatants were tested for WNV replication by TaqMan reverse transcription-polymerase chain reaction (RT-PCR), with primers for the envelope and/or 3'NC

regions, and by cDNA-PCR to detect WNV minus-strand RNA and for the presence of functional virions by infectivity assays in Vero cells. RESULTS: RT-PCR TaqMan of supernatants demonstrated productive infection of MDMs. Viral load reached 2 to 5 log above baseline in 3 to 6 days and then declined, with detectable viral replication persisting for up to 47 days. WNV minus-strand RNA was detected in Day 4 cultures, indicating active viral replication. Infected MDM cultures showed no cytopathic changes. Supernatants that were TaqMan-positive for the presence of WNV-infected Vero cells and produced cytopathic effects within 3 to 5 days of culture. CONCLUSION: The susceptibility of monocytes-macrophages to productive infection in vitro is compatible with a potential role in initial WNV replication and propagation after transmission by transfusion.
Descriptors: blood transfusion, macrophages, monocytes, West Nile fever prevention and control, West Nile virus isolation and purification, cultured cells, reverse transcriptase polymerase chain reaction.

Samuel, M.A. and M.S. Diamond (2006). **Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion.** *Journal of Virology* 80(19): 9349-9360. ISSN: 0022-538X.
Descriptors: immunity, natural immunology, West Nile fever, West Nile virus, central nervous system, disease susceptibility.

Sanchez Guerrero, S.A., S. Romero Estrella, A. Rodriguez Ruiz, L. Infante Ramirez, A. Gomez, E. Villanueva Vidales, M. Garcia Torres , A.M. Dominguez, J.A. Vazquez, E.D. Calderon, L. Valiente Banuet, J.M. Linnen, A. Broulik, W. Harel, and R.A. Marin Y Lopez (2006). **Detection of West Nile virus in the Mexican blood supply.** *Transfusion* 46(1): 111-117.
Abstract: BACKGROUND: West Nile virus (WNV) is the etiologic agent of an emerging disease in the Western Hemisphere that can be transmitted to humans by blood transfusion. WNV first appeared in the United States in 1999, in Canada in 2001, and in Mexico in 2002. The aim of this nationwide study was to determine the prevalence of WNV in blood donors in Mexico as a first step in preventing its transfusion-associated transmission. STUDY DESIGN AND METHODS: In July and August 2004, a total of 3856 fresh plasma specimens collected from each state's center for blood transfusion in 29 of 31 Mexican states were screened with an investigational WNV assay (Procleix,(R) Gen-Probe Inc. and Chiron Corp.), a nucleic acid test based on transcription-mediated amplification (TMA). Reactive specimens were confirmed with a second TMA-based test, the alternative WNV assay (Gen-Probe), and with WNV capture enzyme-linked immunosorbent assays (ELISAs) for detection of immunoglobulin M (IgM) and IgG antibodies. In addition, 3714 frozen plasma samples collected in 2002 and 2003 were similarly tested. RESULTS: One of 3856 fresh samples from an asymptomatic donor from Chihuahua was reactive by

both TMA-based tests and IgM ELISA, suggesting a recently acquired infection. The observed percentage of viremic donors blood donors was 0.03 percent. Results from frozen samples were not included in the prevalence calculation and none were TMA-reactive for WNV. CONCLUSIONS: WNV is present in the Mexican blood supply and measures should be taken to reduce the risk of transfusion transmission.
Descriptors: viral antibodies in blood, blood banks, blood donors, communicable diseases, West Nile fever, West Nile virus, blood transfusion, emerging diseases prevention and control, enzyme linked immunosorbent assay, Mexico, reverse transcriptase polymerase chain reaction.

Schweitzer, B.K., W.L. Kramer, A.R. Sambol, J.L. Meza, S.H. Hinrichs, and P.C. Iwen (2006). **Geographic factors contributing to a high seroprevalence of West Nile virus-specific antibodies in humans following an epidemic.** *Clinical and Vaccine Immunology* 13(3): 314-318. ISSN: 1556-6811.
Abstract: Sera of 624 blood donors were evaluated to determine seroprevalence of West Nile virus (WNV) antibodies following the 2003 WNV epidemic in Nebraska. Geographic factors contributing to differences in WNV seropositivity were evaluated. The overall prevalence of WNV in Nebraska was higher than reported previously in other U.S. locations (9.5% WNV immunoglobulin G seroprevalence rate), with the highest prevalence identified in the western part of the state (19.7%), followed by the central (13.8%) and the eastern (4.2%) parts. Regions of the state with the highest WNV-positive mosquito rates correlated with the highest human WNV seroprevalence rates. The results showed that both the western and central parts of the state, where mosquito positivity rates were highest, had significantly higher seroprevalence rates than the eastern region. Additional studies are needed to determine whether the high prevalence rates in Nebraska will be reflected in other states and what impact environmental and geographical factors may have on future outbreaks of WNV infection.
Descriptors: viral blood antibodies, disease outbreaks, West Nile fever, West Nile virus, Nebraska, seroepidemiologic studies.

Secko, D. (2005). **Immunotherapy for West Nile virus infection.** *Canadian Medical Association Journal* 173(6): 591.
Descriptors: monoclonal antibodies, immunotherapy trends, West Nile fever, West Nile virus, mice, survival analysis.

Skupski, D.W., G.S. Eglinton, A.D. Fine, E.B. Hayes, and D.R. O'Leary (2006). **West Nile virus during pregnancy: A case study of early second trimester maternal infection.** *Fetal Diagnosis and Therapy* 21(3): 293-295.
Abstract: A woman who contracted West Nile virus (WNV) neuroinvasive illness during her second trimester subsequently elected to terminate her pregnancy due to

concerns of possible adverse effects of WNV on her developing fetus. Consent was obtained to test maternal and post-mortem fetal tissues for WNV infection. Fetal blood, liver, kidneys, spleen, umbilicus and amniotic fluid were negative for WNV RNA by polymerase chain reaction and negative for WNV IgM antibodies by ELISA, indicating that in this case there was no evidence of WNV transmission to the fetus. Until further information regarding outcomes of WNV infection during pregnancy is available, pregnant women in areas where WNV is transmitted should take precautions to avoid mosquito bites. Women with WNV illness during pregnancy should undergo regular prenatal checkups including ultrasound examinations to assess fetal development, and healthcare providers should promptly report cases of WNV in pregnant women to their state or local health department or to CDC. Copyright 2006 S. Karger AG, Basel.
Descriptors: pregnancy complications, West Nile fever, induced abortion, amniotic fluid, vertical disease transmission, fetal blood, gestational age, kidney, liver.

Teehee, M.L., M.L. Bunning, S. Stevens, and R.A. Bowen (2005). **Experimental infection of pigs with West Nile virus.** *Archives of Virology* 150(6): 1249-12. ISSN: 0304-8608.
Abstract: Young adult and weanling pigs were challenged with the New York 99 strain of West Nile virus through the bite of infected mosquitoes. Each of six adult pigs seroconverted, but virus was isolated from serum of only one pig following challenge. Three of five weanling pigs developed viremia, with peak titers of 10(1.9) and 10(3.1) PFU/mL. Clinical signs attributable to West Nile virus infection were not observed in any of these animals. An additional four pigs were challenged by feeding West Nile virus-infected mice, and none of the four developed a detectable viremia or seroconverted. These results suggest that pigs are unlikely to play a significant role as amplifying hosts of West Nile virus.
Descriptors: West Nile fever transmission, West Nile virus, infected mosquitoes, viral blood antibodies, mice, neutralization tests, swine, viremia.

Tiawsirisup, S., K.B. Platt, F. Fabiosa, and W.A. Rowley (2006). **West Nile virus titers in *Aedes vexans* (Meigen) and *Culex pipiens* (L.) saliva**. *Thai Journal of Veterinary Medicine* 36(1): 87. ISSN: 0125-6491.
Descriptors: West Nile virus titers, mosquito saliva, blood meals, virus isolation, titration plaque assay, *Aedes vexans*, *Culex pipiens*, birds.
Notes: Meeting Information: Proceedings of the Annual Conference of the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand; April 27-28 , 2006.

Turell, M., D. Dohm, M. Sardelis, M. O' Guinn, T. Andreadis, and J. Blow (2005). **An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus.** *Journal of Medical Entomology.* 42(1): 57-62. ISSN: 0022-2585.

Abstract: Since first discovered in the New York City area in 1999, West Nile virus (WNV) has become established over much of the continental United States and has been responsible for >10,000 cases of severe disease and 400 human fatalities, as well as thousands of fatal infections in horses. To develop appropriate surveillance and control strategies, the identification of which mosquito species are competent vectors and how various factors influence their ability to transmit this virus must be determined. Therefore, we evaluated numerous mosquito species for their ability to transmit WNV under laboratory conditions. This report contains data for several mosquito species not reported previously, as well as a summary of transmission data compiled from previously reported studies. Mosquitoes were allowed to feed on chickens infected with WNV isolated from a crow that died during the 1999 outbreak in New York City. These mosquitoes were tested approximately equal to 2 wk later to determine infection, dissemination, and transmission rates. All Culex species tested were competent vectors in the laboratory and varied from highly efficient vectors (e.g., *Culex tarsalis Coquillett*) to moderately efficient ones (e.g., *Culex nigripalpus Theobald*). Nearly all of the Culex species tested could serve as efficient enzootic or amplifying vectors for WNV. Several container-breeding Aedes and Ochlerotatus species were highly efficient vectors under laboratory conditions, but because of their feeding preferences, would probably not be involved in the maintenance of WNV in nature. However, they would be potential bridge vectors between the avian-Culex cycle and mammalian hosts. In contrast, most of the surface pool-breeding Aedes and Ochlerotatus species tested were relatively inefficient vectors under laboratory conditions and would probably not play a significant role in transmitting WNV in nature. In determining the potential for a mosquito species to become involved in transmitting WNV, it is necessary to consider not only its laboratory vector competence but also its abundance, host-feeding preference, involvement with other viruses with similar transmission cycles, and whether WNV has been isolated from this species under natural conditions.

Descriptors: Culicidae, Culex, mosquitoes, insect vectors, West Nile virus, vector potential, vector competence, disease transmission, infection, vectorial capacity, infection-rate, dissemination-rate, transmission-rate.

Vamvakas, E.C., S. Kleinman, H. Hume, and G.D. Sher (2006). **The development of West Nile virus safety policies by Canadian blood services: Guiding principles and a comparison between Canada and the United States.** *Transfusion Medicine Reviews* 20(2): 97-109.

Abstract: To address the emerging threat of West Nile virus (WNV) to the blood supply, Canadian Blood Services (CBS) made a series of policy decisions that were either similar to those adopted in the United States or more stringent than policies formulated in the United States at the same time. More stringent Canadian policies included the development of an in-house WNV RNA assay, the stockpiling of frozen plasma components in the winter for transfusion in WNV-affected areas in the summer, a special recruitment campaign for red blood cell collections before the start of the 2003 WNV season, and an inventory exchange (ie, WNV-tested for untested red blood cells) initiated 2 weeks after the onset of WNV screening, as well as the implementation of targeted individual-donation WNV testing on August 2, 2004, in the absence of any positive donors or clinical cases of WNV infection in Canada. The general principles that guided CBS decision making with regard to WNV safety included application of the precautionary principle, harmonization with policies in the United States, a consideration of logistic issues, compliance with Health Canada requests, responsiveness to public expectations about transfusion safety, and transparency in decision making with timely communication to stakeholders. Before implementing WNV blood safety policies, CBS assessed their impact on blood availability. When policies were implemented, data were obtained quickly to ensure that the prior impact assessments were accurate. This review discusses the guiding principles affecting CBS policy development and compares CBS WNV safety policies to policies adopted in the United States.
Descriptors: blood bank standards, blood transfusion safety, West Nile fever prevention and control, West Nile virus, blood specimen collection standards, blood transfusion, Canada, health policy, United States.

van der Meulen, K.M., M.B. Pensaert, and H.J. Nauwynck (2005). **West Nile virus in the vertebrate world.** *Archives of Virology* 150(4): 637-657. ISSN: 0304-8608.
Abstract: West Nile virus (WNV), an arthropod-borne virus belonging to the family Flaviviridae, had been recognized in Africa, Asia and the south of Europe for many decades. Only recently, it has been associated with an increasing number of outbreaks of encephalitis in humans and equines as well as an increasing number of infections in vertebrates of a wide variety of species. In this article, the data available on the incidence of WNV in vertebrates are reviewed. Moreover, the role of vertebrates in the transmission of WNV, the control of WNV infections in veterinary medicine as well as future perspectives are discussed. A wide variety of vertebrates, including more than 150 bird species and at least 30 other vertebrate species, are susceptible to WNV infection. The outcome of infection depends on the species, the age of the animal, its immune status and the pathogenicity of the WNV isolate. WNV infection of various birds, especially passeriforms, but also of young chickens and domestic geese, results in high-titred viremia that allows arthropod-borne transmission. For other verte-

brate species, only lemurs, lake frogs and hamsters develop suitable viremia levels to support arthropod-borne transmission. The role of vertebrates in direct, non-arthropod-borne transmission, such as via virus-contaminated organs, tissues or excretions is less well characterized. Even though direct transmission can occur among vertebrates of several species, data are lacking on the exact amounts of infectious virus needed. Finally, the increased importance of WNV infections has led to the development of killed, live-attenuated, DNA-recombinant and chimeric veterinary vaccines.
Descriptors: animal diseases, West Nile fever, horse diseases, horses.

van der Meulen, K.M., M.B. Pensaert, and H.J. Nauwynck (2005). **West Nile virus in the vertebrate world**. *Archives of Virology* 150(4): 637-657. ISSN: 0304-8608.
Online: http://springerlink.metapress.com/link.asp?id=100423
Descriptors: West Nile virus, vertebrates, disease transmission, encephalitis, species vectors, viremia, arthropods, vaccine development.

Ward, M.P. (2005). **Epidemic West Nile virus encephalomyelitis: A temperature-dependent, spatial model of disease dynamics.** *Preventive Veterinary Medicine* 71(3-4): 253-264.
Abstract: Since first being detected in New York in 1999, West Nile virus (WNV) has spread throughout the United States and more than 20,000 cases of equine WNV encephalomyelitis have been reported. A spatial model of disease occurrence was developed, using data from an outbreak of serologically confirmed disease in an unvaccinated population of horses at 108 locations in northern Indiana between 3 August and 17 October 2002. Daily maximum temperature data were recorded at meteorological stations surrounding the study area. The distribution of the total number of degree-days elapsing between July 4 and the date of diagnosis of each case was best described by a normal distribution (mean=5243 degrees F, S.D.=1047). The days on which the average risk was >25, >50 and >75% were predicted (versus observed) to occur on August 23 (August 9), August 31 (September 2) and September 9 (September 9). The epidemic was predicted to occur 3 days earlier, or 4 days later, than observed if temperatures in the study area were uniformly increased, or decreased, by 5 degrees F, respectively. Maps indicated that WNV encephalomyelitis risk always remained greater in the northwest quadrant of the study area. Since WNV might exist at a hypoendemic level of infection, and occasionally re-emerge as a cause of epidemics in equine populations, by identifying factors that contributed to this epidemic, the potential impact of future epidemics can be reduced. Such studies rely on a GIS framework, availability of meteorological and possibly remotely sensed data and information on host and landscape factors. An early-warning system for WNV transmission in equine populations could be developed.
Descriptors: animal disease outbreaks, horse diseases, West Nile fever, horses, Indiana, statistical models, space-time clustering, West Nile virus.

Ward, M.P., J.A. Schuermann, L.D. Highfield, and K.O. Murray (2006). **Characteristics of an outbreak of West Nile virus encephalomyelitis in a previously uninfected population of horses.** *Veterinary Microbiology* 118(3-4): 255-259.

Abstract: Equine West Nile virus (WNV) encephalomyelitis cases - based on clinical signs and ELISA serology test results - reported to Texas disease control authorities during 2002 were analyzed to provide insights into the epidemiology of the disease within a previously disease-free population. The epidemic occurred between June 27 and December 17 (peaking in early October) and 1,698 cases were reported. Three distinct epidemic phases were identified, occurring mostly in southeast, northwest and then central Texas. Significant (P<0.05) disease clusters were identified in northwest and northern Texas. Most (91.1%) cases had no recent travel history, and most (68.9%) cases had not been vaccinated within the previous 12 months. One-third of cases did not survive, 71.2% of which were euthanatized. The most commonly reported presenting signs included ataxia (69%), abnormal gait (52%), muscle fasciculations (49%), depression (32%) and recumbency (28%). Vaccination status, ataxia, falling down, recumbency and lip droop best explained the risk of not surviving WNV disease. Results suggest that the peak risk period for encephalomyelitis caused by WNV may vary substantially among regions within Texas. Recumbent horses have a poor prognosis for survival. Vaccines, even if not administered sufficiently in advance of WNV infection within a district, may reduce the risk of death by at least 44%.

Descriptors: West Nile virus, horses, infection, clinical signs, Texas, prognosis, vaccines, death, depression, ataxia, recumbancy.

Ward, M. (2005). **Epidemic West Nile virus encephalomyelitis: A temperature-dependent, spatial model of disease dynamics.** *Preventive Veterinary Medicine.* 71(3-4): 253-264. ISSN: 0167-5877.

Descriptors: West Nile virus, viral encephalitis, disease outbreaks, ambient temperature, epidemiological studies, epidemiology, spatial distribution, horses, horse diseases, heat sums, risk factors, geographic information systems, biogeography, disease transmission, models, Indiana.

Notes: In the Special Issue: Proceedings of GISVET'04/edited by W. Martin and P. Durr. Proceedings of a conference held June 23-25, 2004, Ontario, Canada.

Warner, R.D., R.C. Kimbrough, J.L. Alexander, J.J. Rush Pierce, T. Ward, and L.P. Martinelli (2006). **Human west nile virus neuroinvasive disease in Texas, 2003 epidemic: Regional differences.** *Annals of Epidemiology* 16(10): 749-755. ISSN: 1047-2797.

Abstract: PURPOSE: Arboviral diseases, such as West Nile virus (WNV) epizootics, tend to be geographically unique because of the biomes that support the vector(s) and reservoir host(s). Understanding such details aids in preventive efforts. We

studied the 2003 epidemic of human West Nile neuroinvasive disease (WNND) in Texas because it initially appeared that incidence was not uniform across regions of the state. METHODS: The epidemic was described by age, sex, and region of residence. These variables were used to compare age-specific incidence, standardized cumulative incidence, and age-adjusted relative risk (RR). We verified case data and used routine software, with population estimates from the US Census Bureau. RESULTS: Regardless of sex, risk increased with age. Males had the greater risk (RR, 1.69); however, males aged 5 to 17 years had the greatest RR. Of the five regions compared, two posed more (RRs, 7.98 and 2.14) and one posed less (RR, 0.40) risk than the remainder of the state. Proportions of Culex vector species differed significantly between regions. CONCLUSIONS: During 2003, the risk for WNND varied considerably across Texas. This suggests that various risks for WNV infection deserve additional research for preventive interventions to be regionally appropriate and effective.
Descriptors: West Nile virus, epizootics, reservoir hosts, preventive efforts, epidemic, variables, risk, Culex vector, Texas.

Yaremych, S.A., R.E. Warner, P.C. Mankin, J.D. Brawn, A. Raim and R. Novak (2005). **West Nile virus and high death rate in American crows.** S.K. Majumdar, J.E. Huffman, F.J. Brenner and A.I.E. Panah *Wildlife Diseases: Landscape Epidemiology, Spatial Distribution and Utilization of Remote Sensing Technology.,* Pennsylvania Academy of Science, Easton., p. Chapter pagination: 200-204, i-ix. ISBN: 0945809190.
Descriptors: *Corvus brachyrhynchos*, dipteran parasites, culicidae, viral diseases, West Nile virus, viral prevalence, epidemiology and associated mortality rate, satellite tracking study, transmission of viruses, mortality, mortality rate, USA.

Zak, I.T., D. Altinok, J.R. Merline, S. Chander, and K.K. Kish (2005). **West Nile virus infection.** *American Journal of Roentgenology* 184(3): 957-961. ISSN: 0361-803X.
Abstract: OBJECTIVE: Our objective is to present a brief review of the clinical aspects of West Nile virus infection with emphasis on the spectrum of MRI findings. CONCLUSION: West Nile virus infection has become endemic in the United States and radiologists should become aware of the diverse imaging appearances in the central nervous system.
Descriptors: West Nile fever diagnosis, West Nile virus, magnetic resonance imaging, literature review.

Zhang, J.S., P.H. Zhang, B.Y. Si, H. Yang, and W.C. Cao (2005). **[Comparison and discrimination of the biological characteristics between West Nile virus and Japanese encephalitis virus]**. *Chinese Journal of Experimental and Clinical Virology* 19(4): 340-343.

Abstract: BACKGROUND: To compare the biological characteristics of West Nile virus (WNV) and Japanese encephalitis virus (JEV), including cells sensitivity, pathogenicity, viral morphology, as well as the results of immunological and molecular biological detection. METHODS: Cytopathic effect (CPE) and pathogenicity were observed in C6/36 cells and in suckling mice inoculated intracerebrally with the WNV or JEV, respectively. The sliced tissue samples for electron microscopic examination were prepared for the morphologic observation of the viruses. Serum antibody to WNV or JEV was detected using indirect immunofluorescence assay (IFA), and the viral RNA was analyzed by RT-PCR method. RESULTS: WNV or JEV-caused CPE was characterized by cell fusion and cell shedding, respectively. There was no significant difference in the pathogenicity to suckling mice between WNV and JEV. The morphologic observation showed that the shape and size of the two virions were similar. WNV and JEV were found to have antigenic cross-reactivity. The viral RNA could be detected from both WNV and JEV samples with universal primer set, but only nucleoside fragments of corresponding virus could be amplified when specific primers were used. CONCLUSION: CPE in C6/36 cell and detection of the viral RNA should be useful in discrimination of WNV and JEV, and simultaneously examining the titers of serum antibodies against WNV and JEV may be helpful to diagnosis of infection with these agents.
Descriptors: West Nile virus, Japanese encephalitis virus, biological characteristics, pathogenicity, morphology, viral RNA, diagnosis.
Language of Text: Chinese.

Zohrabian, A., E.B. Hayes, and L.R. Petersen (2006). **Cost-effectiveness of West Nile virus vaccination.** *Emerging Infectious Diseases* 12(3): 375-380. ISSN: 1080-6040 .
Abstract: West Nile virus (WNV) was first detected in the Western Hemisphere in 1999 in New York City. From 1999 through 2004, >16,600 cases of WNV-related illnesses were reported in the United States, of which >7,000 were neuroinvasive disease and >600 were fatal. Several approaches are under way to develop a human vaccine. Through simulations and sensitivity analysis that incorporated uncertainties regarding future transmission patterns of WNV and costs of health outcomes, we estimated that the range of values for the cost per case of WNV illness prevented by vaccination was US 20,000 dollars-59,000 dollars(mean 36,000 dollars). Cost-effectiveness was most sensitive to changes in the risk for infection, probability of symptomatic illness, and vaccination cost. Analysis indicated that universal vaccination against WNV disease would be unlikely to result in societal monetary savings unless disease incidence increases substantially over what has been seen in the past 6 years.
Descriptors: vaccination economics, viral vaccines, West Nile fever prevention and control, cost benefit analysis, decision trees, health care costs, mortality.

Research

Bai, F., T. Wang, U. Pal, F. Bao, L.H. Gould, and E. Fikrig (2005). **Use of RNA interference to prevent lethal murine West Nile virus infection**. *Journal of Infectious Diseases* 191(7): 1148-1154. ISSN: 0022-1899.
Descriptors: mice, molecular genetics, West Nile virus, encephalitis, genetic techniques, virus replication.

Benzaghou, I., I. Bougie, F. Picard Jean, and M. Bisaillon (2006). **Energetics of RNA binding by the West Nile virus RNA triphosphatase.** *FEBS Letters* 580(3): 867-877.
Abstract: The West Nile virus (WNV) RNA genome harbors the characteristic methylated cap structure present at the 5' end of eukaryotic mRNAs. In the present study, we report a detailed study of the binding energetics and thermodynamic parameters involved in the interaction between RNA and the WNV RNA triphosphatase, an enzyme involved in the synthesis of the RNA cap structure. Fluorescence spectroscopy assays revealed that the initial interaction between RNA and the enzyme is characterized by a high enthalpy of association and that the minimal RNA binding site of NS3 is 13 nucleotides. In order to provide insight into the relationship between the enzyme structure and RNA binding, we also correlated the effect of RNA binding on protein structure using both circular dichroism and denaturation studies as structural indicators. Our data indicate that the protein undergoes structural modifications upon RNA binding, although the interaction does not significantly modify the stability of the protein.
Descriptors: acid anhydride hydrolases, viral RNA, West Nile virus genetics.

Bogachek, M.V., E.V. Protopopova, and V.A. Ternovoi (2005). **Immunochemical Properties of Recombinant Polypeptides Mimicking Domains I and II of West Nile Virus Glycoprotein E**. *Molecular Biology* P.: 710-18.
Descriptors: West Nile virus, immunochemical properties, recombinant polypeptides, glycoprotein E, domains I and II.

Chappell, K.J., M.J. Stoermer, D.P. Fairlie, and P.R. Young (2006). **Insights to Substrate Binding and Processing by West Nile Virus NS3 Protease through Combined Modeling, Protease Mutagenesis, and Kinetic Studies.** *Journal of Biological Chemistry* 281(50): 38448-58.
Abstract: West Nile Virus is becoming a widespread pathogen, infecting people on at least four continents with no effective treatment for these infections or many of their associated pathologies. A key enzyme that is essential for viral replication is the

viral protease NS2B-NS3, which is highly conserved among all flaviviruses. Using a combination of molecular fitting of substrates to the active site of the crystal structure of NS3, site-directed enzyme and cofactor mutagenesis, and kinetic studies on proteolytic processing of panels of short peptide substrates, we have identified important enzyme-substrate interactions that define substrate specificity for NS3 protease. In addition to better understanding the involvement of S2, S3, and S4 enzyme residues in substrate binding, a residue within cofactor NS2B has been found to strongly influence the preference of flavivirus proteases for lysine or arginine at P2 in substrates. Optimization of tetrapeptide substrates for enhanced protease affinity and processing efficiency has also provided important clues for developing inhibitors of West Nile Virus infection.

Descriptors: West Nile virus, substrate binding, protease, combined modeling, kinetic studies, treatment, infections, pathology, enxyme.

Chappell, K.J., T.A. Nall, M.J. Stoermer, N.X. Fang, J.D.A. Tyndall, D.P. Fairlie, and P.R. Young (2005). **Site-directed Mutagenesis and Kinetic Studies of the West Nile Virus NS3 Protease Identify Key Enzyme-Substrate Interactions.** *Journal of Biological Chemistry.* 280(4): 2896-2903. ISSN: 0021-9258.

Abstract: The flavivirus West Nile virus (WNV) has spread rapidly throughout the world in recent years causing fever, meningitis, encephalitis, and fatalities. Because the viral protease NS2B/NS3 is essential for replication, it is attracting attention as a potential therapeutic target, although there are currently no antiviral inhibitors for any flavivirus. This paper focuses on elucidating interactions between a hexapeptide substrate (Ac-KPGLKR-p-nitroanilide) and residues at S1 and S2 in the active site of WNV protease by comparing the catalytic activities of selected mutant recombinant proteases in vitro. Homology modeling enabled the predictions of key mutations in WNV NS3 protease at S1 (V115A/F, D129A/E/N, S135A, Y150A/F, S160A, and S163A) and S2 (N152A) that might influence substrate recognition and catalytic efficiency. Key conclusions are that the substrate P1 Arg strongly interacts with S1 residues Asp-129, Tyr-150, and Ser-163 and, to a lesser extent, Ser-160, and P2 Lys makes an essential interaction with Asn-152 at S2. The inferred substrate-enzyme interactions provide a basis for rational protease inhibitor design and optimization. High sequence conservation within flavivirus proteases means that this study may also be relevant to design of protease inhibitors for other flavivirus proteases.

Descriptors: West Nile virus, studies, mutagenesis, kinetic, NS3 protease, enzyme substrate, replication, therapeutic target, protease inhibitors.

Cheeran, M.C., S. Hu, W.S. Sheng, A. Rashid, P.K. Peterson, and J.R. Lokensgard (2005). **Differential responses of human brain cells to West Nile virus infection.** *Journal of Neurovirology* 11(6): 512-24.

Abstract: In recent years, West Nile virus (WNV) has emerged as a major cause of

encephalitis in the United States. However, the neuropathogenesis of this flavivirus is poorly understood. In the present study, the authors used primary human brain cell cultures to investigate two neuropathogenic features: viral replication and induction of cytokines. Although neurons and astrocytes were found to support productive WNV infection, viral growth was poorly permissive in microglial cells. Compared to neuronal cultures that sustained viral growth for at least 2 weeks, replication peaked in astrocytes by 72 h post infection. In response to viral infection, astrocytes produced chemokines (CXCL10 and CCL5), but none of the cytokines (tumor necrosis factor [TNF]-alpha, interleukin [IL]-1beta, IL-6, interferon alpha or gamma) tested could be detected. Although microglial cells failed to support viral replication, WNV induced production of the proinflammatory cytokines IL-6 and TNF-alpha. Microglial cells also released robust amounts of the chemokines CXCL10 and CCL2, as well as lower levels of CCL5, in response to WNV infection. WNV-induced chemokine and cytokine production by microglia was coupled with activation of mitogen-activated protein kinase (MAPK) intracellular signaling pathways. Inhibition of p38 MAPK decreased chemokine production in response to WNV. Taken together, these findings suggest that microglial cell responses may influence the neuropathogenesis of WNV infection.

Descriptors: brain pathology, cytokines immunology, neurons pathology, West Nile virus, astrocytes pathology, astrocytes virology, brain virology, cells, cultured, *Cercopithecus aethiops*, chemokines, cxc biosynthesis, chemotaxis immunology, cytokines biosynthesis, neurons virology, vero cells.

Chen, Y.P., Y.J. Yang, X.D. Wu, and Z.L. Wang (2006). **[Preparation of monoclonal antibodies against West Nile virus envelope protein domain.]**. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi; Zhonghua Shiyan He Linchuang Bingduxue Zazhi; Chinese Journal of Experimental and Clinical Virology* 20(3): 213-5.

Abstract: BACKGROUND: To prepare monoclonal antibodies against West Nile virus (WNV) envelope protein domain. METHODS: BALB/c mice were immunized with recombinant antigen of West Nile virus envelope protein domain, and the spleen cells of the mice were used to prepare the monoclonal antibodies (McAb) by hybridoma technique. RESULTS: Three hybridoma cell strains secreting McAbs against WNV envelope protein domain, designated as 4F7, 6H3 and 8E4, respectively, were obtained and were identified by indirect enzyme linked immunosorbent assay (ELISA), they belonged to IgG1, IgG1 and Ig2a, respectively. Two epitopes of envelope protein domain were determined, among them, 4F7 and 6H3 were against the same epitope and 8E4 to another one. CONCLUSIONS: The results of indirect ELISA, Western blot and indirect immunofluorescence experiment indicated that these three McAbs were specific for West Nile virus envelope protein domain and did not cross-react with Japanese encephalitis virus and other viruses, so they can be used

for specific detection of West Nile virus.
Descriptors: West Nile virus, envelope protein, spleen cells, mice, monoclonal antibodies, western blot, enzyme linked immunosorbent assay, Japanese encephalitis.
Language of Text: Chinese.

Chu, J.J., P.W. Leong, and M.L. Ng (2005). **Characterization of plasma membrane-associated proteins from *Aedes albopictus* mosquito (C6/36) cells that mediate West Nile virus binding and infection.** *Virology* 339(2): 249-60.
Abstract: This study isolated and characterized the West Nile virus (WNV) putative receptor molecule(s) from *Aedes albopictus* mosquito (C6/36) cells. The binding of WNV to C6/36 cells was saturated with 5000 particles per cell. The entry of WNV into C6/36 cells was strongly inhibited when pretreated with proteinase K and to a lesser extent with sodium periodate. However, pretreatment of C6/36 cells with phospholipases, glycosidases, heparinases and neurimidase had no effect on virus entry. By using virus overlay protein blot assay, WNV was observed to bind to the 140-kDa, 95-kDa, 70-kDa and 55-kDa plasma membrane-associated molecules isolated from C6/36 cells. Murine antibodies generated against the 95-kDa and 70-kDa membrane proteins effectively blocked WNV, Japanese encephalitis virus (JEV) and Dengue virus (DV) serotype 2 infection in C6/36 cells. In addition, the binding of the recombinant-WNV envelope domain III protein to C6/36 cells can be inhibited by the anti-95-kDa and anti-70-kDa membrane protein antibodies. These data strongly supported the possibility that the 95-kDa and 70-kDa plasma membrane-associated proteins are part of a receptor complex for mosquito-borne flaviviruses (WNV, JEV and DV) on mosquito cells.
Descriptors: aedes metabolism, membrane proteins metabolism, West Nile fever metabolism, West Nile virus, aedes cytology, aedes virology, cell line, cell membrane metabolism, culicidae, gene expression, protein binding.

Chu, J.J., R. Rajamanonmani, J. Li, R. Bhuvanakantham, J. Lescar, and M.L. Ng (2005). **Inhibition of West Nile virus entry by using a recombinant domain III from the envelope glycoprotein.** *Journal of General Virology* 86(Pt 2): 405-12.
Abstract: The envelope glycoprotein located at the outermost surface of the flavivirus particle mediates entry of virus into host cells. In this study, the involvement of domain III of West Nile virus (WNV-DIII) envelope protein in binding to host cell surface was investigated. WNV-DIII was first expressed as a recombinant protein and purified after a solubilization and refolding procedure. The refolded WNV-DIII protein displays a content of beta-sheets consistent with known homologous structures of other flavivirus envelope DIII, shown by using circular dichroism analysis. Purified recombinant WNV-DIII protein was able to inhibit WNV entry into Vero cells and C6/36 mosquito cells. Recombinant WNV-DIII only partially blocked the entry of dengue-2 (Den 2) virus into Vero cells. However, entry of Den 2 virus into

C6/36 was blocked effectively by recombinant WNV-DIII. Murine polyclonal serum produced against recombinant WNV-DIII protein inhibited infection with WNV and to a much lesser extent with Den 2 virus, as demonstrated by plaque neutralization assays. Together these results provided strong evidence that immunoglobulin-like DIII of WNV envelope protein is responsible for binding to receptor on the surface of host cells. The data also suggest that similar attachment molecule(s) or receptor(s) were used by WNV and Den 2 virus for entry into C6/36 mosquito cells.
Descriptors: antigens, viral pharmacology, viral envelope proteins pharmacology, virus replication drug effects, West Nile virus drug effects, amino acid sequence, viral biosynthesis, cell line, *Cercopithecus aethiops*, culicidae, dengue virus drug effects, immune sera immunology, mice, molecular sequence data, neutralization tests, protein structure, tertiary, recombinant proteins pharmacology, sequence alignment, solubility, vero cells, viral envelope proteins biosynthesis, viral envelope proteins chemistry, West Nile virus.

Chung, K.M., M.K. Liszewski, G. Nybakken, A.E. Davis, R.R. Townsend, D.H. Fremont, J.P. Atkinson, and M.S. Diamond (2006). **West Nile virus nonstructural protein NS1 inhibits complement activation by binding the regulatory protein factor H.** *Proceedings of the National Academy of Sciences of the United States of America* 103(50): 19111-6.
Abstract: The complement system, by virtue of its dual effector and priming functions, is a major host defense against pathogens. Flavivirus nonstructural protein (NS)-1 has been speculated to have immune evasion activity, because it is a secreted glycoprotein, binds back to cell surfaces, and accumulates to high levels in the serum of infected patients. Herein, we demonstrate an immunomodulatory function of West Nile virus NS1. Soluble and cell-surface-associated NS1 binds to and recruits the complement regulatory protein factor H, resulting in decreased complement activation in solution and attenuated deposition of C3 fragments and C5b-9 membrane attack complexes on cell surfaces. Accordingly, extracellular NS1 may function to minimize immune system targeting of West Nile virus by decreasing complement recognition of infected cells.
Descriptors: West Nile virus, nonstructural protein, complement activation, protein factor H, host defense, immune system.

Chung, K.M., G.E. Nybakken, and B.S. Thompson (2006). **Antibodies against West Nile Virus Nonstructural Protein NS1 Prevent Lethal Infection through Fc g Receptor-Dependent and -Independent Mechanisms**. *Journal of Virology* P.: 1340-51.
Descriptors: West Nile virus, antibodies, nonstructural protein, lethal infection, Fc g receptor, NS1.

Cinatl, J.J., M. Michaelis, C. Fleckenstein, G. Bauer, H. Kabickova, M. Scholz, H.F. Rabenau, and H.W. Doerr (2006). **West Nile virus infection induces interferon signalling in human retinal pigment epithelial cells.** *Investigative Ophthalmology and Visual Science* 47(2): 645-51.

Abstract: PURPOSE: In addition to neuroinvasive disease, West Nile virus (WNV) infection is frequently associated with self-limiting chorioretinitis and vitritis. However, the mechanisms of ophthalmic WNV infection are rarely investigated, in part because of the lack of reliable in vitro models. The authors therefore established the first model of ocular WNV infection and investigated interaction of WNV with IFN signal-transduction mechanisms. METHODS: Human retinal pigment epithelial (RPE) cells were infected with WNV strain NY385-99 at a multiplicity of infection of 5. Virus replication was evaluated by virus titers at different times after infection. The susceptibility of RPE cells to WNV infection was confirmed by transmission electron microscopy. IFN-beta expression was assessed by quantitative real-time PCR and by measurements of antiviral activity in cell culture supernatants. IFN signaling was evaluated by phosphorylation of transducer and activator of transcription 1 and 2 (STAT1/2) proteins, with immunoblot analysis. RESULTS: RPE cells appeared to be highly sensitive to WNV infection. Maximum viral titers were found 24 hours after infection, followed by a continuous decline during the course of infection. WNV infection of RPE cells was followed by increased IFN-beta expression associated with IFN signaling and subsequent inhibition of WNV replication. CONCLUSIONS: In this study, the first cell culture model of ophthalmic WNV infection was developed and characterized in RPE cells, and the molecular mechanisms of WNV infection were studied. The data suggest that WNV induces a general antiviral state in RPE cells. This general antiviral state correlates with WNV-induced IFN signaling in retinal cells.

Descriptors: interferon beta biosynthesis, pigment epithelium of eye virology, signal transduction physiology, West Nile virus, blotting, western, cell culture techniques, *Cercopithecus aethiops*, interferon beta genetics, microscopy, electron, transmission, models, biological, phosphorylation, pigment epithelium of eye metabolism, pigment epithelium of eye ultrastructure, reverse transcriptase polymerase chain reaction, stat1 transcription factor metabolism, stat2 transcription factor metabolism, vero cells, virus replication physiology, West Nile virus ultrastructure.

Cosnier, S., R.E. Ionescu, S. Herrmann, L. Bouffier, M. Demeunynck, and R.S. Marks (2006). **Electroenzymatic polypyrrole-intercalator sensor for the determination of West Nile virus cDNA.** *Analytical Chemistry* 78(19): 7054-7.

Abstract: The chemical binding of a redox acridone derivative onto a polypyrrole film functionalized by N-hydroxysuccinimide groups provided an electrode capable of anchoring DNA duplex by simple insertion of the grafted acridone intercalator

into the dsDNA solution. This electrode was applied for the detection of a ssDNA derived from a West Nile virus sequence. The latter was thus amperometrically detected after its hybridization in solution with a biotinylated complementary oligonucleotide followed by its anchoring and labeling by a glucose oxidase at 1 pg/mL.
Descriptors: West Nile virus, DNA, determination, virus sequence, hybridization, chemical binding, redox acridone derivative, electrode.

Cruz Pacheco, G., L. Esteva, J.A. Montano Hirose , and C. Vargas (2005). **Modelling the dynamics of West Nile Virus.** *Bulletin of Mathematical Biology* 67(6): 1157-72.
Abstract: In this work we formulate and analyze a mathematical model for the transmission of West Nile Virus (WNV) infection between vector (mosquito) and avian population. We find the Basic Reproductive Number R0 in terms of measurable epidemiological and demographic parameters. R0 is the threshold condition that determines the dynamics of WNV infection: if R0< or =1 the disease fades out, and for R0 >1 the disease remains endemic. Using experimental and field data we estimate R0 for several species of birds. Numerical simulations of the temporal course of the infected bird proportion show damped oscillations approaching the endemic value.
Descriptors: bird diseases transmission, bird diseases virology, culex virology, insect vectors virology, models, biological, West Nile fever transmission, West Nile fever, West Nile virus growth and development, bird diseases epidemiology, birds, computer simulation, United States epidemiology, West Nile fever epidemiology, West Nile fever, zoonoses epidemiology, zoonoses virology.

Davis, A., M. Bunning, P. Gordy, N. Panella, B. Blitvich, and R. Bowen (2005). **Experimental and natural infection of North American bats with West Nile virus.** *American Journal of Tropical Medicine and Hygiene* 73(2): 467-469. ISSN: 0002-9637.
Abstract: Big brown (*Eptesicus fuscus*) and Mexican free-tailed (*Tadarida brasiliensis*) bats were inoculated with the New York 99 strain of West Nile virus to assess their potential to serve as amplifying hosts and determine the clinical effect of infection. Groups of three or four bats were bled at daily intervals between 1 and 6 days after inoculation to determine the pattern of viremia. Beginning 2 days after inoculation, virus was isolated each day from one or more *E. fuscus* bats, in titers ranging from 10 to 180 plaque-forming units per milliliter of serum. Virus was not isolated from any of the sera collected from *T brasiliensis bats*. None of the bats from either species showed clinical signs associated with exposure to virus. Sera from an additional 149 bats collected in Louisiana in 2002 during an epizootic of West Nile fever were tested for antibodies to virus, and two were found to be positive. These data suggest that bats from these two widely distributed species are unlikely to serve as amplifying hosts for West Nile virus.

Descriptors: *Eptesicus fuscus*, *Tadarida brasiliensis*, transmission of viruses, West Nile virus, experimental and natural infection, USA.

Davis, C.W., H.Y. Nguyen, S.L. Hanna, M.D. Sanchez, R.W. Doms, and T.C. Pierson (2006). **West Nile virus discriminates between DC-SIGN and DC-SIGNR for cellular attachment and infection.** *Journal of Virology* 80(3): 1290-1301.

Abstract: The C-type lectins DC-SIGN and DC-SIGNR bind mannose-rich glycans with high affinity. In vitro, cells expressing these attachment factors efficiently capture, and are infected by, a diverse array of appropriately glycosylated pathogens, including dengue virus. In this study, we investigated whether these lectins could enhance cellular infection by West Nile virus (WNV), a mosquito-borne flavivirus related to dengue virus. We discovered that DC-SIGNR promoted WNV infection much more efficiently than did DC-SIGN, particularly when the virus was grown in human cell types. The presence of a single N-linked glycosylation site on either the prM or E glycoprotein of WNV was sufficient to allow DC-SIGNR-mediated infection, demonstrating that uncleaved prM protein present on a flavivirus virion can influence viral tropism under certain circumstances. Preferential utilization of DC-SIGNR was a specific property conferred by the WNV envelope glycoproteins. Chimeras between DC-SIGN and DC-SIGNR demonstrated that the ability of DC-SIGNR to promote WNV infection maps to its carbohydrate recognition domain. WNV virions and subviral particles bound to DC-SIGNR with much greater affinity than DC-SIGN. We believe this is the first report of a pathogen interacting more efficiently with DC-SIGNR than with DC-SIGN. Our results should lead to the discovery of new mechanisms by which these well-studied lectins discriminate among ligands.

Descriptors: cell adhesion molecules, C-type lectins, cell surface receptors, West Nile virus, cell adhesion molecules.

Dhingra, V., Q. Li, A.B. Allison, D.E. Stallknecht, and Z.F. Fu (2005). **Proteomic profiling and neurodegeneration in West Nile virus-infected neurons.** *Journal of Biomedicine and Biotechnology* 2005(3): 271-279.

Abstract: West Nile virus, a mosquito-borne flavivirus, is a human, equine, and avian pathogen. High-resolution two-dimensional differential-gel electrophoresis (2D-DIGE) was used to characterize protein expression in primary rat neurons and to examine the proteomic profiling to understand the pathogenesis of West-Nile-associated meningoencephalitis. Three pH ranges, 3-10, 4-7, and 5-6, were used to analyze the protein spots. The proteins are labeled with fluorescent dyes Cy3 and Cy5 before being separated on the basis of charge and size respectively on a two-dimensional platform. About 55 proteins showed altered expression levels. These were then subsequently digested and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis using peptide mass

fingerprinting and database searching. These cellular proteins could represent distinct roles during infection related to apoptosis. Our findings show that two-dimensional differential gel electrophoresis combined with mass spectrometry is a powerful approach that permits the identification of proteins whose expression was altered due to West Nile virus infection.
Descriptors: West Nile virus, neurodegeneration, infected neurons, differential gel electrophoresis, pathogenesis, cellular proteins.

Diamond, M.S. (2005). **Development of effective therapies against West Nile virus infection.** *Expert Review of Anti Infective Therapy* 3(6): 931-944.
Abstract: Since its entry into North America in 1999, West Nile virus has spread throughout the USA and Canada, and now annually causes a clinical spectrum of human disease ranging from a self-limiting acute febrile illness to potentially lethal encephalitis. Although no therapy is currently approved for use in humans, several strategies are being pursued to develop effective prophylaxis and treatments. This review describes the epidemiology, clinical presentation and pathogenesis of West Nile virus infection, and highlights recent progress towards an effective therapy.
Descriptors: antiviral agents, pharmaceutical trends, West Nile fever, West Nile virus drug effects, literature review.

Diamond, M.S. and R.S. Klein (2006). **A genetic basis for human susceptibility to West Nile virus.** *Trends in Microbiology* 14(7): 287-289.
Abstract: West Nile virus (WNV) infects thousands of humans annually and causes a spectrum of disease ranging from an acute febrile illness to lethal encephalitis. A new study suggests a link between CCR5Delta32 (a common mutant allele of the chemokine and HIV receptor CCR5) and fatal WNV infection. The study highlights a possible risk in targeting this receptor for the prevention and/or treatment of infectious diseases.
Descriptors: genetic predisposition to disease, CCR5 receptors genetics, West Nile fever, mice, West Nile virus, risk factors.

Ding, X., X. Wu, T. Duan, M. Siirin, H. Guzman, Z. Yang, R.B. Tesh, and S.Y. Xiao (2005). **Nucleotide and amino acid changes in West Nile virus strains exhibiting renal tropism in hamsters.** *American Journal of Tropical Medicine and Hygiene* 73(4): 803-807.
Abstract: Recent studies have shown that West Nile virus (WNV) can induce an asymptomatic persistent infection in the kidneys of experimentally infected hamsters. The chronically infected rodents shed virus in their urine for up to 8 months, despite the disappearance of viremia and the development of high levels of neutralizing antibodies. WNV, like most members of the Japanese encephalitis virus complex (Flavivirus; Flaviviridae), is assumed to be mainly neurotropic; little is known about

the genetic basis for its renal tropism. In this study, complete sequence analyses were done to compare four WNV isolates from the urines of persistently infected hamsters with the wild-type parent virus (NY 385-99). Nucleotide changes, ranging from 0.05% to 0.09%, were identified in all of the WNV isolates from urine; most of the changes were in coding regions, causing amino acid substitutions in the E, NS1, NS2B, and NS5 proteins. The genetic changes associated with renal tropism were also accompanied by a loss of virulence for hamsters and a change in plaque morphology.

Descriptors: amino acid substitution, kidney, West Nile fever, West Nile virus, chronic disease, animal disease models, kidney pathology, molecular sequence data, urine virology, virulence.

Diniz, J.A., A.P. Da Rosa, H. Guzman, F. Xu, S.Y. Xiao, V.L. Popov, P.F. Vasconcelos, and R.B. Tesh (2006). **West Nile virus infection of primary mouse neuronal and neuroglial cells: The role of astrocytes in chronic infection.** *American Journal of Tropical Medicine and Hygiene* 75(4): 691-696.

Abstract: Primary cultures of embryonic murine neurons and newborn mouse astrocytes were inoculated with West Nile virus (WNV) strain NY385-99 to compare the pathogenesis of WNV infection in these types of CNS cells. Two different outcomes were observed. WNV infection in the neurons was rapidly progressive and destructive; within 5 days, all of the neurons were destroyed through apoptosis. WNV infection in the astrocytes evolved more slowly and did not seem to be highly lethal to the cells. The infected astrocytes continued to produce infectious virus (10(4.6)-10(6.5) PFU/mL) for 114 days, in a permissive, persistent infection. During this period, WNV antigen could be shown in the cytoplasm of the infected astrocytes by immunocytochemical assay, transmission electron microscopy of ultrathin sections, and in the cell culture medium by complement fixation test. Our results with this in vitro experimental murine cell model indicate that astrocytes can develop chronic or persistent infection with WNV, suggesting that these cells may play a role in the maintenance of WNV in the CNS.

Descriptors: newborn mouse astrocytes, embryonic murine neurons, West Nile virus, complement fixation tests, cytopathogenic effect, viral hemagglutination tests, in situ nick end labeling, mice, virus replication.

Elghonemy, S., W.G. Davis, and M.A. Brinton (2005). **The majority of the nucleotides in the top loop of the genomic 3 terminal stem loop structure are cis-acting in a West Nile virus infectious clone**. *Virology* P.: 238-46.

Descriptors: West Nile virus, infectious clone, nucleotides, genomic 3, terminal loop stem structure.

Faraj, C., M. Elkohli, and M. Lyagoubi (2006). **The gonotrophic cycle of *Culex pipiens* (Diptera: Culicidae), West Nile virus potential vector, in Morocco: Evaluation of its duration in laboratory. [Cycle gonotrophique de *Culex pipiens* (Diptera: culicidae), vecteur potentiel du virus West Nile, au Maroc: Estimation de la duree en laboratoire]**. *Bulletin De La Societe De Pathologie Exotique* 99(2): 119-121.
Abstract: *Culex pipiens* gonotrophic cycle has been studied, for the first time in Morocco, under experimental conditions. Its average duration was estimated at 5.54 +/- 1.73 days.
Descriptors: culex growth and development, insect vectors, West Nile virus, Morocco.
Language of Text: French.

Fayzulin, R., F. Scholle, O. Petrakova, I. Frolov, and P.W. Mason (2006). **Evaluation of replicative capacity and genetic stability of West Nile virus replicons using highly efficient packaging cell lines.** *Virology* 351(1): 196-209. ISSN: 0042-6822.
Abstract: A stable cell system for high-efficiency packaging of West Nile virus (WNV) subgenomic replicons into virus-like particles (VLPs) was developed. VLPs could be propagated on these packaging cells and produced infectious foci similar to foci produced by WNV. Focus size correlated with the replicative capacity of WNV replicons, indicating that genome copy number, rather than amount of trans-complementing structural proteins, was rate-limiting in packaging of VLPs. Comparison of VLP production from replicon genomes encoding partial or complete C genes indicated that portions of C downstream of the cyclization sequence could improve genome replication or that cis expression of C could enhance packaging. Interestingly, a rapid loss of replicon-encoded reporter gene activity was detected within two serial passages of reporter gene-containing VLPs. The loss of reporter activity correlated with gene deletion and better VLP growth, indicating a powerful selection pressure for WNV genomes lacking reporter genes.
Descriptors: variation genetics, virus assembly, virus replication physiology, West Nile virus, *Cercopithecus aethiops*, cricetinae, vero cells.

Formosinho, P. and M.M. Santos Silva (2006). **Experimental infection of *Hyalomma marginatum* ticks with West Nile virus.** *Acta Virologica* 50(3): 175-180.
Abstract: To define the possible role of *Hyalomma marginatum* ticks in the transmission of West Nile virus (WNV) in Portugal an experimental infection was established. Ticks were fed on viremic rabbits previously infected with WNV. In different developmental stage of *H. marginatum* virus isolation and detection of viral antigen and viral RNA were attempted. The oral infection rates were 3%, 33% and 75% for engorged larvae, nymphs and females after oviposition, respectively. Transstadial transmission rates for nymphs exposed to virus as larvae, for adults exposed as larvae, and for adults exposed as nymphs were 33%, 11% and 46%, respectively.

No evidence of transovarial transmission was obtained. Ticks in the stages of nymphs and adults were able to transmit the infection to uninfected hosts. This study demonstrated that *H. marginatum* could be involved in the natural circulation of WNV in Portugal.

Descriptors: West Nile virus, ticks, experimental infection, vector, *Hyalomma marginatum*, viremic rabbits, infection rates, transviral transmission.

Fredericksen, B.L. and M.J. Gale (2006). **West Nile virus evades activation of interferon regulatory factor 3 through RIG-I-dependent and -independent pathways without antagonizing host defense signaling.** *Journal of Virology* 80(6): 2913-2923. ISSN: 0022-538X.

Abstract: The ability of viruses to control and/or evade the host antiviral response is critical to the establishment of a productive infection. We have previously shown that West Nile virus NY (WNV-NY) delays activation of interferon regulatory factor 3 (IRF-3), a transcription factor critical to the initiation of the antiviral response. Here we demonstrate that the delayed activation of IRF-3 is essential for WNV-NY to achieve maximum virus production. Furthermore, WNV-NY utilizes a unique mechanism to control activation of IRF-3. In contrast to many other viruses that impose a nonspecific block to the IRF-3 pathway, WNV-NY eludes detection by the host cell at early times postinfection. To better understand this process, we assessed the role of the pathogen recognition receptor (PRR) retinoic acid-inducible gene I (RIG-I) in sensing WNV-NY infection. RIG-I null mouse embryo fibroblasts (MEFs) retained the ability to respond to WNV-NY infection; however, the onset of the host response was delayed compared to wild-type (WT) MEFs. This suggests that RIG-I is involved in initially sensing WNV-NY infection, while other PRRs sustain and/or amplify the host response later in infection. The delayed initiation of the host response correlated with an increase in WNV-NY replication in RIG-I null MEFs compared to WT MEFs. Our data suggest that activation of the host response by RIG-I early in infection is important for controlling replication of WNV-NY. Furthermore, pathogenic strains of WNV may have evolved to circumvent stimulation of the host response until after replication is well under way.

Descriptors: interferon regulatory factor 3 antagonists and inhibitors, trans activation genetics, trans activators metabolism, West Nile fever immunology, West Nile virus, cell line, interferon regulatory factor 3 metabolism, mice, plaque assay, virus replication.

Garcia Tapia, D., C.M. Loiacono, and S.B. Kleiboeker (2006). **Replication of West Nile virus in equine peripheral blood mononuclear cells.** *Veterinary Immunology and Immunopathology* 110(3-4): 229-244.

Abstract: A cell model of primary monocytes and other mononuclear cells isolated from equine blood was used to study the kinetics of West Nile virus (WNV)

replication in a natural host. West Nile virus has emerged on the North American continent as a significant cause of morbidity and mortality in a wide range of avian and mammalian species. While other flaviviruses are known to infect monocytes and lymphocytes, the ability of WNV to productively replicate in specific immune cells of peripheral blood has not been assessed. In this study, enriched populations of monocytes and lymphocytes as well as purified monocytes, CD4+, CD8+ and B lymphocytes were obtained from equine blood. Productive WNV replication was demonstrated by viral growth curves, quantitative RT-PCR for WNV RNA, and indirect immunofluorescence detection of a non-structural WNV protein. Enriched and purified monocytes consistently supported productive viral replication in blood from nine of nine horses tested while a minor subset of CD4+ lymphocytes supported productive replication in cells from three of the nine horses tested. Peak viral titers of 3.2-6.6 log10 PFU/ml were reached at 6 days post-inoculation (p.i.) and titers were maintained through 10-15 days p.i. Activation of monocytes with bacterial lipopolysaccharide, which resulted in activation of nuclear transcription factor kappaB (NF-kappaB) plus elevation of nitric oxide and type I interferon levels, reduced or eliminated WNV replication. These results suggest that immune cells of the peripheral blood may serve as target cells for initial replication of WNV and may play a role in subsequent viral dissemination. Furthermore, primary equine immune cell cultures represent a potentially useful model of a natural WNV host when testing compounds such as antivirals for use in WNV treatment.
Descriptors: horses, mononuclear leukocytes, virus replication, West Nile virus, antigens, CD metabolism, cell adhesion, interferon type I metabolism, mononuclear metabolism, lipopolysaccharides, primary equine immune cell cultures, model of natural WNV host.

Glass, W.G., J.K. Lim, R. Cholera, A.G. Pletnev, J.L. Gao, and P.M. Murphy (2005). **Chemokine receptor CCR5 promotes leukocyte trafficking to the brain and survival in West Nile virus infection.** *Journal of Experimental Medicine* 202(8): 1087-1098.

Abstract: The molecular immunopathogenesis of West Nile virus (WNV) infection is poorly understood. Here, we characterize a mouse model for WNV using a subcutaneous route of infection and delineate leukocyte subsets and immunoregulatory factors present in the brains of infected mice. Central nervous system (CNS) expression of the chemokine receptor CCR5 and its ligand CCL5 was prominently up-regulated by WNV, and this was associated with CNS infiltration of CD4+ and CD8+ T cells, NK1.1+ cells and macrophages expressing the receptor. The significance of CCR5 in pathogenesis was established by mortality studies in which infection of CCR5-/- mice was rapidly and uniformly fatal. In the brain, WNV-infected CCR5-/- mice had increased viral burden but markedly reduced NK1.1+

cells, macrophages, and CD4+ and CD8+ T cells compared with WNV-infected CCR5+/+ mice. Adoptive transfer of splenocytes from WNV-infected CCR5+/+ mice into infected CCR5-/- mice increased leukocyte accumulation in the CNS compared with transfer of splenocytes from infected CCR5-/- mice into infected CCR5-/- mice, and increased survival to 60%, the same as in infected CCR5+/+ control mice. We conclude that CCR5 is a critical antiviral and survival determinant in WNV infection of mice that acts by regulating trafficking of leukocytes to the infected brain.

Descriptors: brain metabolism, cell movement, mononuclear leukocytes, CCR5 metabolism, West Nile fever, West Nile virus , adoptive transfer, brain immunology and pathology, cytokines, DNA primers, enzyme linked immunosorbent assay (ELISA), flow cytometry, knockout mice, reverse transcriptase polymerase chain reaction.

Glass, W.G., J.K. Lim, D.H. Mcdermott, A. Pletnev, R. Cholera, J. Gao, S. Lekhong, S.F. Yu, W.A. Frank, J. Pape, R.C. Cheshier, and P.M. Murphy (2006). **CCR5 saves lives: The protective role of CCR5 during West Nile virus infection**. *Journal of Neurochemistry* 96(Suppl. 1): 94. ISSN: 0022-3042.

Descriptors: West Nile virus infection, CCR5 protective role, immune system, blood lymphatics, susceptibility, immunopathogenic mechanism.

Notes: Meeting Information: Annual Meeting of the American Society for Neurochemistry, Portland, Oregon, USA; March 11 -15, 2006.

Glass, W.G., D.H. McDermott, J.K. Lim, S. Lekhong, S.F. Yu, W.A. Frank, J. Pape, R.C. Cheshier, and P.M. Murphy (2006). **CCR5 deficiency increases risk of symptomatic West Nile virus infection.** *Journal of Experimental Medicine* 203(1): 35-40.

Abstract: West Nile virus (WNV) is a reemerging pathogen that causes fatal encephalitis in several species, including mouse and human. Recently, we showed that the chemokine receptor CCR5 is critical for survival of mice infected with WNV, acting at the level of leukocyte trafficking to the brain. To test whether this receptor is also protective in man, we determined the frequency of CCR5Delta32, a defective CCR5 allele found predominantly in Caucasians, in two independent cohorts of patients, one from Arizona and the other from Colorado, who had laboratory-confirmed, symptomatic WNV infection. The distribution of CCR5Delta32 in a control population of healthy United States Caucasian random blood donors was in Hardy-Weinberg equilibrium and CCR5Delta32 homozygotes represented 1.0% of the total group (n = 1,318). In contrast, CCR5Delta32 homozygotes represented 4.2% of Caucasians in the Arizona cohort (odds ratios [OR] = 4.4 [95% confidence interval [CI], 1.6-11.8], P = 0.0013) and 8.3% of Caucasians in the Colorado cohort (OR = 9.1 [95% CI, 3.4-24.8], P < 0.0001). CCR5Delta32 homozygosity was significantly associated with fatal outcome in the Arizona cohort (OR = 13.2 [95% CI, 1.9-89.9],

P = 0.03). We conclude that CCR5 mediates resistance to symptomatic WNV infection. Because CCR5 is also the major HIV coreceptor, these findings have important implications for the safety of CCR5-blocking agents under development for HIV/AIDS.

Descriptors: genetic predisposition to disease, CCR5 receptors deficiency, West Nile fever, homozygote, odds ratio.

Goodell, J.R., F. Puig Basagoiti, B.M. Forshey, P.Y. Shi, and D.M. Ferguson (2006). **Identification of compounds with anti-West Nile Virus activity.** *Journal of Medicinal Chemistry* 49(6): 2127-2137. ISSN: 0022-2623.

Abstract: The lack of antiviral compounds targeting flaviviruses represents a significant problem in the development of strategies for treating West Nile Virus (WNV), Dengue, and Yellow Fever infections. Using WNV high-throughput screening techniques developed in our laboratories, we report the identification of several small molecule anti-WNV compounds belonging to four different structural classes including pyrazolines, xanthanes, acridines, and quinolines. The initial set of "hits" was further refined using cell viability-cytotoxicity assays to two 1,3,5-triaryl pyrazoline compounds: 1-(4-chlorophenylacetyl)-5-(4-nitrophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole and 1-benzoyl-5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole. On the basis of their activity and favorable therapeutic indexes, these compounds were identified as viable leads and subjected to additional evaluation using an authentic viral titer reduction assay employing an epidemic strain of WNV. The compounds were further evaluated in a transient replicon reporting system to gain insight into the mechanism of action by identifying the step at which inhibition takes place during viral replication. The results indicate the pyrazolines inhibit RNA synthesis, pointing to viral RNA polymerase, RNA helicase, or other viral replication enzymes as potential targets. Progress was also made in understanding the structural requirements for activity by synthesizing a focused chemical library of substituted pyrazolines. Preliminary SAR data are presented that show the aryl-rings are required for activity against WNV. More importantly, the results indicate WNV activity is tolerant to aryl-substitutions paving the way for the design and development of much larger combinatorial libraries with varied physicochemical properties.

Descriptors: acridines, chemical synthesis, antiviral agents, pyrazoles, quinolines, West Nile virus, xanthenes, virus replication.

Gould, L.H., J. Sui, H. Foellmer, T. Oliphant, T. Wang, M. Ledizet, A. Murakami, K. Noonan, C. Lambeth, K. Kar, J.F. Anderson, A.M. de Silva, M.S. Diamond, R.A. Koski, W.A. Marasco, and E. Fikrig (2005). **Protective and therapeutic capacity of human single-chain Fv-Fc fusion proteins against West Nile virus.** *Journal of Virology* 79(23): 14606-14613. ISSN: 0022-538X.

Abstract: West Nile virus has spread rapidly across the United States, and there is currently no approved human vaccine or therapy to prevent or treat disease. Passive immunization with antibodies against the envelope protein represents a promising means to provide short-term prophylaxis and treatment for West Nile virus infection. In this study, we identified a panel of 11 unique human single-chain variable region antibody fragments (scFvs) that bind the envelope protein of West Nile virus. Selected scFvs were converted to Fc fusion proteins (scFv-Fcs) and were tested in mice for their ability to prevent lethal West Nile virus infection. Five of these scFv-Fcs, 11, 15, 71, 85, and 95, protected 100% of mice from death when given prior to infection with virus. Two of them, 11 and 15, protected 80% of mice when given at days 1 and 4 after infection. In addition, four of the scFv-Fcs cross-neutralized dengue virus, serotype 2. Binding assays using yeast surface display demonstrated that all of our scFvs bind to sites within domains I and II of West Nile virus envelope protein. These recombinant human scFvs are potential candidates for immunoprophylaxis and therapy of flavivirus infections.
Descriptors: viral antibodies, viral vaccines , West Nile fever prevention and control, West Nile virus, antibody specificity, immunoglobulin fragments, recombinant fusion proteins.

Gu, B., S. Ouzunov, L. Wang, P. Mason, N. Bourne , A. Cuconati, and T.M. Block (2006). **Discovery of small molecule inhibitors of West Nile virus using a high-throughput sub-genomic replicon screen.** *Antiviral Research* 70(2): 39-50.

Abstract: West Nile virus (WNV) is a positive-sense, single-stranded RNA virus of the family Flaviviridae. WNV persistently infects insect cells, but can causes acute cytopathic infection of mammalian cells and is an etiologic agent of viral encephalitis in humans. By using a cell line expressing a WNV subgenomic replicon [Rossi, S.L., Zhao, Q., O'Donnell, V.K., Mason, P.W., 2005. Adaptation of West Nile virus replicons to cells in culture and use of replicon-bearing cells to probe antiviral action. Virology 331 (2), 457-470], we developed a high-throughput assay and used it to screen a library of small molecule compounds for inhibitors of WNV replication in the absence of live virus. Here we report the identification of novel small molecule inhibitors for WNV replicon replication. We demonstrate that the compounds inhibited WNV replication-dependent luciferase expression in the replicon cells and reduced WNV viral protein accumulation and viral RNA copy number in the replicon cells. Two classes of compounds with multiple hits, parazolotrahydrothophenes and pyrozolopyrimidines, showed preliminary structure-activity relationships. In WNV infection assays, one pyrozolopyrimidine compound was confirmed to have antiviral activity. These compounds should be valuable for developing anti-WNV therapeutic drugs as well as research tools to study the mechanism of WNV replication.

Descriptors: antiviral agents, West Nile virus, WNV replication, preclinical methods, viral genetics, luciferases, replicon drug effects.

Gu, B., P. Mason, L. Wang, N. Bourne, S. Rossi, S. Ouzounov, A. Cuconati, A. Mehta, and T. Block (2005). **Discovery of West Nile virus inhibitors**. *Antiviral Research* 65(3): A68. ISSN: 0166-3542.
Descriptors: West Nile virus, viral disease inhibitors, drug therapy, cell based replicon assay.
Notes: Meeting Information: 18th International Conference on Antiviral Research, Barcelona, Spain; April 11 -14, 2005.

Guo, J.T., J. Hayashi, and C. Seeger (2005). **West Nile Virus Inhibits the Signal Transduction Pathway of Alpha Interferon**. *Journal of Virology* P.: 1343-50.
Descriptors: West Nile virus, signal transduction pathway, alpha interferon, inhibits.

Hahn, D.C., N.M. Nemeth, E. Edwards, P.R. Bright, and N. Komar (2006). **Passive West Nile virus antibody transfer from maternal Eastern screech-owls (*Megascops asio*) to progeny.** *Avian Diseases* 50(3): 454-455. ISSN: 0005-2086.
Abstract: Transovarial antibody transfer in owls has not been demonstrated for West Nile virus (WNV). We sampled chicks from captive adult WNV-antibody-positive Eastern Screech-Owls (*Megascops asio*) to evaluate the prevalence of transovarial maternal antibody transfer, as well as titers and duration of maternal antibodies. Twenty-four owlets aged 1 to 27 days old circulated detectable antibodies with neutralizing antibody titers ranging from 20 to 1600 (median 1:40). Demonstrating that WNV antibodies are passively transferred transovarially is important for accurate interpretation of serologic data from young birds.
Descriptors: viral blood antibodies, bird diseases, maternally acquired immunity, West Nile virus, screech owls.

Higgs, S., B. Schneider, D. Vanlandingham, K. Klingler, and E. Gould (2005). **Nonviremic transmission of West Nile virus.** *Proceedings of the National Academy of Sciences of the United States of America.* 102(25): 8871-8874. ISSN: 0027-8424.
Abstract: West Nile virus (WNV) is now the predominant circulating arthropod-borne virus in the United States with >15,000 human cases and >600 fatalities since 1999. Conventionally, mosquitoes become infected when feeding on viremic birds and subsequently transmit the virus to susceptible hosts. Here, we demonstrate non-viremic transmission of WNV between cofeeding mosquitoes. Donor, *Culex pipiens quinquefasciatus* mosquitoes infected with WNV were fed simultaneously with uninfected "recipient" mosquitoes on naive mice. At all times, donor and recipient mosquitoes were housed in separate sealed containers, precluding the possibility of

mixing. Recipients became infected in all five trials, with infection rates as high as 5.8% and no detectable viremia in the hosts. Remarkably, a 2.3% infection rate was observed when 87 uninfected mosquitoes fed adjacent to a single infected mosquito. This phenomenon could potentially enhance virus survival, transmission, and dispersion and obviate the requirement for viremia. All vertebrates, including immune and insusceptible animals, might therefore facilitate mosquito infection. Our findings question the status of dead-end hosts in the WNV transmission cycle and may partly explain the success with which WNV established and rapidly dispersed throughout North America.

Descriptors: West Nile virus, disease transmission, *Culex quinquefasciatus*, *Culex pipiens*, mosquitoes , insect vectors, hematophagy, mice, *Culex-pipiens-quinquefasciatus.*

Hunsperger, E.A. and J.T. Roehrig (2006). **Temporal analyses of the neuropathogenesis of a West Nile virus infection in mice.** *Journal of Neurovirology* 12(2): 129-139.

Abstract: A West Nile virus (WNV) infection in humans can produce neurological symptoms including acute flaccid paralysis, encephalitis, meningitis and myelitis. To investigate the pathogenesis of WNV in the peripheral and the central nervous system (PNS and CNS), the authors used a murine footpad inoculation model of WNV infection. Survival curves of virus-infected animals of ages 4- to 6-weeks-old demonstrated age-dependent mortality where older animals (6-weeks-old) had a higher mortality rate compared to younger animals (4- and 5-weeks-old). The mice that survived the virus infection formed WNV-reactive antibodies, confirming viral infection and clearance. The localization of viral RNA (vRNA) and antigen in infected murine tissues was investigated using TaqMan and immunohistochemistry (IHC) respectively. During a nine day infection, vRNA levels in the spinal cord and brainstem fluctuated, suggesting early viral clearance from these tissues by days 3-4 p.i. with later re-introduction. Viral antigens detected using IHC were primarily observed in three main regions of the brain: cortex, hippocampus and brainstem. Additionally, the dorsal root ganglion neurons of the PNS stained positive for viral antigens. These data are consistent with multiple routes of neuroinvasion following a peripheral inoculation of virus and do not preclude the previous observation that virus-infected peripheral neurons can introduce virus into the CNS by a retrograde transport mechanism.

Descriptors: West Nile fever, West Nile virus isolation and purification, disease models, disease progression, inbred C57BL mice, viral RNA analysis, viral genetics, reverse transcriptase polymerase chain reaction, time factors.

Iglesias, M.C., M.P. Frenkiel, K. Mollier, P. Souque, P. Despres, and P. Charneau (2006). **A single immunization with a minute dose of a lentiviral vector-based vaccine is highly effective at eliciting protective humoral immunity against West Nile virus.**

Journal of Gene Medicine 8(3): 265-274.
Abstract: BACKGROUND: Lentiviral vectors, due to their capacity to transduce non-dividing cells, have become precious and worldwide used gene transfer systems. Their ability to efficiently and stably transduce dendritic cells (DCs) has led to their successful use as vaccination vectors for eliciting strong, specific and protective cellular immune responses mostly in anti-tumoral but also in anti-viral applications. However, the ability of lentiviral vectors to elicit an antibody-based protective immunity has, to date, not been evaluated. In the present study, we evaluated the potential of a lentiviral vector-based vaccine to elicit humoral immunity against West Nile virus (WNV). WNV is a mosquito-borne flavivirus that emerged in North America and causes encephalitis in humans, birds and horses. Neutralizing anti-WNV antibodies have been shown to be crucial for protection against WNV encephalitis. METHODS: The ability of lentiviral vector TRIP/sE(WNV), expressing the secreted soluble form of the envelope E-glycoprotein (sE(WNV)) from the highly virulent IS-98-ST1 strain of WNV, to induce a specific humoral response and protection against WNV infection was assessed in a mouse model of WNV encephalitis. RESULTS: Remarkably, a single immunization with a minute dose of TRIP/sE(WNV) was efficient at eliciting a long-lasting, protective and sterilizing humoral immunity, only 1 week after priming. CONCLUSIONS: This study broadens the applicability of lentiviral vectors as efficient non-replicating vaccines against pathogens for which a neutralizing humoral response is one active arm of the protective immunity. The TRIP/sE(WNV) lentiviral vector appears to be a promising tool for veterinary vaccination against zoonotic WNV.
Descriptors: antibody formation, lentivirus, viral vaccines, West Nile fever, West Nile virus, genetic vectors, immunization schedule, mice, transduction, viral envelope proteins.

Johnson, B.W., O. Kosoy, D.A. Martin, A.J. Noga, B.J. Russell, A.A. Johnson, and L.R. Petersen (2005). **West Nile virus infection and serologic response among persons previously vaccinated against yellow fever and Japanese encephalitis viruses.** *Vector Borne and Zoonotic Diseases* 5(2): 137-145. ISSN: 1530-3667.
Abstract: It is hypothesized that previous heterologous flaviviral exposure may modulate clinical illness among persons infected with West Nile virus (WNV). Little is known about the serological response in such persons. In summer 2003, a WNV outbreak occurred in Colorado, the location of the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases (DVBID). DVBID employees, most previously vaccinated with yellow fever virus (YFV) or Japanese encephalitis virus (JEV) vaccines, were studied to determine whether previous vaccination affected symptom development among those subsequently infected with WNV during the outbreak, as well as their serological response. Serum samples collected in December

2003 and previously banked samples were tested using the plaque reduction neutralization test (PRNT) against WNV, Saint Louis encephalitis virus, dengue- 4 virus, JEV, and YFV. Specimens shown to have WNV antibody by PRNT were tested by IgM and IgG enzymelinked immunosorbent assays (ELISAs). Ten (9%) of 113 serosurvey participants had WNV neutralizing antibody titers in December 2003. PRNT titers from previous specimens showed that one of the ten had seroconverted to WNV before 2003. Of the remaining nine participants, seven reported illness in the summer of 2003, two of which were unvaccinated and five previously vaccinated. In the December 2003 specimens, five persons previously unvaccinated or vaccinated only against YFV had a fourfold or greater neutralizing titer with WNV than with other flaviviruses, whereas no persons previously vaccinated against JEV or JEV and YFV showed a similar difference in neutralizing titers. Eight of nine persons infected in 2003 had negative or indeterminate WNV MAC-ELISA results in the December 2003 sample; the ninth person was vaccinated against YFV one month previously, and was also YFV positive by MAC-ELISA. We conclude that previous flaviviral vaccination does not markedly affect the development of WNV fever and that the IgM antibody response in patients without neuroinvasive WNV disease is transient.
Descriptors: Japanese encephalitis vaccines, adverse effects, West Nile fever immunology, West Nile virus, yellow fever vaccine, viral biosynthesis, Colorado, enzyme linked immunosorbent assay.

Julander, J.G., Q.A. Winger, A.L. Olsen, C.W. Day, R.W. Sidwell, and J.D. Morrey (2005). **Treatment of West Nile virus-infected mice with reactive immunoglobulin reduces fetal titers and increases dam survival.** *Antiviral Research* 65(2): 79-85. ISSN: 0166-3542.
Abstract: The objectives of this study were to determine if injection of West Nile virus (WNV) into timed-pregnant mice would result in fetal infection and if administration of WNV-reactive immunoglobulin would increase dam survival and reduce fetal viral titers. Dams injected on 7.5 days post-coitus (dpc) had detectable viral titers in the placenta 10.5dpc with a mean titer of 10(4.9) 50% cell-culture infectious doses per gram of tissue (CCID(50)/g tissue). The mean placental titer increased to 10(8.6)CCID(50)/g tissue at 12.5dpc. Infectious virus was detectable 12.5dpc in 10 of 10 fetuses with a mean titer of 10(7.5)CCID(50)/g tissue. Treatment of dams (challenged with WNV on 7.5dpc) with WNV-reactive human immunoglobulin (Ig) on 8.5 and 9.5dpc resulted in a significant reduction of virus in fetuses as compared with non-reactive human Ig-treated females on 12.5dpc (P< or =0.001). Treatment also resulted in survival of dams to term. Treatment of dams with WNV-reactive human Ig on 12.5 and 13.5dpc also resulted in reduction of viral titer on 14.5dpc, indicating that later treatment may also be efficacious. This suggests that Ig treatment may be useful in treating fetal WNV infection in women.

Descriptors: viral antibodies, immunoglobulins, pregnancy complications, West Nile fever , West Nile virus, disease transmission, mice, infectious immunology.

Kajaste Rudnitski, A., T. Mashimo, M.P. Frenkiel , J.L. Guenet, M. Lucas, and P. Despres (2006). **The 2',5'-Oligoadenylate Synthetase 1b Is a Potent Inhibitor of West Nile Virus Replication Inside Infected Cells.** *Journal of Biological Chemistry.* 281(8): 4624-4637. ISSN: 0021-9258.
Abstract: The 2',5'-oligoadenylate synthetase (OAS) proteins associated with endoribonuclease RNase L are components of the interferon-regulated OAS/RNase L system, which is an RNA decay pathway known to play an important role in the innate antiviral immunity. A large body of evidence suggests a critical role for the 1b isoform of the mouse Oas gene (Oas1b) in resistance to West Nile virus (WNV) infection in vivo. WNV is a positive, single-stranded RNA virus responsible for severe encephalitis in a large range of animal species and humans. To investigate the molecular basis for the sensitivity of WNV to the Oas1b antiviral pathway, we established a stable mouse fibroblastic cell clone that up-regulates Oas1b protein expression under the control of the Tet-Off expression system. We showed that murine cells respond to Oas1b expression by efficiently inhibiting WNV replication. The antiviral action of Oas1b was essentially restricted to the early stages in virus life cycle. We found that the inability of WNV to productively infect the Oas1b-expressing cells was attributable to a dramatic reduction in positive-stranded viral RNA level. Thus, Oas1b represents an antiviral pathway that exerts its inhibitory effect on WNV replication by preventing viral RNA accumulation inside infected cells.
Descriptors: Internet resource, West Nile virus, oligoadenylate synthase, OAS, RNaseL, proteins, antiviral immunity.

Kalitzky, M., H. Rohde and P. Borowski (2005). **West Nile virus: New targets for potential antivirals**. In: P.F. Torrence *Antiviral Drug Discovery for Emerging Diseases and Bioterrorism Threats,* John Wiley & Sons Ltd: Chichester, UK, p. 263-278. ISBN: 0471668273.
Descriptors: West Nile virus, anti-viral drug discovery, emerging diseases, protein synthesis, enzyme inhibition, virus replication.

Kanai, R., K. Kar, K. Anthony, L.H. Gould, M. Ledizet, E. Fikrig, W.A. Marasco, R.A. Koski, and Y. Modis (2006). **Crystal structure of west nile virus envelope glycoprotein reveals viral surface epitopes.** *Journal of Virology* 80(22): 11000-11008. ISSN: 0022-538X.
Abstract: West Nile virus, a member of the Flavivirus genus, causes fever that can progress to life-threatening encephalitis. The major envelope glycoprotein, E, of these viruses mediates viral attachment and entry by membrane fusion. We have determined the crystal structure of a soluble fragment of West Nile virus E. The structure

adopts the same overall fold as that of the E proteins from dengue and tick-borne encephalitis viruses. The conformation of domain II is different from that in other prefusion E structures, however, and resembles the conformation of domain II in postfusion E structures. The epitopes of neutralizing West Nile virus-specific antibodies map to a region of domain III that is exposed on the viral surface and has been implicated in receptor binding. In contrast, we show that certain recombinant therapeutic antibodies, which cross-neutralize West Nile and dengue viruses, bind a peptide from domain I that is exposed only during the membrane fusion transition. By revealing the details of the molecular landscape of the West Nile virus surface, our structure will assist the design of antiviral vaccines and therapeutics.
Descriptors: epitopes, viral envelope proteins chemistry, West Nile virus, amino acid sequence, crystallography, x ray, dengue virus, encephalitis viruses, tick borne, epitope mapping, molecular sequence data, protein binding, tertiary protein structure, sequence alignment.

Karaca, K., R. Bowen, L.E. Austgen, M. Teehee, L. Siger, D. Grosenbaugh, L. Loosemore, J.C. Audonnet, R. Nordgren, and J.M. Minke (2005). **Recombinant canarypox vectored West Nile virus (WNV) vaccine protects dogs and cats against a mosquito WNV challenge.** *Vaccine* 23(29): 3808-3813.
Abstract: The safety and efficacy of a canarypox vector expressing PrM and E genes of West Nile virus (WNV) (ALVAC-WNV) was evaluated in dogs and cats. One group of 17 dogs (vaccinated with 10(5.6) TCID(50)) and two groups of cats (groups 1 [n=14] vaccinated with 10(7.5) TCID(50) and 2 [n=8] 10(5.6) TCID(50)) were vaccinated twice at 28-day intervals. Fifteen dogs and eleven cats served as negative controls. The cats and dogs were challenged 120 and 135 days after the second immunization, respectively via the bites of Aedes albopictus mosquitoes infected with WNV. The first dose of vaccine induced a detectable antibody response in four dogs and five cats (one immunized with low and four with high doses). After the second dose, all the vaccinated dogs and all of the cats, immunized with high dose had detectable antibody titers, whereas only four of eight cats in the low dose group were seropositive. None of the vaccinated dogs and one vaccinated cat developed viremia following the WNV mosquito-challenge. In contrast, 14 of the 15 control dogs and 9 of the 11 control cats developed viremia. The experimental vaccine described in this study may be of value in the prevention of WNV infection in dogs and cats.
Descriptors: canarypox vector, cat diseases , dog diseases, viral vaccines, West Nile fever, West Nile virus, viral blood antibodies, synthetic genetics, viral envelope proteins, viral vaccines, West Nile fever prevention and control.

Kaufmann, B., G.E. Nybakken, P.R. Chipman, W. Zhang, M.S. Diamond, D.H. Fremont, R.J. Kuhn, and M.G. Rossmann (2006). **West Nile virus in complex with the Fab fragment of a neutralizing monoclonal antibody.** *Proceedings of the National*

Academy of Sciences of the United States of America 103(33): 12400-12404.
Abstract: Flaviviruses, such as West Nile virus (WNV), are significant human pathogens. The humoral immune response plays an important role in the control of flavivirus infection and disease. The structure of WNV complexed with the Fab fragment of the strongly neutralizing mAb E16 was determined to 14.5-Angstrom resolution with cryo-electron microscopy. E16, an antibody with therapeutic potential, binds to domain III of the WNV envelope glycoprotein. Because of steric hindrance, Fab E16 binds to only 120 of the 180 possible binding sites on the viral surface. Fitting of the previously determined x-ray structure of the Fab-domain III complex into the cryo-electron microscopy density required a change of the elbow angle between the variable and constant domains of the Fab. The structure suggests that the E16 antibody neutralizes WNV by blocking the initial rearrangement of the E glycoprotein before fusion with a cellular membrane.
Descriptors: monoclonal antibodies, immunoglobulin Fab fragments, West Nile virus chemistry, binding sites, cell line, cricetinae, cryoelectron microscopy, crystallography, x ray, hydrogen ion concentration, models, protein conformation, viral fusion proteins.

Keller, B.C., B.L. Fredericksen, M.A. Samuel, R.E. Mock, P.W. Mason, M.S. Diamond, and M.J. Gale (2006). **Resistance to alpha/beta interferon is a determinant of West Nile virus replication fitness and virulence.** *Journal of Virology* 80(19): 9424-9434. ISSN: 0022-538X.
Abstract: The emergence of West Nile virus (WNV) in the Western Hemisphere is marked by the spread of pathogenic lineage I strains, which differ from typically avirulent lineage II strains. To begin to understand the virus-host interactions that may influence the phenotypic properties of divergent lineage I and II viruses, we compared the genetic, pathogenic, and alpha/beta interferon (IFN-alpha/beta)-regulatory properties of a lineage II isolate from Madagascar (MAD78) with those of a new lineage I isolate from Texas (TX02). Full genome sequence analysis revealed that MAD78 clustered, albeit distantly, with other lineage II strains, while TX02 clustered with emergent North American isolates, more specifically with other Texas strains. Compared to TX02, MAD78 replicated at low levels in cultured human cells, was highly sensitive to the antiviral actions of IFN in vitro, and demonstrated a completely avirulent phenotype in wild-type mice. In contrast to TX02 and other pathogenic forms of WNV, MAD78 was defective in its ability to disrupt IFN-induced JAK-STAT signaling, including the activation of Tyk2 and downstream phosphorylation and nuclear translocation of STAT1 and STAT2. However, replication of MAD78 was rescued in cells with a nonfunctional IFN-alpha/beta receptor (IFNAR). Consistent with this finding, the virulence of MAD78 was unmasked upon infection of mice lacking IFNAR. Thus, control of the innate host response and

IFN actions is a key feature of WNV pathogenesis and replication fitness.
Descriptors: interferon alpha immunology, interferon beta immunology, virus replication, West Nile fever, mice, molecular sequence data, phenotype, phylogeny, protein tyrosine kinase metabolism, stat transcription factors metabolism, signal transduction, drug effects, West Nile virus isolation and purification.

Khairallah, M., S. Ben Yahia, S. Attia, S. Zaouali, B. Jelliti, S. Jenzri, A. Ladjimi, and R. Messaoud (2006). **Indocyanine green angiographic features in multifocal chorioretinitis associated with West Nile virus infection.** *Retina* 26(3): 358-359.
Descriptors: chorioretinitis, diagnostic use of coloring agents, eye infections, indocyanine green, West Nile fever diagnosis, viral blood antibodies, chorioretinitis, viral virology, fluorescein angiography, immunoglobulin M blood.

Kilpatrick, A.M., L.D. Kramer, S.R. Campbell, E.O. Alleyne, A.P. Dobson, and P. Daszak (2005). **West Nile virus risk assessment and the bridge vector paradigm.** *Emerging Infectious Diseases* 11(3): 425-429. ISSN: 1080-6040.
Abstract: In the northeast United States, control of West Nile virus (WNV) vectors has been unfocused because of a lack of accurate knowledge about the roles different mosquitoes play in WNV transmission. We analyzed the risk posed by 10 species of mosquitoes for transmitting WNV to humans by using a novel risk-assessment measure that combines information on the abundance, infection prevalence, vector competence, and biting behavior of vectors. This analysis suggests that 2 species (*Culex pipiens* L. and *Cx. restuans* Theobald [Diptera: Cilicidae]) not previously considered important in transmitting WNV to humans may be responsible for up to 80% of human WNV infections in this region. This finding suggests that control efforts should be focused on these species which may reduce effects on nontarget wetland organisms. Our risk measure has broad applicability to other regions and diseases and can be adapted for use as a predictive tool of future human WNV infections.
Descriptors: culex mosquitoes physiology, insect vectors, West Nile fever transmission, New England, risk, West Nile virus.

Kinney, R.M., C.Y. Huang, M.C. Whiteman, R.A. Bowen, S.A. Langevin, B.R. Miller, and A.C. Brault (2006). **Avian virulence and thermostable replication of the North American strain of West Nile virus.** *Journal of General Virology* 87(Pt 12): 3611-3622.
Abstract: The NY99 genotype of West Nile virus (WNV) introduced into North America has demonstrated high virulence for American crows (AMCRs), whilst a closely related WNV strain (KEN-3829) from Kenya exhibits substantially reduced virulence in AMCRs [Brault, A. C., Langevin, S. A., Bowen, R. A., Panella, N. A., Biggerstaff, B. J., Miller, B. R. & Nicholas, K. (2004). Emerg Infect Dis 10,

2161-2168]. Viruses rescued from infectious cDNA clones of both the NY99 and KEN-3829 strains demonstrated virulence comparable to that of their parental strains in AMCRs. To begin to define parameters that might explain the different virulence phenotypes between these two viruses, temperature-sensitivity assays were performed for both viruses at the high temperatures experienced in viraemic AMCRs. Growth curves of the two WNV strains were performed in African green monkey kidney (Vero; 37-42 degrees C) and duck embryonic fibroblast (DEF; 37-45 degrees C) cells cultured at temperatures that were tolerated by the cell line. Unlike the NY99 virus, marked decreases in KEN-3829 viral titres were detected between 36 and 120 h post-infection (p.i.) at temperatures above 43 degrees C. Replication of KEN-3829 viral RNA was reduced 6500-fold at 72 h p.i. in DEF cells incubated at 44 degrees C relative to levels of intracellular virus-specific RNA measured at 37 degrees C. In contrast, replication of virus derived from the NY99 infectious cDNA at 44 degrees C demonstrated only a 17-fold reduction in RNA level. These results indicated that the ability of WNV NY99 to replicate at the high temperatures measured in infected AMCRs could be an important factor leading to the increased avian virulence and emergence of this strain of WNV.
Descriptors: bird diseases, crows, virus replication, West Nile fever, West Nile virus, body temperature, cell line, *Cercopithecus aethiops*, ducks, plaque assay, viral RNA biosynthesis, survival analysis, temperature, vero cells.

Koh, W.L. and M.L. Ng (2005). **Molecular mechanisms of West Nile virus pathogenesis in brain cell.** *Emerging Infectious Diseases* 11(4): 629-362. ISSN: viral blood.
Abstract: We analyzed the response of human glioma cells to West Nile virus infection by investigating host transcriptional changes. Changes in expression of 23 genes showed similarities to those in other neurodegenerative diseases. These changes may be useful as potential biomarkers and elucidate novel mechanisms behind the neuropathology of infection with this virus.
Descriptors: brain virology, West Nile virus, biological markers, tumor cell line, cytopathogenic effect, viral gene expression, glioblastoma, microarray analysis, reproducibility of results, reverse transcriptase polymerase chain reaction, West Nile fever physiopathology.

Lee, J.W.M., J.J.H. Chu, and M.L. Ng (2006). **Quantifying the Specific Binding between West Nile Virus Envelope Domain III Protein and the Cellular Receptor [alpha] V[beta]3 Integrin.** *Journal of Biological Chemistry.* 281(3): 1352-1360. ISSN: 0021-9258.
Abstract: A previous study has illustrated that the [alpha]V[beta]3 integrin served as the functional receptor for West Nile virus (WNV) entry into cells. Domain III (DIII) of WNV envelope protein (E) was postulated to mediate virus binding to the cellular receptor. In this study, the specificity and affinity binding of WNV E DIII

protein to [alpha]V[beta]3 integrin was confirmed with co-immunoprecipitation and receptor competition assay. Binding of WNV E DIII protein to [alpha]V[beta]3 integrin induced the phosphorylation of focal adhesion kinase that is required to mediate ligand-receptor internalization into cells. A novel platform was then developed using the atomic force microscopy to measure this specific binding force between WNV E DIII protein and the cellular receptor, [alpha]V[beta]3 integrin. The single protein pair-interacting force measured was in the range of 45 +/- 5 piconewtons. This interacting force was highly specific as minimal force was measured in the WNV E DIII protein interaction with [alpha]V[beta]5 integrin molecules and heparan sulfate. These experiments provided an insight to quantitate virus-receptor interaction. Force measurement using atomic force microscopy can serve to quantitatively analyze the effect of candidate drugs that modulate virus-host receptor affinity.
Descriptors: Internet-resource, West Nile virus, Domain III protein, binding, cellular receptor, virus binding.

Lewis, M., J. Renclawowicz, and P. van den Driessche (2006). **Traveling waves and spread rates for a West Nile virus model.** *Bulletin of Mathematical Biology* 68(1): 3-23.
Abstract: A reaction-diffusion model for the spatial spread of West Nile virus is developed and analysed. Infection dynamics are based on a modified version of a model for cross infection between birds and mosquitoes (Wonham et al., 2004, An epidemiological model for West-Nile virus: Invasion analysis and control application. Proc. R. Soc. Lond. B 271), and diffusion terms describe movement of birds and mosquitoes. Working with a simplified version of the model, the cooperative nature of cross-infection dynamics is utilized to prove the existence of traveling waves and to calculate the spatial spread rate of infection. Comparison theorem results are used to show that the spread rate of the simplified model may provide an upper bound for the spread rate of a more realistic and complex version of the model.
Descriptors: biological models, West Nile fever, epidemiology algorithms , animal migration, birds, culicidae virology, North America.

Lewis, M.A., J. Renclawowicz, P. van den Driessche, and M. Wonham (2006). **A comparison of continuous and discrete-time West Nile virus models.** *Bulletin of Mathematical Biology* 68(3): 491-509.
Abstract: The first recorded North American epidemic of West Nile virus was detected in New York state in 1999, and since then the virus has spread and become established in much of North America. Mathematical models for this vector-transmitted disease with cross-infection between mosquitoes and birds have recently been formulated with the aim of predicting disease dynamics and evaluating possible control methods. We consider discrete and continuous time versions of the West Nile virus models proposed by Wonham et al. [Proc. R. Soc. Lond. B 271:501-507, 2004] and by Thomas and Urena [Math. Comput. Modell. 34:771-781, 2001], and

evaluate the basic reproduction number as the spectral radius of the next-generation matrix in each case. The assumptions on mosquito-feeding efficiency are crucial for the basic reproduction number calculation. Differing assumptions lead to the conclusion from one model [Wonham, M.J. et al., [Proc. R. Soc. Lond. B] 271:501-507, 2004] that a reduction in bird density would exacerbate the epidemic, while the other model [Thomas, D.M., Urena, B., Math. Comput. Modell. 34:771-781, 2001] predicts the opposite: a reduction in bird density would help control the epidemic.
Descriptors: biological models, West Nile virus, birds, culicidae virology, feeding behavior, North America, population density.

Li, J., R. Bhuvanakantham, J. Howe, and M.L. Ng (2006). **The glycosylation site in the envelope protein of West Nile virus (Sarafend) plays an important role in replication and maturation processes.** *Journal of General Virology* 87(Pt 3): 613-622.
Abstract: The complete genome of West Nile (Sarafend) virus [WN(S)V] was sequenced. Phylogenetic trees utilizing the complete genomic sequence, capsid gene, envelope gene and NS5 gene/3' untranslated region of WN(S)V classified WN(S)V as a lineage II virus. A full-length infectious clone of WN(S)V with a point mutation in the glycosylation site of the envelope protein (pWNS-S154A) was constructed. Both growth kinetics and the mode of maturation were affected by this mutation. The titre of the pWNS-S154A virus was lower than the wild-type virus. This defect was corrected by the expression of wild-type envelope protein in trans. The pWNS-S154A virus matured intracellularly instead of at the plasma membrane as shown for the parental WN(S)V.
Descriptors: viral genome, viral envelope proteins, West Nile virus, amino acid sequence, cell line, *Cercopithecus aethiops*, glycosylation, molecular sequence data, sequence alignment, species specificity, vero cells, viral envelope proteins.

Li, L., A. Barrett, and D. Beasley (2005). **Differential expression of domain III neutralizing epitopes on the envelope proteins of West Nile virus strains.** *Virology* 335(1): 99-105. ISSN: 0042-6822.
Descriptors: West Nile virus, viral proteins, viral antigens, epitopes, neutralization, amino acid sequences, antigenic variation, nucleotide sequences, structure activity relationships, viral-envelope-protein, viral-antibodies, molecular-sequence-data, Internet-resource.

Lim, J.K., W.G. Glass, D.H. McDermott, and P.M. Murphy (2006). **CCR5: No longer a "good for nothing" gene--chemokine control of West Nile virus infection.** *Trends in Immunology* 27(7): 308-312.
Abstract: The chemokine receptor CCR5 was identified in 1996 as a crucial host factor exploited by HIV for cell entry. CCR5 presumably functions normally in antimicrobial host defense because it generally mediates leukocyte chemotactic responses;

however, evidence of antimicrobial functions for CCR5 in humans has been elusive. Recently, genetic analyses in mice and humans have provided strong evidence for the CCR5 control of infection by West Nile virus (WNV), a re-emerging pathogen capable of causing fatal encephalitis. Thus, the same receptor can benefit or harm the host, depending on the virus. Although CCR5 might be a logical target for new drug development in HIV/AIDS, the benefits of blocking CCR5 could carry the cost of an increased risk of WNV disease in co-infected patients.
Descriptors: CCR5 receptor, West Nile fever, anti-HIV agents, drug therapy, drug development, chemokine receptor.

Liu, R., J. Shuai, J. Wu, and H. Zhu (2006). **Modeling spatial spread of West Nile virus and impact of directional dispersal of birds**. *Mathematical Biosciences and Engineering* 3(1): 145-160. ISSN: 1547-1063.
Descriptors: West Nile virus spread, modeling, bird dispersal, disease transmission, spatial spread model, long range dispersal patterns.

Liu, W.J., X.J. Wang, D.C. Clark, M. Lobigs, R.A. Hall, and A.A. Khromykh (2006). **A single amino acid substitution in the West Nile virus nonstructural protein NS2A disables its ability to inhibit alpha/beta interferon induction and attenuates virus virulence in mice.** *Journal of Virology* 80(5): 2396-2404. ISSN: 0022-538X.
Abstract: Alpha/beta interferons (IFN-alpha/beta) are key mediators of the innate immune response against viral infection. The ability of viruses to circumvent IFN-alpha/beta responses plays a crucial role in determining the outcome of infection. In a previous study using subgenomic replicons of the Kunjin subtype of West Nile virus (WNV(KUN)), we demonstrated that the nonstructural protein NS2A is a major inhibitor of IFN-beta promoter-driven transcription and that a single amino acid substitution in NS2A (Ala30 to Pro [A30P]) dramatically reduced its inhibitory effect (W. J. Liu, H. B. Chen, X. J. Wang, H. Huang, and A. A. Khromykh, J. Virol. 78:12225-12235). Here we show that incorporation of the A30P mutation into the WNV(KUN) genome results in a mutant virus which elicits more rapid induction and higher levels of synthesis of IFN-alpha/beta in infected human A549 cells than that detected following wild-type WNV(KUN) infection. Consequently, replication of the WNV(KUN)NS2A/A30P mutant virus in these cells known to be high producers of IFN-alpha/beta was abortive. In contrast, both the mutant and the wild-type WNV(KUN) produced similar-size plaques and replicated with similar efficiency in BHK cells which are known to be deficient in IFN-alpha/beta production. The mutant virus was highly attenuated in neuroinvasiveness and also attenuated in neurovirulence in 3-week-old mice. Surprisingly, the mutant virus was also partially attenuated in IFN-alpha/betagamma receptor knockout mice, suggesting that the A30P mutation may also play a role in more efficient activation of other antiviral pathways in addition to the IFN response. Immunization of wild-type mice with the

mutant virus resulted in induction of an antibody response of similar magnitude to that observed in mice immunized with wild-type WNV(KUN) and gave complete protection against challenge with a lethal dose of the highly virulent New York 99 strain of WNV. The results confirm and extend our previous original findings on the role of the flavivirus NS2A protein in inhibition of a host antiviral response and demonstrate that the targeted disabling of a viral mechanism for evading the IFN response can be applied to the development of live attenuated flavivirus vaccine candidates.

Descriptors: interferon alpha biosynthesis, interferon beta biosynthesis, viral nonstructural proteins genetics, amino acid substitution, antibodies, viral blood, cell line, cricetinae, animal disease models, knockout mice, plaque assay, survival analysis, virulence genetics, virus replication, West Nile fever, West Nile virus.

Long, M.T., W. Jeter, J. Hernandez, D.C. Sellon, D. Gosche, K. Gillis, E. Bille, and E.P. Gibbs (2006). **Diagnostic performance of the equine IgM capture ELISA for serodiagnosis of West Nile virus infection.** *Journal of Veterinary Internal Medicine* 20(3): 608-613.

Abstract: The objectives of these studies were to assess the diagnostic performance (sensitivity and specificity) of the IgM capture enzyme-linked immunosorbent assay (ELISA; MAC) for diagnosis of West Nile (WN) virus in horses and to examine the performance of this test by using different criteria for seropositivity. A total of 36 horses classified as WN virus infected (group 1) and 383 horses from 4 subpopulations of hoses classified as noninfected (groups 2, 3, 4, and 5) were used in the study. The sensitivity (proportion of infected horses that tested positive for WN virus IgM antibodies) and specificity (proportion of noninfected horses that tested negative) were calculated at different cutoff points by using receiver operating curve (ROC) analysis. Using a selected cutoff point = 2.0, the sensitivity and specificity of the MAC were 91.7 and 99.2%, respectively. The area under the ROC curve = 0.95 (95% confidence interval [CI], 0.89 to 1.0), suggesting that the MAC is a useful tool for diagnosis of recent WN virus exposure in horses. In fulfillment of the 2nd objective, 2 other indices were developed and these indices approached 1.0 for the AUC with smaller 95% CIs. These indices were then used to test 602 additional diagnostic samples submitted from suspect horses between 2002 and 2004. Using the standard cutoff, 194 (32%) of the horses were interpreted as positive. Utilizing newly predicted cutoff criteria from each index, additional horses were identified as positive. In conclusion, the MAC as used for identification of WN virus-diseased horses undergoing recent exposure performs reliably at the standard cutoff for seropositivity. A negative test might not completely rule out WN virus disease, but horses that test negative were most likely not exposed to WNV. Performance of the test can be further improved by investigation of other indexes of seropositivity.

Descriptors: sensitivity and specificity of the IgM capture enzyme-linked immunosorbent assay (ELISA), diagnosis of West Nile virus, horses, seropositivity, predictive value of tests, serologic tests, West Nile fever.

Macdonald, J., J. Tonry, R.A. Hall, B. Williams, G. Palacios, M.S. Ashok, O. Jabado, D. Clark, R.B. Tesh, T. Briese, and W.I. Lipkin (2005). **NS1 protein secretion during the acute phase of West Nile virus infection.** *Journal of Virology* 79(22): 13924-13933. ISSN: 0022-538X.

Abstract: The West Nile virus (WNV) nonstructural protein NS1 is a protein of unknown function that is found within, associated with, and secreted from infected cells. We systematically investigated the kinetics of NS1 secretion in vitro and in vivo to determine the potential use of this protein as a diagnostic marker and to analyze NS1 secretion in relation to the infection cycle. A sensitive antigen capture enzyme-linked immunosorbent assay (ELISA) for detection of WNV NS1 (polyclonal-ACE) was developed, as well as a capture ELISA for the specific detection of NS1 multimers (4G4-ACE). The 4G4-ACE detected native NS1 antigens at high sensitivity, whereas the polyclonal-ACE had a higher specificity for recombinant forms of the protein. Applying these assays we found that only a small fraction of intracellular NS1 is secreted and that secretion of NS1 in tissue culture is delayed compared to the release of virus particles. In experimentally infected hamsters, NS1 was detected in the serum between days 3 and 8 postinfection, peaking on day 5, the day prior to the onset of clinical disease; immunoglobulin M (IgM) antibodies were detected at low levels on day 5 postinfection. Although real-time PCR gave the earliest indication of infection (day 1), the diagnostic performance of the 4G4-ACE was comparable to that of real-time PCR during the time period when NS1 was secreted. Moreover, the 4G4-ACE was found to be superior in performance to both the IgM and plaque assays during this time period, suggesting that NS1 is a viable early diagnostic marker of WNV infection.

Descriptors: viral nonstructural proteins, West Nile fever, West Nile virus, cell line, *Cercopithecus aethiops*, cricetinae, animal disease models, gene expression regulation, Sprague Dawley rats.

Mateo, R., S.Y. Xiao, H. Guzman, H. Lei, A.P. Da Rosa, and R.B. Tesh (2006). **Effects of immunosuppression on West Nile virus infection in hamsters.** *American Journal of Tropical Medicine and Hygiene* 75(2): 356-362. ISSN: 0002-9637.

Abstract: A research study, comparing the pathogenesis of experimental West Nile virus (WNV) infection in immunocompetent and immunosuppressed golden hamsters, is described. Cyclophosphamide was used to immunosuppress the animals. The immunosuppressed hamsters had a prolonged period of viremia, depressed humoral immune response, more extensive and severe pathology, and higher fatality rate than the untreated immunocompetent animals. Histopathological and immunohis-

tochemical studies of tissues from the two groups showed that pathologic changes in the untreated infected animals were confined to the brain and spinal cord, whereas the histopathological changes and WNV antigen distribution in the immunosuppressed animals were much more extensive and diffuse, involving the adrenal, kidney, heart and lung, and brain and spinal cord. Results of this study in the hamster model provide insight into the increased severity of WNV infection observed in immunosuppressed people.
Descriptors: central nervous system, West Nile fever, West Nile virus, antibody formation, viral isolation and purification, *Cercopithecus aethiops*, cyclophosphamide, disease models, animal immunocompetence, immunocompromised host, immunosuppressive agents.

Mattheus, A., L. Coleman, J.-S. Temple, and M.-B. Doughty (2006). **Purification and enzymatic characterization of West Nile virus RNA dependent RNA polymerase**. *FASEB Journal* 20(5, Part 2): A903. ISSN: 0892-6638.
Descriptors: West Nile virus, purification and enzymatic characterization, RNA, polymerase chain reaction, laboratory techniques, gel filtration chromatography.
Notes: Meeting Information: Experimental Biology 2006 Meeting, San Francisco, California, USA; April 01 -05, 2006.

Mcmurtrey, C., W. Bardet, A. Fleshman, and W. Hildebrand (2006). **Direct HLA class I epitope discovery in West Nile virus infection**. *Journal of Immunology* 176(Suppl. S): S211. ISSN: 0022-1767.
Descriptors: West Nile virus infection, epitope discovery, western blot, electrophoretic techniques, immunologic techniques, mass spectrometry, quantitative polymerase chain reaction.
Notes: Meeting Information: Annual Meeting of the American-Association-of-Immunologists, Boston, Massachusettes, USA; May 12 -16, 2006.

Mehlhop, E. and M.S. Diamond (2006). **Protective immune responses against West Nile virus are primed by distinct complement activation pathways.** *Journal of Experimental Medicine* 203(5): 1371-1381.
Abstract: West Nile virus (WNV) causes a severe infection of the central nervous system in several vertebrate animals including humans. Prior studies have shown that complement plays a critical role in controlling WNV infection in complement (C) 3(-/-) and complement receptor 1/2(-/-) mice. Here, we dissect the contributions of the individual complement activation pathways to the protection from WNV disease. Genetic deficiencies in C1q, C4, factor B, or factor D all resulted in increased mortality in mice, suggesting that all activation pathways function together to limit WNV spread. In the absence of alternative pathway complement activation, WNV disseminated into the central nervous system at earlier times and was associated

with reduced CD8+ T cell responses yet near normal anti-WNV antibody profiles. Animals lacking the classical and lectin pathways had deficits in both B and T cell responses to WNV. Finally, and somewhat surprisingly, C1q was required for productive infection in the spleen but not for development of adaptive immune responses after WNV infection. Our results suggest that individual pathways of complement activation control WNV infection by priming adaptive immune responses through distinct mechanisms.

Descriptors: CD8 positive T lymphocytes, West Nile fever, West Nile virus antibodies, B lymphocytes immunology, central nervous system, knockout mice, spleen.

Mehlhop, E., K. Whitby, T. Oliphant, A. Marri, M. Engle, and M.S. Diamond (2005). **Complement activation is required for induction of a protective antibody response against West Nile virus infection.** *Journal of Virology* 79(12): 7466-7477. ISSN: 0022-538X.

Abstract: Infection with West Nile virus (WNV) causes a severe infection of the central nervous system (CNS) with higher levels of morbidity and mortality in the elderly and the immunocompromised. Experiments with mice have begun to define how the innate and adaptive immune responses function to limit infection. Here, we demonstrate that the complement system, a major component of innate immunity, controls WNV infection in vitro primarily in an antibody-dependent manner by neutralizing virus particles in solution and lysing WNV-infected cells. More decisively, mice that genetically lack the third component of complement or complement receptor 1 (CR1) and CR2 developed increased CNS virus burdens and were vulnerable to lethal infection at a low dose of WNV. Both C3-deficient and CR1- and CR2-deficient mice also had significant deficits in their humoral responses after infection with markedly reduced levels of specific anti-WNV immunoglobulin M (IgM) and IgG. Overall, these results suggest that complement controls WNV infection, in part through its ability to induce a protective antibody response.

Descriptors: viral blood antibodies, complement activation, West Nile fever, West Nile virus, complement c3 genetics, cricetinae, mice, neutralization tests, complement genetics, West Nile fever.

Morrey, J.D., V. Siddharthan, A.L. Olsen, G.Y. Roper, H. Wang, T.J. Baldwin, S. Koenig, S. Johnson, J.L. Nordstrom, and M.S. Diamond (2006). **Humanized monoclonal antibody against West Nile virus envelope protein administered after neuronal infection protects against lethal encephalitis in hamsters.** *Journal of Infectious Diseases* 194(9): 1300-1308.

Abstract: Humans infected with West Nile virus (WNV) may clinically present with symptoms that are suggestive of neurological infection. Nearly all treatments of WNV disease have been effective in animal models only if administered before or soon after viral challenge. Here, we evaluated whether a potent neutralizing anti-

WNV humanized monoclonal antibody (MAb), hE16, could improve the course of disease in a hamster model when administered after the virus had infected neurons in the brain. Five days after viral injection, WNV was detected in the brains of hamsters by cytopathic assay, quantitative reverse-transcription polymerase chain reaction, and immunohistochemical staining of WNV envelope in neurons. Notably, 80%-90% of the hamsters treated 5 days after viral injection by intraperitoneal injection with hE16 survived the disease, compared with 37% of the placebo-treated hamsters (P< or =.001). The hamsters that received hE16 directly in the brain also exhibited markedly improved survival rates, compared with those in the placebo-treated hamsters. In prospective experiments, hamsters with high levels of infectious WNV in their cerebrospinal fluid were also protected by hE16 when administered 5 days after viral injection. These experiments suggest that humanized MAbs with potent neutralizing activity are a possible treatment for human patients after WNV has infected neurons in the central nervous system.

Descriptors: administration of monoclonal antibodies, West Nile fever, drug therapy, West Nile virus, antiviral agents, therapeutic use, cricetinae, dose-response relationship.

Murgai, M., M. Mayda, M. Rios, R. Hammamieh, and M. Jett (2006). **Gene expression analysis of the West Nile virus and the Dengue serotypes**. *FASEB Journal* 20(4, Part 1): A62. ISSN: 0892-6638.

Descriptors: West Nile virus, Dengue fever, vector borne diseases, meningitis, encephalitis, microarray gene expression analysis, genetic technique, cDNA microarrays.

Notes: Meeting Information: Experimental Biology 2006 Meeting, San Francisco, California, USA; April 01 -05, 2006.

Nemeth, N., D. Hahn, D. Gould, and R. Bowen (2006). **Experimental West Nile Virus Infection in Eastern Screech Owls (*Megascops asio*).** *Avian Diseases.* 50(2): 252-258. ISSN: 0005-2086.

Abstract: Eastern Screech Owls (EASOs) were experimentally infected with the pathogenic New York 1999 strain of West Nile virus (WNV) by subcutaneous injection or per os. Two of nine subcutaneously inoculated birds died or were euthanatized on 8 or 9 days postinfection (DPI) after <24 hr of lethargy and recumbency. All subcutaneously inoculated birds developed levels of viremia that are likely infectious to mosquitoes, with peak viremia levels ranging from 105.0 to 109.6 plaque-forming units/ml. Despite the viremia, the remaining seven birds did not display signs of illness. All birds alive beyond 5 DPI seroconverted, although the morbid birds demonstrated significantly lower antibody titers than the clinically normal birds. Cagemates of infected birds did not become infected. One of five orally exposed EASOs became viremic and seroconverted, whereas WNV infection in the remaining

four birds was not evident. All infected birds shed virus via the oral and cloacal route. Early during infection, WNV targeted skin, spleen, esophagus, and skeletal muscle. The two morbid owls had myocardial and skeletal muscle necrosis and mild encephalitis and nephritis, whereas some of the clinically healthy birds that were sacrificed on 14 DPI had myocardial arteritis and renal phlebitis. WNV is a significant pathogen of EASOs, causing pathologic lesions with varying clinical outcomes.
Descriptors: Strigiformes, wild birds, West Nile virus, pathogenicity, vertebrate viruses, subcutaneous injection, drug delivery systems, viremia, disease transmission, insect vectors, symptoms, seroconversion, morbidity, oral administration, disease course, disease severity, hosts, new host records, pathogen-shedding, Internet-resource.
Language of Text: Summary in Spanish.

Nybakken, G.E., C.A. Nelson, B.R. Chen, M.S. Diamond, and D.H. Fremont (2006). **Crystal structure of the West Nile virus envelope glycoprotein.** *Journal of Virology* 80(23): 11467-11474. ISSN: 0022-538X.
Abstract: The envelope glycoprotein (E) of West Nile virus (WNV) undergoes a conformational rearrangement triggered by low pH that results in a class II fusion event required for viral entry. Herein we present the 3.0-A crystal structure of the ectodomain of WNV E, which reveals insights into the flavivirus life cycle. We found that WNV E adopts a three-domain architecture that is shared by the E proteins from dengue and tick-borne encephalitis viruses and forms a rod-shaped configuration similar to that observed in immature flavivirus particles. Interestingly, the single N-linked glycosylation site on WNV E is displaced by a novel alpha-helix, which could potentially alter lectin-mediated attachment. The localization of histidines within the hinge regions of E implicates these residues in pH-induced conformational transitions. Most strikingly, the WNV E ectodomain crystallized as a monomer, in contrast to other flavivirus E proteins, which have crystallized as anti-parallel dimers. WNV E assembles in a crystalline lattice of perpendicular molecules, with the fusion loop of one E protein buried in a hydrophobic pocket at the DI-DIII interface of another. Dimeric E proteins pack their fusion loops into analogous pockets at the dimer interface. We speculate that E proteins could pivot around the fusion loop-pocket junction, allowing virion conformational transitions while minimizing fusion loop exposure.
Descriptors: West Nile cirus, crystal structure, envelope glycoprotein, low pH, conformational rearrangement.

Nybakken, G.E., T. Oliphant, S. Johnson, S. Burke, M.S. Diamond, and D.H. Fremont (2005). **Structural basis of West Nile virus neutralization by a therapeutic antibody**. *Nature* 437(7059): 764-768. ISSN: 0028-0836.

Descriptors: West Nile virus neutralization, therapeutic antibody, monoclonal antibody, E16 DIII epitope, immune response.

O'Leary, D.R., S. Kuhn, K.L. Kniss, A.F. Hinckley, S.A. Rasmussen, W.J. Pape, L.K. Kightlinger, B.D. Beecham, T.K. Miller, D.F. Neitzel, S.R. Michaels, G.L. Campbell, R.S. Lanciotti, and E.B. Hayes (2006). **Birth outcomes following West Nile Virus infection of pregnant women in the United States: 2003-2004.** *Pediatrics* 117(3): E537-E545.

Abstract: BACKGROUND: Congenital West Nile virus (WNV) infection was first described in a single case in 2002. The proportion of maternal WNV infections resulting in congenital infection and clinical consequences of such infections are unknown. METHODS: In 2003 and 2004, women in the United States who acquired WNV infection during pregnancy were reported to the Centers for Disease Control and Prevention by state health departments. Data on pregnancy outcomes were collected. One of the maternal WNV infections was identified retrospectively after the infant was born. Maternal sera, placenta, umbilical cord tissue, and cord serum were tested for WNV infection by using serologic assays and reverse-transcription polymerase chain reaction. Infant health was assessed at delivery and through 12 months of age. RESULTS: Seventy-seven women infected with WNV during pregnancy were clinically followed in 16 states. A total of 71 women delivered 72 live infants; 4 women had miscarriages, and 2 had elective abortions. Of the 72 live infants, 67 were born at term, and 4 were preterm; gestational age was unknown for 1. Of 55 live infants from whom cord serum was available, 54 tested negative for anti-WNV IgM. One infant born with umbilical hernia and skin tags had anti-WNV IgM in cord serum but not in peripheral serum at age 1 month. An infant who had no anti-WNV IgM in cord blood, but whose mother had WNV illness 6 days prepartum, developed WNV meningitis at age 10 days. Another infant, whose mother had acute WNV illness at delivery, was born with a rash and coarctation of the aorta and had anti-WNV IgM in serum at 1 month of age; cord serum was not available. A fourth infant, whose mother had onset of WNV illness 3 weeks prepartum that was not diagnosed until after delivery, had WNV encephalitis and underlying lissencephaly detected at age 17 days and subsequently died; cord serum was not available. The following major malformations were noted among live-born infants: aortic coarctation (n = 1); cleft palate (n = 1); Down syndrome (n = 1); lissencephaly (n = 1); microcephaly (n = 2); and polydactyly (n = 1). One infant had glycogen storage disease type 1. Abnormal growth was noted in 8 infants. CONCLUSIONS: Of 72 infants followed to date in 2003 and 2004, almost all seemed normal, and none had conclusive laboratory evidence of congenital WNV infection. Three infants had WNV infection that could have been congenitally acquired. Seven infants had major malformations, but only 3 of these had defects that could have been caused

by maternal WNV infection based on the timing of the infections and the sensitive developmental period for the specific malformations, and none had any conclusive evidence of WNV etiology. However, the sensitivity and specificity of IgM testing of cord blood to detect congenital WNV infection are currently unknown, and congenital WNV infection among newborns with IgM-negative serology cannot be ruled out. Prospective studies comparing pregnancy outcomes of WNV-infected and -uninfected women are needed to better define the outcomes of WNV infection during pregnancy.
Descriptors: pregnancy complications, West Nile fever, children, vertical disease transmission, fetal blood, immunoglobulin M analysis , human milk, viral RNA analysis, West Nile virus isolation and purification.
Notes: Comment In: Pediatrics. 2006 Mar;117(3):936-9.

Oh, W., M.R. Yang, E.W. Lee, K.M. Park, S. Pyo, J.S. Yang, H.W. Lee, and J. Song (2006). **Jab1 mediates cytoplasmic localization and degradation of West Nile virus capsid protein.** *Journal of Biological Chemistry* 281(40): 30166-30174.
Abstract: The clinical manifestations of West Nile virus (WNV), a member of the Flavivirus family, include febrile illness, sporadic encephalitis, and paralysis. The capsid (Cp) of WNV is thought to participate in these processes by inducing apoptosis through mitochondrial dysfunction and activation of caspase-9 and caspase-3. To further identify the molecular mechanism of the WNV capsid protein (WNVCp), yeast two-hybrid assays were employed using WNV-Cp as bait. Jab1, the fifth subunit of the COP9 signalosome, was subsequently identified as a molecule that interacts with WNVCp. Immunoprecipitation and glutathione S-transferase pulldown assays confirmed that direct interaction could occur between WNVCp and Jab1. Immunofluorescence microscopy demonstrated that the overexpressed WNVCp, which localized to the nucleolus, was translocated to the cytoplasm upon its co-expression with Jab1. When treated with leptomycin B, Jab1-facilitated nuclear exclusion of WNVCp was prevented, which indicated that the CRM1 complex is required for Jab1-facilitated nuclear export of WNVCp. Moreover, Jab1 promoted the degradation of WNVCp in a proteasome-dependent way. Consistent with this, WNVCp-mediated cell cycle arrest at the G(2) phase in H1299 was prevented by exogenous Jab1. Finally, an analysis of WNVCp deletion mutants indicated that the first 15 amino acids were required for interaction with Jab1. Furthermore, the double-point mutant of the WNVCp, P5A/P8A, was incapable of binding to Jab1. These results indicate that Jab1 has a potential protective effect against pathogenic WNVCp and might provide a novel target site for the treatment of disease caused by WNV.
Descriptors: capsid proteins, cytoplasm metabolism, intracellular signaling peptides and proteins, peptide hydrolases, West Nile virus, active transport, cell nucleus genetics, amino acid sequence, cell line, tumor, cell nucleus, enzymology, gene expression

regulation, protein genetics, molecular sequence data, signal transduction genetics, two hybrid system techniques.

Oh, W.K. and J. Song (2006). **Hsp70 functions as a negative regulator of West Nile virus capsid protein through direct interaction.** *Biochemical and Biophysical Research Communications* 347(4): 994-1000. ISSN: 0006-291X.
Abstract: West Nile virus (WNV) is a member of the Flavivirus family and induces febrile illness, sporadic encephalitis, and paralysis. The capsid (Cp) of WNV is thought to play a role in inducing these symptoms through caspase-3- and caspase-9-dependent apoptosis. Using WNVCp as bait for a yeast two-hybrid assay, we identified that Hsp70 interacted with WNVCp. The interaction between Hsp70 and WNVCp was further substantiated using purified proteins. Deletion analysis of Hsp70 indicated that WNVCp could bind to the substrate binding domain of Hsp70. The presence of WNVCp in the Hsp70-dependent folding system inhibited the refolding of beta-galactosidase (beta-gal), which showed that WNVCp might function as a negative regulator of Hsp70. Finally, the cytotoxic effect of WNVCp in 293T cells was prevented by ectopic Hsp70, suggesting a negative regulatory role of Hsp70 on WNVCp. Our findings suggest a possible negative regulatory role of Hps70 in the pathway of WNV infection.
Descriptors: capsid protein metabolism, Hsp70 heat shock proteins, West Nile virus, apoptosis, tumor cell line, protein binding, two hybrid system techniques.

Oliphant, T., M. Engle, G.E. Nybakken, C. Doane, S. Johnson, L. Huang, S. Gorlatov, E. Mehlhop, A. Marri, K.M. Chung, G.D. Ebel, L.D. Kramer, D.H. Fremont, and M.S. Diamond (2005). **Development of a humanized monoclonal antibody with therapeutic potential against West Nile virus.** *Nature Medicine* 11(5): 522-530.
Abstract: Neutralization of West Nile virus (WNV) in vivo correlates with the development of an antibody response against the viral envelope (E) protein. Using random mutagenesis and yeast surface display, we defined individual contact residues of 14 newly generated monoclonal antibodies against domain III of the WNV E protein. Monoclonal antibodies that strongly neutralized WNV localized to a surface patch on the lateral face of domain III. Convalescent antibodies from individuals who had recovered from WNV infection also detected this epitope. One monoclonal antibody, E16, neutralized 10 different strains in vitro, and showed therapeutic efficacy in mice, even when administered as a single dose 5 d after infection. A humanized version of E16 was generated that retained antigen specificity, avidity and neutralizing activity. In postexposure therapeutic trials in mice, a single dose of humanized E16 protected mice against WNV-induced mortality, and may therefore be a viable treatment option against WNV infection in humans.
Descriptors: monoclonal antibodies, viral envelope (E) protein, neutralization of West Nile virus in vivo, mice, treatment option.

Oliphant, T., G.E. Nybakken, M. Engle, Q. Xu, C.A. Nelson, S. Sukupolvi Petty, A. Marri, B.E. Lachmi, U. Olshevsky, D.H. Fremont, T.C. Pierson, and M.S. Diamond (2006). **Antibody recognition and neutralization determinants on domains I and II of west Nile Virus envelope protein.** *Journal of Virology* 80(24): 12149-12159. ISSN: 0022-538X.

Abstract: Previous studies have demonstrated that monoclonal antibodies (MAbs) against an epitope on the lateral surface of domain III (DIII) of the West Nile virus (WNV) envelope (E) strongly protect against infection in animals. Herein, we observed significantly less efficient neutralization by 89 MAbs that recognized domain I (DI) or II (DII) of WNV E protein. Moreover, in cells expressing Fc gamma receptors, many of the DI- and DII-specific MAbs enhanced infection over a broad range of concentrations. Using yeast surface display of E protein variants, we identified 25 E protein residues to be critical for recognition by DI- or DII-specific neutralizing MAbs. These residues cluster into six novel and one previously characterized epitope located on the lateral ridge of DI, the linker region between DI and DIII, the hinge interface between DI and DII, and the lateral ridge, central interface, dimer interface, and fusion loop of DII. Approximately 45% of DI-DII-specific MAbs showed reduced binding with mutations in the highly conserved fusion loop in DII: 85% of these (34 of 40) cross-reacted with the distantly related dengue virus (DENV). In contrast, MAbs that bound the other neutralizing epitopes in DI and DII showed no apparent cross-reactivity with DENV E protein. Surprisingly, several of the neutralizing epitopes were located in solvent-inaccessible positions in the context of the available pseudoatomic model of WNV. Nonetheless, DI and DII MAbs protect against WNV infection in mice, albeit with lower efficiency than DIII-specific neutralizing MAbs.

Descriptors: West Nile virus, monoclonal antibodies, epitope, envelope, MAbs, neutralization, gamma receptors, mice.

Olsen, A., V. Siddharthan, and J. Morrey (2006). **Transforming growth factor-beta 1 improves blood-brain barrier properties in mice infected with West Nile virus**. *Antiviral Research* 70(1): A78. ISSN: 0166-3542.

Descriptors: mice, West Nile virus, growth factor beta 1, blood brain barrier, neural coordination, drug therapy.

Notes: Meeting Information: 19th International Conference on Antiviral Research, San Juan, Puerto Rico; May 7 -11, 2006.

Olsen, A.L., D. Chen, and J.D. Morrey (2005). **Presumptive identification of a protein associated with West Nile virus encephalitis in CSF of hamsters**. *Antiviral Research* 65(3): A39. ISSN: 0166-3542.

Descriptors: West Nile virus encephalitis, hamsters, presumptive identification, nervous system disease, proteomics approach, mass spectrophotometer analysis, cere-

brospinal fluid.
Notes: Meeting Information: 18th International Conference on Antiviral Research, Barcelona, Spain; April 11 -14, 2005.

Ong, S.P., B.G. Choo, J.J. Chu, and M.L. Ng (2006). **Expression of vector-based small interfering RNA against West Nile virus effectively inhibits virus replication.** *Antiviral Research* 72(3): 216-223. ISSN: 0166-3542.
Abstract: RNA interference is one of the effective emerging anti-viral strategies to inhibit virus infection in cells. In this study, a small interfering RNA expressing vector (pSilencer-NS5) targeting the NS5 gene of West Nile virus (WNV) was employed to target and destroy WNV transcripts. Real-time PCR revealed drastic reduction in WNV RNA transcripts in pSilencer-NS5-transfected Vero cells. The virus infectious titre was also significantly reduced by 90% as determined by plaque assays. The resulting decrease in virus replication was shown to be specific since both scrambled and nucleotide(s) mismatch siRNA against WNV NS5 gene did not have any effect on WNV productive yields. Furthermore, Western immunoblot analysis on the expression of viral NS5 and envelope (E) proteins showed significant down-regulation on the expression of viral NS5 and envelope (E) proteins in virus-infected cells that were pre-transfected with pSilencer-NS5. These data clearly supported the notion that the expression of vector-based siRNA against WNV NS5 gene is able to exert its silencing effect on WNV-infected cells without inducing cytotoxicity, hence holding promise in therapeutic treatment of this important emerging infectious disease.
Descriptors: West Nile virus, virus replication, small interfering RNA, anti viral strategies, western immunoblot analysis.

Owen, J., F. Moore, N. Panella, E. Edwards, R. Bru, M. Hughes, and N. Komar (2006). **Migrating birds as dispersal vehicles for West Nile virus**. *EcoHealth* 3(2): 79-85. ISSN: 1612-92021612-9210.
Descriptors: West Nile virus, migrating birds, dispersal vehicles, locomotor activity, virus spread.

Paisley, J.E., A.F. Hinckley, D.R. O'Leary, W.C. Kramer, R.S. Lanciotti, G.L. Campbell, and E.B. Hayes (2006). **West Nile virus infection among pregnant women in a northern Colorado community, 2003 to 2004.** *Pediatrics* 117(3): 814-820.
Abstract: OBJECTIVE: Since West Nile virus (WNV) was first detected in New York in 1999, it has spread across North America and become a major public health concern. In 2002, the first documented case of intrauterine WNV infection was reported, involving an infant with severe brain abnormalities. To determine the frequencies of WNV infections during pregnancy and of intrauterine WNV infections, we measured WNV-specific antibodies in cord blood from infant deliveries after a

community-wide epidemic of WNV disease. METHODS: Five hundred sixty-six pregnant women who presented to Poudre Valley Hospital (Fort Collins, CO) for delivery between September 2003 and May 2004 provided demographic and health history data through self-administered questionnaires and hospital admission records. Umbilical cord blood was collected from 549 infants and screened for WNV-specific IgM and IgG antibodies with enzyme-linked immunosorbent assays, with confirmation by plaque-reduction neutralization tests. Newborn growth parameters, Apgar scores, and hearing test results were recorded. RESULTS: Four percent (95% confidence interval: 2.4-5.7%) of cord blood samples tested positive for WNV-specific IgG antibodies. No cord blood samples were positive for WNV-specific IgM antibodies. There were no significant differences between infants of seropositive and seronegative mothers with respect to any of the growth parameters or outcomes measured. CONCLUSIONS: Intrauterine WNV infections seemed to be infrequent. In our study, WNV infection during pregnancy did not seem to affect adversely infant health at birth. Larger prospective studies are necessary to measure more completely the effects of maternal WNV infection on pregnancy and infant health outcomes.
Descriptors: pregnancy complications, infectious epidemiology, West Nile fever, Colorado, fetal blood, immunoglobulin A blood, immunoglobulin G blood, children, seroepidemiologic studies, West Nile virus isolation and purification.

Papin, J.F., R.A. Floyd, and D.P. Dittmer (2005). **Methylene blue photoinactivation abolishes West Nile virus infectivity in vivo.** *Antiviral Research* 68(2): 84-87. ISSN: 0166-3542.
Abstract: The prevalence of West Nile virus (WNV) infections and associated morbidity has accelerated in recent years. Of particular concern is the recent demonstration that this virus can be transmitted by blood products and can cause severe illness and mortality in transfusion recipients. We have evaluated methylene blue (MB)+light as a safe and cost-effective means to inactivate WNV in vitro. This regimen inactivated WNV with an IC50 of 0.10 microM. Up to 10(7)pfu/ml of WNV could be inactivated by MB+light with no residual infectivity. MB+light inactivated three primary WNV isolates from the years 1999, 2002 and 2003 and prevented mortality in a murine model for WNV infection. Since MB is already approved for human use at a dose of 100mg/kg/day, we conjecture that MB+light treatment of blood products for high-risk patients will be efficacious and suitable for use in resource-limited settings.
Descriptors: methylene blue, West Nile virus, blood transfusion, light, inbred BalbC mice, plaque assay, sterilization, West Nile fever.

Paterson, R. (2005). **How West Nile virus crosses the blood-brain barrier.** *Lancet. Neurology* 4(1): 18.
Descriptors: membrane glycoproteins, adverse effects , cell surface receptors biosyn-

thesis, viral load, West Nile fever, West Nile virus isolation and purification, blood brain barrier, toll like receptors.

Pierson, T.C., M.S. Diamond, A.A. Ahmed, L.E. Valentine, C.W. Davis, M.A. Samuel, S.L. Hanna, B.A. Puffer, and R.W. Doms (2005). **An infectious West Nile virus that expresses a GFP reporter gene**. *Virology* 334(1): 28-40. ISSN: 0042-6822.
Descriptors: West Nile virus, GFP reporter gene , cell transfection, plasmid encoding, DNA, viral replication.

Pierson, T.C., M.D. Sanchez, B.A. Puffer, A.A. Ahmed, B.J. Geiss, L.E. Valentine, L.A. Altamura, M.S. Diamond, and R.W. Doms (2006). **A rapid and quantitative assay for measuring antibody-mediated neutralization of West Nile virus infection.** *Virology* 346(1): 53-65. ISSN: 0042-6822.
Abstract: West Nile virus (WNV) is a neurotropic flavivirus within the Japanese encephalitis antigenic complex that is responsible for causing West Nile encephalitis in humans. The surface of WNV virions is covered by a highly ordered icosahedral array of envelope proteins that is responsible for mediating attachment and fusion with target cells. These envelope proteins are also primary targets for the generation of neutralizing antibodies in vivo. In this study, we describe a novel approach for measuring antibody-mediated neutralization of WNV infection using virus-like particles that measure infection as a function of reporter gene expression. These reporter virus particles (RVPs) are produced by complementation of a sub-genomic replicon with WNV structural proteins provided in trans using conventional DNA expression vectors. The precision and accuracy of this approach stem from an ability to measure the outcome of the interaction between antibody and viral antigens under conditions that satisfy the assumptions of the law of mass action as applied to virus neutralization. In addition to its quantitative strengths, this approach allows the production of WNV RVPs bearing the prM-E proteins of different WNV strains and mutants, offering considerable flexibility for the study of the humoral immune response to WNV in vitro. WNV RVPs are capable of only a single round of infection, can be used under BSL-2 conditions, and offer a rapid and quantitative approach for detecting virus entry and its inhibition by neutralizing antibody.
Descriptors: viral antibodies immunology, West Nile virus, cell line, *Cercopithecus aethiops*, cricetinae, reporter genes, neutralization tests, time factors, viral envelope proteins, West Nile fever immunology.

Pierson, T., M. Diamond, A. Ahmed, L. Valentine, C. Davis, M. Samuel, S. Hanna, B. Puffer , and R. Doms (2005). **An infectious West Nile Virus that expresses a GFP reporter gene.** *Virology* 334(1): 28-40. ISSN: 0042-6822.
Descriptors: West Nile virus, genetic vectors, green fluorescent protein, reporter genes, gene expression regulation, neutralization, virus replication, humans, cultured

cells, nucleotide sequences, viral-antibodies, molecular-sequence-data, Internet-resource.

Prince, H.E., M. Lape Nixon, M.P. Busch, L.H. Tobler, G.A. Foster, and S.L. Stramer (2005). **Utilization of follow-up specimens from viremic blood donors to assess the value of west nile virus immunoglobulin G avidity as an indicator of recent infection.** *Clinical and Diagnostic Laboratory Immunology* 12(9): 1123-1126.
Abstract: The value of West Nile virus immunoglobulin G avidity for distinguishing recent from past infection was investigated using 348 follow-up specimens from 170 viremic blood donors. Low avidity accurately indicated infection within the previous 4 months. However, due to rapid avidity maturation in some individuals, high avidity did not accurately indicate past infection.
Descriptors: blood donors, West Nile fever, West Nile virus antibodies, antibody affinity, follow-up studies, blood immunoglobulin G.

Prince, H.E., L.H. Tobler, M. Lape Nixon, G.A. Foster, S.L. Stramer, and M.P. Busch (2005). **Development and Persistence of West Nile Virus-Specific Immunoglobulin M (IgM), IgA, and IgG in Viremic Blood Donors.** *Journal of Clinical Microbiology.* 43(9): 4316-20. ISSN: 0095-1137.
Abstract: West Nile Virus (WNV) antibody development and persistence were investigated in blood donors who made WNV RNA-positive (viremic) donations in 2003. Plasma samples from the index donations and follow-up serum or plasma samples were tested for WNV immunoglobulin M (IgM), IgA, and IgG by using enzyme-linked immunosorbent assays. Antibody development was investigated with 154 samples collected from 84 donors 1 to 21 days after their RNA-positive, antibody-negative, index donation. WNV IgM and IgA were first detected on day 3, and all samples collected after day 9 were WNV IgM and IgA positive; WNV IgG was first detected on day 4, and all samples collected after day 16 were positive. Antibody persistence in this donor group (index donations antibody negative) was evaluated by using 128 samples collected from 89 donors on days 22 to 440 of follow-up; 88% of samples were WNV IgM positive, 86% were WNV IgA positive, and 100% were WNV IgG positive. In linear regression analysis, trendlines for WNV IgM and IgA reached the value discriminating positive from negative results at 218 days and 232 days of follow-up, respectively. Similar WNV IgM and IgA persistence trends characterized 27 donors whose index samples were positive for WNV IgM and IgA, as well as 14 donors whose index samples were positive for WNV IgG but negative for WNV IgM. These findings show that WNV IgG emerges after WNV IgM and IgA and that both WNV IgM and IgA typically persist for at least 6 months after infection. Thus, unlike some other flavivirus infections, WNV infection is not characterized by a relatively rapid disappearance of virus-specific IgA.

Descriptors: West Nile virus, viremic blood donors, specific immunoglobulin M, IgM, persistence, plasma samples, enzyme linked immunosorbent assays.

Puig Basagoiti, F., T.S. Deas, P. Ren, M. Tilgner, D.M. Ferguson, and P.Y. Shi (2005). **High-throughput assays using a luciferase-expressing replicon, virus-like particles, and full-length virus for West Nile virus drug discovery**. *Antimicrobial Agents and Chemotherapy* 49(12): 4980-4988. ISSN: 0066-4804.
Online: http://aac.asm.org/
Descriptors: West Nile virus, high throughput assays, lucifrase, drug discovery, genetic systems.

Ramanathan, M.P., J.A. Chambers, P. Pankhong, M. Chattergoon, W. Attatippaholkun, K. Dang, N. Shah, and D.B. Weiner (2006). **Host cell killing by the West Nile Virus NS2B-NS3 proteolytic complex: NS3 alone is sufficient to recruit caspase-8-based apoptotic pathway.** *Virology* 345(1): 56-72. ISSN: 0042-6822.
Abstract: The West Nile Virus (WNV) non-structural proteins 2B and 3 (NS2B-NS3) constitute the proteolytic complex that mediates the cleavage and processing of the viral polyprotein. NS3 recruits NS2B and NS5 proteins to direct protease and replication activities. In an effort to investigate the biology of the viral protease, we cloned cDNA encoding the NS2B-NS3 proteolytic complex from brain tissue of a WNV-infected dead crow, collected from the Lower Merion area (Merion strain). Expression of the NS2B-NS3 gene cassette induced apoptosis within 48 h of transfection. Electron microscopic analysis of NS2B-NS3-transfected cells revealed ultra-structural changes that are typical of apoptotic cells including membrane blebbing, nuclear disintegration and cytoplasmic vacuolations. The role of NS3 or NS2B in contributing to host cell apoptosis was examined. NS3 alone triggers the apoptotic pathways involving caspases-8 and -3. Experimental results from the use of caspase-specific inhibitors and caspase-8 siRNA demonstrated that the activation of caspase-8 was essential to initiate apoptotic signaling in NS3-expressing cells. Downstream of caspase-3 activation, we observed nuclear membrane ruptures and cleavage of the DNA-repair enzyme, PARP in NS3-expressing cells. Nuclear herniations due to NS3 expression were absent in the cells treated with a caspase-3 inhibitor. Expression of protease and helicase domains themselves was sufficient to trigger apoptosis generating insight into the apoptotic pathways triggered by NS3 from WNV.
Descriptors: apoptosis, caspases, viral nonstructural proteins, West Nile virus, amino acid sequence, cell line, crow, DNA helicases, enzyme inhibitors, gene silencing, molecular sequence data, peptide hydrolases, protein structure, tertiary genetics, vacuoles, West Nile fever.

Ramanathan, M.P., J.A. Chambers, J. Taylor, B.T. Korber, M.D. Lee, A. Nalca, K. Dang, P. Pankhong, W. Attatippaholkun, and D.B. Weiner (2005). **Expression and evolutionary analysis of West Nile virus (Merion strain).** *Journal of Neurovirology* 11(6): 544-556.

Abstract: The authors report a new strain of West Nile virus (WNV) with the expression analysis of its individual open reading frames. Since its sudden appearance in the summer of 1999 in New York City, the virus has spread rapidly across the continental United States into Canada and Mexico. Besides, its rapid transmission by various vectors, the spread of this virus through organ transplantation, blood transfusion, and mother-child transmission through breast milk is of concern. In order to understand molecular variations of WNV in North America and to generate new tools for understanding WNV biology, a complete clone of WNV has been constructed. Investigations so far have focused only on half of its genes products and a detailed molecular and cell biological aspects on all of WNV gene have yet to be clearly established. The open reading frames of WNV were recovered through an reverse transcriptase-polymerase chain reaction (RT-PCR)-PCR using brain tissue from a dead crow collected in Merion, PA, and cloned into a mammalian expression vector. The deduced amino acid sequences of individual open reading frames were analyzed to determine various structural motifs and functional domains. Expression analysis shows that in neuronal cells, C, NS1, and NS5 proteins are nuclear localized whereas the rest of the antigens are confined to the cytoplasm when they are expressed in the absence of other viral antigens. This is the first report that provides an expression analysis as well as intracellular distribution pattern for all of WNV gene products, cloned from an infected bird. Evolutionary analysis of Merion strain sequences indicates that this strain is distinct phylogenetically from the previously reported WNV strains.

Descriptors: viral gene expression regulation, West Nile fever, West Nile virus, amino acid sequence, bird diseases, birds, molecular sequence data, phylogeny, reverse transcriptase polymerase chain reaction.

Ramanathan, M.P., P. Pankhong, M.A. Kutzler, C.A. Chambers, J.J. Kim, G. Gunasekaran, T.C. Pierson, and D.B. Weiner (2006). **Protein kinase CK2: A potential target in the development of novel therapeutics against West Nile virus**. *Journal of Neurovirology* 12(Suppl. 1): 69. ISSN: 1355-0284.

Descriptors: West Nile virus, protein kinase CK2, potential target in therapeutics developmen.

Notes: Meeting Information: 7th International Symposium on NeuroVirology, Philadelphia, Pennsylvania, USA; May 31 -June 03, 2006.

Ramanathan, M., J. Chambers, P. Pankhong, M. Chattergoon, W. Attatippaholkun, K. Dang, N. Shah, and D. Weiner (2006). **Host cell killing by the West Nile Virus NS2B-NS3 proteolytic complex: NS3 alone is sufficient to recruit caspase-8-based apoptotic pathway.** *Virology* 345(1): 56-72. ISSN: 0042-6822.
Descriptors: West Nile virus, viral proteins, proteolysis, cysteine proteinases, Corvidae, crows, cloning (DNA), complementary DNA, gene expression regulation, apoptosis, amino acid sequences, enzyme activation, nucleotide sequences , viral-nonstructural-proteins, caspase- molecular-sequence-data, Internet-resource.

RASGON, J.L., M. VENKATESAN, C.J. WESTBROOK, and M.C. HAUER (2006). **Polymorphic microsatellite loci from the West Nile virus vector *Culex tarsalis*.** *Molecular Ecology Notes.* 6(3): 680-682. ISSN: 1471-8278.
Abstract: Since its introduction in 1999, West Nile virus (WNV) has spread across North America. *Culex tarsalis* is a highly efficient WNV vector species. Many traits such as virus susceptibility, autogeny and host preference vary geographically and temporally in *C. tarsalis*. *Culex tarsalis* genomic libraries were developed and were highly enriched for microsatellite inserts (42-96%). We identified 12 loci that were polymorphic in wild *C. tarsalis* populations. These microsatellites are the first DNA-based genetic markers developed for *C. tarsalis* and will be useful for investigating population structure and constructing genetic maps in this mosquito.
Descriptors: Internet-resource, West Nile virus, vectors, *C. tarsalis*, genomic libraries, loci, microsatellites, genetic markers.

Rawal, A., P.J. Gavin, and C.D. Sturgis (2006). **Cerebrospinal fluid cytology in seasonal epidemic West Nile virus meningo-encephalitis.** *Diagnostic Cytopathology* 34(2): 127-129.
Abstract: The incidence of West Nile Virus (WNV) infection has progressively increased in North America since the first epidemic in 1999. Formal scholarly documentation of cerebrospinal fluid (CSF) cytology changes in patients with WNV infection is limited. We report our experience with CSF cytospins from a population of consecutive patients with documented CSF WNV-specific IgM. Thirty-two patients (12 male, 20 female) with a median age of 52 yr (range, 19-88) diagnosed with WNV meningo-encephalitis were studied. Symptoms were present for a mean of 5 days (range, 1-14) prior to lumbar puncture. CSF proteins were elevated in 94% of patients (30/32) with a mean value of 79 mg/dl (range, 36-185). CSF glucose was normal to elevated in all cases. All cytomorphologically adequate samples demonstrated a pleocytosis with a mean of 156 cells/mm3 (range, 13-683). Nearly, all (26/28) patients showed increased CSF neutrophils--mean 43% (range, 1-83). Mean lymphocyte and monocyte fractions were 44% (range, 8-85) and 14% (range, 2-27), respectively. Three cases showed 1-4% plasma cells. Mean total leukocyte counts (TLC) (197 cells/mm3) and mean neutrophil fractions (50%) were greater in

patients sampled within the first 3 days of symptoms than in those sampled beyond day 3 (mean TLC, 126 cells/mm3; mean neutrophil fraction, 37%). Relative lymphocyte proportions increased from a mean of 39 to 48% after 3 days of illness. WNV should be considered as a potential etiology of infectious CSF pleocytosis in the North American late summer and early fall seasons. 2006 Wiley-Liss, Inc.
Descriptors: cerebrospinal fluid, meningoencephalitis, West Nile virus, immunoglobulin M metabolism, seasons, West Nile virus.

Rios, M., M.J. Zhang, A. Grinev, K. Srinivasan, S. Daniel, O. Wood, I.K. Hewlett, and A.I. Dayton (2006). **Monocytes-macrophages are a potential target in human infection with West Nile virus through blood transfusion.** *Transfusion* 46(4): 659-667.
Abstract: BACKGROUND: West Nile virus (WNV) transmission by transfusion was documented in 2002. Approximately 80 percent of WNV infections are asymptomatic and 1 percent develop severe neurological illness. In animals, Langerhans-dendritic cells support initial viral replication, followed by replication in lymphoid tissues and dissemination to organs and possibly to the CNS. The cellular tropism of WNV infection after transfusion and the particular human blood cells that sustain viral replication remain largely unknown. Whether primary monocyte-derived macrophages (MDMs) support WNV infection-replication and produce infectious virions, with an in vitro system, was investigated. STUDY DESIGN AND METHODS: Elutriated monocytes (CD33+/CD14+) from suitable blood donors were cultured in the presence of macrophage-colony-stimulating factor, infected with WNV-NY99 at different time points, washed, and cultivated for up to 47 days. Supernatants were tested for WNV replication by TaqMan reverse transcription-polymerase chain reaction (RT-PCR), with primers for the envelope and/or 3'NC regions, and by cDNA-PCR to detect WNV minus-strand RNA and for the presence of functional virions by infectivity assays in Vero cells. RESULTS: RT-PCR TaqMan of supernatants demonstrated productive infection of MDMs. Viral load reached 2 to 5 log above baseline in 3 to 6 days and then declined, with detectable viral replication persisting for up to 47 days. WNV minus-strand RNA was detected in Day 4 cultures, indicating active viral replication. Infected MDM cultures showed no cytopathic changes. Supernatants that were TaqMan-positive for the presence of WNV-infected Vero cells and produced cytopathic effects within 3 to 5 days of culture. CONCLUSION: The susceptibility of monocytes-macrophages to productive infection in vitro is compatible with a potential role in initial WNV replication and propagation after transmission by transfusion.
Descriptors: blood transfusion, macrophages, monocytes, West Nile fever prevention and control, West Nile virus isolation and purification, cultured cells, reverse transcriptase polymerase chain reaction.

Roosendaal, J., E.G. Westaway, A. Khromykh, and J.M. Mackenzie (2006). **Regulated cleavages at the West Nile virus NS4A-2K-NS4B junctions play a major role in rearranging cytoplasmic membranes and Golgi trafficking of the NS4A protein.** *Journal of Virology* 80(9): 4623-4632. ISSN: 0022-538X.

Abstract: A common feature associated with the replication of most RNA viruses is the formation of a unique membrane environment encapsulating the viral replication complex. For their part, flaviviruses are no exception, whereupon infection causes a dramatic rearrangement and induction of unique membrane structures within the cytoplasm of infected cells. These virus-induced membranes, termed paracrystalline arrays, convoluted membranes, and vesicle packets, all appear to have specific functions during replication and are derived from different organelles within the host cell. The aim of this study was to identify which protein(s) specified by the Australian strain of West Nile virus, Kunjin virus (KUNV), are responsible for the dramatic membrane alterations observed during infection. Thus, we have shown using immunolabeling of ultrathin cryosections of transfected cells that expression of the KUNV polyprotein intermediates NS4A-4B and NS2B-3-4A, as well as that of individual NS4A proteins with and without the C-terminal transmembrane domain 2K, resulted in different degrees of rearrangement of cytoplasmic membranes. The formation of the membrane structures characteristic for virus infection required coexpression of an NS4A-NS4B cassette with the viral protease NS2B-3pro which was shown to be essential for the release of the individual NS4A and NS4B proteins. Individual expression of NS4A protein retaining the C-terminal transmembrane domain 2K resulted in the induction of membrane rearrangements most resembling virus-induced structures, while removal of the 2K domain led to a less profound membrane rearrangement but resulted in the redistribution of the NS4A protein to the Golgi apparatus. The results show that cleavage of the KUNV polyprotein NS4A-4B by the viral protease is the key initiation event in the induction of membrane rearrangement and that the NS4A protein intermediate containing the uncleaved C-terminal transmembrane domain plays an essential role in these membrane rearrangements.

Descriptors: cell membrane, golgi apparatus, viral nonstructural proteins, West Nile virus metabolism, cell line, cell membrane ultrastructure, *Cercopithecus aethiops*, genetic vectors, electron microscopy, protein binding, protein transport, replicon genetics.

Rossi, S.L., Q. Zhao, V.K. O' Donnell, and P.W. Mason (2005). **Adaptation of West Nile virus replicons to cells in culture and use of replicon-bearing cells to probe antiviral action**. *Virology* 331(2): 457-470. ISSN: 0042-6822.

Descriptors: West Nile virus, virus replicons, cell culture, hamsters, humans, monkeys, WNV genomes.

Saldanha, J., S. Shead, A. Heath, and M. Drebot (2005). **Collaborative study to evaluate a working reagent for West Nile virus RNA detection by nucleic acid testing**. *Transfusion* 45(1): 97-102. ISSN: 0041-1132.
Online: http://www.blackwellpublishing.com/journal.asp?ref=0041-1132
Descriptors: West Nile virus, RNA detection, nucleic acid testing, assays.

Samuel, M.A. and M.S. Diamond (2005). **Alpha/beta interferon protects against lethal West Nile virus infection by restricting cellular tropism and enhancing neuronal survival.** *Journal of Virology* 79(21): 13350-13361. ISSN: 0022-538X.
Abstract: West Nile virus (WNV) is a mosquito-borne flavivirus that is neurotropic in humans, birds, and other animals. While adaptive immunity plays an important role in preventing WNV spread to the central nervous system (CNS), little is known about how alpha/beta interferon (IFN-alpha/beta) protects against peripheral and CNS infection. In this study, we examine the virulence and tropism of WNV in IFN-alpha/beta receptor-deficient (IFN- alpha/betaR-/-) mice and primary neuronal cultures. IFN-alpha/betaR-/- mice were acutely susceptible to WNV infection through subcutaneous inoculation, with 100% mortality and a mean time to death (MTD) of 4.6 +/- 0.7 and 3.8+/- 0.5 days after infection with 10(0) and 10(2) PFU, respectively. In contrast, congenic wild-type 129Sv/Ev mice infected with 10(2) PFU showed 62% mortality and a MTD of 11.9 +/- 1.9 days. IFN-alpha/betaR-/- mice developed high viral loads by day 3 after infection in nearly all tissues assayed, including many that were not infected in wild-type mice. IFN-alpha/betaR-/- mice also demonstrated altered cellular tropism, with increased infection in macrophages, B cells, and T cells in the spleen. Additionally, treatment of primary wild-type neurons in vitro with IFN-beta either before or after infection increased neuronal survival independent of its effect on WNV replication. Collectively, our data suggest that IFN-alpha/beta controls WNV infection by restricting tropism and viral burden and by preventing death of infected neurons.
Descriptors: interferon alpha analysis, interferon beta analysis, West Nile fever , West Nile virus, antiviral agents, brain immunology, cultured cells, cultured, inbred mice, skeletal muscle, organ specificity, messenger RNA genetics, reverse transcriptase polymerase chain reaction, spinal cord , spleen, virus replication.

Samuel, M.A., K. Whitby, B.C. Keller, A. Marri, W. Barchet, B.R. Williams, R.H. Silverman, M.J. Gale, and M.S. Diamond (2006). **PKR and RNase L contribute to protection against lethal West Nile Virus infection by controlling early viral spread in the periphery and replication in neurons.** *Journal of Virology* 80(14): 7009-7019. ISSN: 0022-538X.
Abstract: West Nile virus (WNV) is a neurotropic, mosquito-borne flavivirus that can cause lethal meningoencephalitis. Type I interferon (IFN) plays a critical role in controlling WNV replication, spread, and tropism. In this study, we begin to

examine the effector mechanisms by which type I IFN inhibits WNV infection. Mice lacking both the interferon-induced, double-stranded-RNA-activated protein kinase (PKR) and the endoribonuclease of the 2',5'-oligoadenylate synthetase-RNase L system (PKR(-/-) x RL(-/-)) were highly susceptible to subcutaneous WNV infection, with a 90% mortality rate compared to the 30% mortality rate observed in congenic wild-type mice. PKR(-/-) x RL(-/-) mice had increased viral loads in their draining lymph nodes, sera, and spleens, which led to early viral entry into the central nervous system (CNS) and higher viral burden in neuronal tissues. Although mice lacking RNase L showed a higher CNS viral burden and an increased mortality, they were less susceptible than the PKR(-/-) x RL(-/-) mice; thus, we also infer an antiviral role for PKR in the control of WNV infection. Notably, a deficiency in both PKR and RNase L resulted in a decreased ability of type I IFN to inhibit WNV in primary macrophages and cortical neurons. In contrast, the peripheral neurons of the superior cervical ganglia of PKR(-/-) x RL(-/-) mice showed no deficiency in the IFN-mediated inhibition of WNV. Our data suggest that PKR and RNase L contribute to IFN-mediated protection in a cell-restricted manner and control WNV infection in peripheral tissues and some neuronal subtypes.
Descriptors: endoribonucleases, meningoencephalitis, neurons, virus replication genetics, West Nile fever, West Nile virus metabolism, cerebellar cortex, endoribonucleases deficiency, type II interferon metabolism, macrophages, meningoencephalitis, knockout mice, organ specificity, superior cervical ganglion.

Sanchez, M.D., T.C. Pierson, D. McAllister, S.L. Hanna, B.A. Puffer, L.E. Valentine, M.M. Murtadha, J.A. Hoxie, and R.W. Doms (2005). **Characterization of neutralizing antibodies to West Nile virus.** *Virology* 336(1): 70-82. ISSN: 0042-6822.
Abstract: We produced nine monoclonal antibodies (MAbs) directed against the West Nile virus E glycoprotein using three different immunization strategies: inactivated virus, naked DNA, and recombinant protein. Most of the MAbs bound to conformation dependent epitopes in domain III of the E protein. Four of the MAbs neutralized WNV infection and bound to the same region of domain III with high affinity. The neutralizing MAbs were obtained from mice immunized with inactivated virus alone or in combination with a DNA plasmid. In contrast, MAbs obtained by immunization with a soluble version of the E glycoprotein did not exhibit neutralizing activity. These non-neutralizing antibodies were cross-reactive with several other flaviviruses, including Saint Louis encephalitis, Japanese encephalitis, Yellow Fever and Powassan viruses. Interestingly, some non-neutralizing MAbs bound with high affinity to domains I or III, indicating that both affinity and the precise epitope recognized by an antibody are important determinants of WNV neutralization.
Descriptors: monoclonal antibodies, viral immunology, West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus, tick borne diseases , enzyme linked

immunosorbent assay, epitope mapping, glycoproteins, mice, tertiary protein structure, yellow fever virus.

Savage, H.M., M. Anderson, E. Gordon, L. McMillen, L. Colton, D. Charnetzky, M. Delorey, S. Aspen, K. Burkhalter, B.J. Biggerstaff, and M. Godsey (2006). **Oviposition activity patterns and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (Diptera: Culicidae).** *Journal of Medical Entomology* 43(6): 1227-1238. ISSN: 0022-2585.
Abstract: Oviposition activity and West Nile virus (family Flaviviridae, genus Flavivirus, WNV) infection rates were assessed for members of the *Culex pipiens* complex from July through December 2002 by using gravid traps placed at four ecologically different sites in the southern portion of the hybrid zone in Shelby County, TN. Molecular assays identified three members of the *Cx. pipiens* complex: *Cx. pipiens pipiens* L., *Cx. p. quinquefasciatus* Say, and *Cx. p. pipiens-Cx. p. quinquefasciatus* hybrids (hybrids). The *Cx. pipiens* complex accounted for 90% of mosquitoes collected in gravid traps. All 285 WNV-positive mosquitoes were Culex mosquitoes, and 277 (97%) were *Cx. pipiens* complex mosquitoes. Infection rates among members of the *Cx. pipiens* complex were not significantly different. Infection rates were significantly higher at two urban sites than at a rural site, and WNV was not detected at a forested site. At urban sites, abundances of members of the *Cx. pipiens* complex corresponded to a simple latitude model of the hybrid zone. *Cx. p. quinquefasciatus* was most abundant (46.4%), followed by hybrids (34.1%) and *Cx. p. pipiens* (19.5%). The relative abundances at a rural site were reversed with *Cx. p. pipiens* (48.4%) being most abundant. This demonstrates that spatial habitat variation may profoundly influence the distribution of members of the *Cx. pipiens* complex within the hybrid zone. Members of the *Cx. pipiens* complex did not display different oviposition patterns. However, oviposition patterns assessed hourly at urban and rural sites were significantly different. At urban sites, oviposition activity of *Cx. pipiens* complex mosquitoes was bimodal with an evening peak associated with sunset and a morning peak associated with sunrise. At the rural site, the evening peak was pronounced and the morning peak weak and similar to nighttime activity.
Descriptors: West Nile virus, Culex mosquitoes, traps, habitat variation, infection rates, oviposition activity, evening peak, morning peak.

Sbrana, E., J.H. Tonry, S.Y. Xiao, A.P. da Rosa, S. Higgs, and R.B. Tesh (2005). **Oral transmission of West Nile virus in a hamster model.** *American Journal of Tropical Medicine and Hygiene* 72(3): 325-329. ISSN: 0002-9637.
Abstract: The results of experiments comparing the pathogenesis of West Nile virus (WNV) following infection by mosquito bite, needle inoculation, and ingestion are reported. Adult hamsters were readily infected by all three routes. The level and

duration of viremia, clinical manifestations, pathology, and antibody response in the hamsters following mosquito infection and needle inoculation were similar; after oral infection, the onset of viremia was delayed and the mortality was lower, but the level and duration of viremia, histopathology, and antibody response were similar to the other routes. The results from this and previously published studies indicate that a wide variety of animal species are susceptible to oral infection with WNV and that orally infected animals develop a viremia and illness similar to that following the bite of infected mosquitoes. Oral infection appears to be an alternative transmission mechanism used by a number of different flaviviruses; its potential role in the natural history of WNV is discussed.

Descriptors: West Nile fever transmission, West Nile virus isolation and purification, viral blood antibodies, *Cercopithecus aethiops*, cricetinae, culex mosquitoes, disease models, animal, liver virology, mesocricetus, vero cells, viremia physiopathology, hamsters.

Scherbik, S.V., J.M. Paranjape, B.M. Stockman, R.H. Silverman, and M.A. Brinton (2006). **RNase L plays a role in the antiviral response to West Nile virus.** *Journal of Virology* 80(6): 2987-2999. ISSN: 0022-538X.

Abstract: Alleles at the Flv locus determine disease outcome after a flavivirus infection in mice. Although comparable numbers of congenic resistant and susceptible mouse embryo fibroblasts (MEFs) are infected by the flavivirus West Nile virus (WNV), resistant MEFs produce approximately 100- to 150-fold lower titers than susceptible ones and flavivirus titers in the brains of resistant and susceptible animals can differ by >10,000-fold. The Flv locus was previously identified as the 2'-5' oligoadenylate synthetase 1b (Oas1b) gene. Oas gene expression is up-regulated by interferon (IFN), and after activation by double-stranded RNA, some mouse synthetases produce 2-5A, which activates latent RNase L to degrade viral and cellular RNAs. To determine whether the lower levels of intracellular flavivirus genomic RNA from resistant mice detected in cells at all times after infection were mediated by RNase L, RNase L activity levels in congenic resistant and susceptible cells were compared. Similar moderate levels of RNase L activation by transfected 2-5A were observed in both types of uninfected cells. After WNV infection, the mRNAs of IFN-beta and three Oas genes were up-regulated to similar levels in both types of cells. However, significant levels of RNase L activity were not detected until 72 h after WNV infection and the patterns of viral RNA cleavage products generated were similar in both types of cells. When RNase L activity was down-regulated in resistant cells via stable expression of a dominant negative RNase L mutant, approximately 5- to 10-times-higher yields of WNV were produced. Similarly, about approximately 5- to 10-times-higher virus yields were produced by susceptible C57BL/6 RNase L-/- cells compared to RNase L+/+ cells that were either left untreated or pretreated

with IFN and/or poly(I) . poly(C). The data indicate that WNV genomic RNA is susceptible to RNase L cleavage and that RNase L plays a role in the cellular antiviral response to flaviviruses. The results suggest that RNase L activation is not a major component of the Oas1b-mediated flavivirus resistance phenotype.
Descriptors: endoribonucleases, West Nile virus, 2',5' oligoadenylate synthetase, adenine nucleotides, cell line, cricetinae, enzyme activation, gene expression regulation, interferons pharmacology, mice, mice congenic, mice, virus replication, West Nile virus.

Scholle, F. and P.W. Mason (2005). **West Nile virus replication interferes with both poly(I:C)-induced interferon gene transcription and response to interferon treatment.** *Virology* 342(1): 77-87. ISSN: 0042-6822.
Abstract: West Nile virus (WNV), the leading cause of viral encephalitis in the United States, is an arthropod-transmitted member of the family Flaviviridae. We have explored the interaction of this positive-strand RNA virus with signaling pathways involved in induction of the host's innate immune response. Phosphorylation of STAT-1 in response to interferon (IFN) treatment and the ability of IFN to establish an antiviral state were reduced in WNV replicon-bearing cell lines. Similarly, the activation of IRF3 and stimulation of IFN-beta transcription in response to the double-stranded RNA (dsRNA) mimetic poly(I:C) were inhibited in replicon-bearing and WNV-infected HeLa cells. In contrast, WNV replicons did not affect IRF3 activation by Sendai virus infection, suggesting that not all IRF3 activating pathways are inhibited by WNV. Taken together, these findings demonstrate that WNV replication in cultured cells interferes with both the response to IFN and synthesis of IFN-beta in response to dsRNA.
Descriptors: interferon beta genetics, West Nile virus, cell nucleus active transport, dimerization, hela cells, natural immunity, interferon regulatory factor 3, viral genetics, signal transduction, transcription, genetic drug effects, virus replication.

Seidah, N.G. (2006). **Unexpected similarity between the cytosolic West Nile virus NS3 and the secretory furin-like serine proteinases.** *Biochemical Journal* 393(Pt 2): E1-E3. ISSN: 0264-6021.
Abstract: Many viral proteins undergo proteolytic processing events that are required for virus infection and virion assembly. In this issue of Biochemical Journal, Strongin and co-workers report that the NS3 protease from West Nile virus unexpectedly cleaves certain substrates at pairs of basic residues, a specificity that resembles that of the furin-like PCs (proprotein convertases). This led to the demonstration that furin/PC inhibitors containing poly(D-arginine) are also potent inhibitors of NS3, and that anthrax toxin protective antigen and myelin basic protein are potential NS3 substrates. Structural modelling based on Dengue virus NS3 provided a possible rationale for the observed cleavage specificity of West Nile virus NS3.

Descriptors: cytosol, furin, viral nonstructural proteins, West Nile virus, RNA helicases antagonists and inhibitors, amino acid sequence, serine endopeptidases, substrate specificity.
Notes: Comment On: Biochem J. 2006 Jan 15;393(Pt 2):503-11.

Seregin, A., R. Nistler, V. Borisevich, G. Yamshchikov, E. Chaporgina, C.W. Kwok, and V. Yamshchikov (2006). **Immunogenicity of West Nile virus infectious DNA and its noninfectious derivatives.** *Virology* 356(1-2): 115-125. ISSN: 0042-6822.
Abstract: The exceptionally high virulence of the West Nile NY99 strain makes its suitability in the development of a live WN vaccine uncertain. The aim of this study is to investigate the immunogenicity of noninfectious virus derivatives carrying pseudolethal mutations, which preclude virion formation without affecting preceding steps of the viral infectious cycle. When administered using DNA immunization, such constructs initiate an infectious cycle but cannot lead to a viremia. While the magnitude of the immune response to a noninfectious replication-competent construct was lower than that of virus or infectious DNA, its overall quality and the protective effect were similar. In contrast, a nonreplicating construct of similar length induced only a marginally detectable immune response in the dose range used. Thus, replication-competent noninfectious constructs derived from infectious DNA may offer an advantageous combination of the safety of noninfectious formulations with the quality of the immune response characteristic of infectious vaccines.
Descriptors: West Nile virus, immunogenicity, DNA, infectious, non infectious, virulence, immune response, NY99 strain.

Sheets, R.L., J. Stein, T.S. Manetz, C. Andrews, R. Bailer, J. Rathmann, and P.L. Gomez (2006). **Toxicological safety evaluation of DNA plasmid vaccines against HIV-1, Ebola, Severe Acute Respiratory Syndrome, or West Nile virus is similar despite differing plasmid backbones or gene-inserts.** *Toxicological Sciences* 91(2): 620-630.
Abstract: The Vaccine Research Center has developed a number of vaccine candidates for different diseases/infectious agents (HIV-1, Severe Acute Respiratory Syndrome virus, West Nile virus, and Ebola virus, plus a plasmid cytokine adjuvant-IL-2/Ig) based on a DNA plasmid vaccine platform. To support the clinical development of each of these vaccine candidates, preclinical studies were performed to screen for potential toxicities (intrinsic and immunotoxicities). All treatment-related toxicities identified in these repeated-dose toxicology studies have been confined primarily to the sites of injection and seem to be the result of both the delivery method (as they are seen in both control and treated animals) and the intended immune response to the vaccine (as they occur with greater frequency and severity in treated animals). Reactogenicity at the site of injection is generally seen to be reversible as the frequency and severity diminished between doses and between the immediate and recovery termination time points. This observation also correlated

with the biodistribution data reported in the companion article (Sheets et al., 2006), in which DNA plasmid vaccine was shown to remain at the site of injection, rather than biodistributing widely, and to clear over time. The results of these safety studies have been submitted to the Food and Drug Administration to support the safety of initiating clinical studies with these and related DNA plasmid vaccines. Thus far, standard repeated-dose toxicology studies have not identified any target organs for toxicity (other than the injection site) for our DNA plasmid vaccines at doses up to 8 mg per immunization, regardless of disease indication (i.e., expressed gene-insert) and despite differences (strengths) in the promoters used to drive this expression. As clinical data accumulate with these products, it will be possible to retrospectively compare the safety profiles of the products in the clinic to the results of the repeated-dose toxicology studies, in order to determine the utility of such toxicology studies for signaling potential immunotoxicities or intrinsic toxicities from DNA vaccines. These data build on the biodistribution studies performed (see companion article, Sheets et al., 2006) to demonstrate the safety and suitability for investigational human use of DNA plasmid vaccine candidates for a variety of infectious disease prevention indications.

Descriptors: DNA vaccines, viral vaccines, acquired immunodeficiency syndrome, ebola like viruses, HIV-1 genetics, hemorrhagic fever, rabbits, severe acute respiratory syndrome, tissue distribution, West Nile fever genetics, West Nile virus.

Sheets, R.L., J. Stein, T.S. Manetz, C. Duffy, M. Nason, C. Andrews, W.P. Kong, G.J. Nabel, and P.L. Gomez (2006). **Biodistribution of DNA plasmid vaccines against HIV-1, Ebola, Severe Acute Respiratory Syndrome, or West Nile virus is similar, without integration, despite differing plasmid backbones or gene inserts.** *Toxicological Sciences* 91(2): 610-619.

Abstract: The Vaccine Research Center has developed a number of vaccine candidates for different diseases/infectious agents (HIV-1, Severe Acute Respiratory Syndrome virus, West Nile virus, and Ebola virus, plus a plasmid cytokine adjuvant-IL-2/Ig) based on a DNA plasmid vaccine platform. To support the clinical development of each of these vaccine candidates, preclinical studies have been performed in mice or rabbits to determine where in the body these plasmid vaccines would biodistribute and how rapidly they would clear. In the course of these studies, it has been observed that regardless of the gene insert (expressing the vaccine immunogen or cytokine adjuvant) and regardless of the promoter used to drive expression of the gene insert in the plasmid backbone, the plasmid vaccines do not biodistribute widely and remain essentially in the site of injection, in the muscle and overlying subcutis. Even though approximately 10(14) molecules are inoculated in the studies in rabbits, by day 8 or 9 (approximately 1 week postinoculation), already all but on the order of 10(4)-10(6) molecules per microgram of DNA extracted from tissue have

been cleared at the injection site. Over the course of 2 months, the plasmid clears from the site of injection with only a small percentage of animals (generally 10-20%) retaining a small number of copies (generally around 100 copies) in the muscle at the injection site. This pattern of biodistribution (confined to the injection site) and clearance (within 2 months) is consistent regardless of differences in the promoter in the plasmid backbone or differences in the gene insert being expressed by the plasmid vaccine. In addition, integration has not been observed with plasmid vaccine candidates inoculated i.m. by Biojector 2000 or by needle and syringe. These data build on the repeated-dose toxicology studies performed (see companion article, Sheets et al., 2006) to demonstrate the safety and suitability for investigational human use of DNA plasmid vaccine candidates for a variety of infectious disease prevention indications.

Descriptors: vaccines, viral vaccines, acquired immunodeficiency syndrome, ebola-like viruses, HIV-1, hemorrhagic fever, inbred strains of mice, plasmids, promoter regions, severe acute respiratory syndrome, DNA administration, West Nile virus.

Shirato, K., H. Miyoshi, H. Kariwa, and I. Takashima (2006). **The kinetics of proinflammatory cytokines in murine peritoneal macrophages infected with envelope protein-glycosylated or non-glycosylated West Nile virus.** *Virus Research* 121(1): 11-16. ISSN: 0168-1702.

Abstract: The envelope (E) protein glycosylation status of the New York strain of West Nile (WN) virus is an important determinant of virus neuroinvasiveness. To elucidate the determinant of the difference between E protein-glycosylated and non-glycosylated WN virus infections, the cytokine expression of murine peritoneal macrophages infected with each virus was examined. Tumor necrosis factor (TNF) alpha and interleukin (IL)-1beta were up-regulated with replication of the E protein-glycosylated virus. Interferon (IFN) beta and IL-6 were up-regulated with the clearance of both viruses. These results suggest that TNFalpha and IL-1beta expression are related to the virulence of E protein-glycosylated WN virus.

Descriptors: interleukin 1 metabolism, peritoneal macrophages, tumor necrosis factor, alpha metabolism, viral envelope proteins, West Nile fever, cultured cells, glycosylation , inbred Balb C mice, up-regulation.

Shiryaev, S.A., B.I. Ratnikov, A.V. Chekanov, S. Sikora, D.V. Rozanov, A. Godzik, J. Wang, J.W. Smith, Z. Huang, I. Lindberg, M.A. Samuel, M.S. Diamond, and A.Y. Strongin (2006). **Cleavage targets and the D-arginine-based inhibitors of the West Nile virus NS3 processing proteinase.** *Biochemical Journal* 393(Pt 2): 503-511. ISSN: 0264-6021.

Abstract: Mosquito-borne WNV (West Nile virus) is an emerging global threat. The NS3 proteinase, which is essential for the proteolytic processing of the viral polyprotein precursor, is a promising drug target. We have isolated and biochemically

characterized the recombinant, highly active NS3 proteinase. We have determined that the NS3 proteinase functions in a manner that is distantly similar to furin in cleaving the peptide and protein substrates. We determined that aprotinin and D-arginine-based 9-12-mer peptides are potent inhibitors of WNV NS3 with K(i) values of 26 nM and 1 nM respectively. Consistent with the essential role of NS3 activity in the life cycle of WNV and with the sensitivity of NS3 activity to the D-arginine-based peptides, we showed that nona-D-Arg-NH2 reduced WNV infection in primary neurons. We have also shown that myelin basic protein, a deficiency of which is linked to neurological abnormalities of the brain, is sensitive to NS3 proteolysis in vitro and therefore this protein represents a convenient test substrate for the studies of NS3. A three-dimensional model of WNV NS3 that we created may provide a structural guidance and a rationale for the subsequent design of fine-tuned inhibitors. Overall, our findings represent a foundation for in-depth mechanistic and structural studies as well as for the design of novel and efficient inhibitors of WNV NS3.

Descriptors: arginine analysis, protease inhibitors, viral nonstructural proteins, West Nile virus, amino acid sequence, cultured cells, furin, mice, molecular sequence data, myelin sheath, inhibitors of WNV NS3 proteinase.

Notes: Comment In: Biochem J. 2006 Jan 15;393(Pt 2):e1-3.

Shrestha, B., M.A. Samuel, and M.S. Diamond (2006). **CD8+ T cells require perforin to clear West Nile virus from infected neurons.** *Journal of Virology* 80(1): 119-129. ISSN: 0022-538X.

Abstract: Injury to neurons after West Nile virus (WNV) infection is believed to occur because of viral and host immune-mediated effects. Previously, we demonstrated that CD8+ T cells are required for the resolution of WNV infection in the central nervous system (CNS). CD8+ T cells can control infection by producing antiviral cytokines (e.g., gamma interferon or tumor necrosis factor alpha) or by triggering death of infected cells through perforin- or Fas ligand-dependent pathways. Here, we directly evaluated the role of perforin in controlling infection of a lineage I New York isolate of WNV in mice. A genetic deficiency of perforin molecules resulted in higher viral burden in the CNS and increased mortality after WNV infection. In the few perforin-deficient mice that survived initial challenge, viral persistence was observed in the CNS for several weeks. CD8+ T cells required perforin to control WNV infection as adoptive transfer of WNV-primed wild-type but not perforin-deficient CD8+ T cells greatly reduced infection in the brain and spinal cord and enhanced survival of CD8-deficient mice. Analogous results were obtained when wild-type or perforin-deficient CD8+ T cells were added to congenic primary cortical neuron cultures. Taken together, our data suggest that despite the risk of immunopathogenesis, CD8+ T cells use a perforin-dependent mechanism to clear WNV from

infected neurons.
Descriptors: West Nile virus, infected neurons, perforin, CD8+T cells, host immune mediated effects, central nervous system, genetic deficiency.

Shrestha, B., T. Wang, M.A. Samuel, K. Whitby, J. Craft, E. Fikrig, and M.S. Diamond (2006). **Gamma interferon plays a crucial early antiviral role in protection against West Nile virus infection.** *Journal of Virology* 80(11): 5338-5348. ISSN: 0022-538X.
Abstract: West Nile virus (WNV) causes a severe central nervous system (CNS) infection in humans, primarily in the elderly and immunocompromised. Prior studies have established an essential protective role of several innate immune response elements, including alpha/beta interferon (IFN-alpha/beta), immunoglobulin M, gammadelta T cells, and complement against WNV infection. In this study, we demonstrate that a lack of IFN-gamma production or signaling results in increased vulnerability to lethal WNV infection by a subcutaneous route in mice, with a rise in mortality from 30% (wild-type mice) to 90% (IFN-gamma(-/-) or IFN-gammaR(-/-) mice) and a decrease in the average survival time. This survival pattern in IFN-gamma(-/-) and IFN-gammaR(-/-) mice correlated with higher viremia and greater viral replication in lymphoid tissues. The increase in peripheral infection led to early CNS seeding since infectious WNV was detected several days earlier in the brains and spinal cords of IFN-gamma(-/-) or IFN-gammaR(-/-) mice. Bone marrow reconstitution experiments showed that gammadelta T cells require IFN-gamma to limit dissemination by WNV. Moreover, treatment of primary dendritic cells with IFN-gamma reduced WNV production by 130-fold. Collectively, our experiments suggest that the dominant protective role of IFN-gamma against WNV is antiviral in nature, occurs in peripheral lymphoid tissues, and prevents viral dissemination to the CNS.
Descriptors: West Nile virus, gamma interferon, infection, antiviral, CNS, mice, alpha-beta interferon, virus replication, dendritic cells.

Siddharthan, V., G.J. Roper, H. Wang, A.L. Olsen, L. Preece, C. Andy, B. Taro, and J.D. Morrey (2006). **Identification of West Nile virus-infected cells in the central nervous system of rodents early in infection: Implications for treatment.** *Antiviral Research* 70(1): A79. ISSN: 0166-3542.
Descriptors: West Nile virus infected cells, central nervous system, rodents, treatment, confocal microscopy, immunofluorescence.
Notes: Meeting Information: 19th International Conference on Antiviral Research, San Juan, Puerto Rico; May 7 -11, 2006.

Siger, L., R. Bowen, K. Karaca, M. Murray, S. Jagannatha, B. Echols, R. Nordgren, and J.M. Minke (2006). **Evaluation of the efficacy provided by a Recombinant Canarypox-Vectored Equine West Nile Virus vaccine against an experimental West Nile Virus**

intrathecal challenge in horses. *Veterinary Therapeutics Research in Applied Veterinary Medicine* 7(3): 249-256.

Abstract: Efficacy of the Recombitek Equine West Nile Virus (WNV) vaccine was evaluated against a WNV intrathecal challenge model that results in WNV-induced clinical disease. Ten vaccinated (twice at days 0 and 35) and 10 control horses were challenged 2 weeks after administration of the second vaccine with a virulent WNV by intrathecal administration. After the challenge, eight of 10 controls developed clinical signs of encephalomyelitis whereas one vaccinate exhibited muscle fasciculation only once. Nine controls and one vaccinate developed a fever. Histopathology revealed mild to moderate nonsuppurative encephalitis in eight controls and one vaccinate. None of the vaccinates and all of the controls developed WNV viremia after challenge. All vaccinated horses developed antibodies to WNV after vaccination. These and results of previous studies demonstrate efficacy of the Recombitek WNV vaccine against WNV-induced clinical disease and natural challenge with WNV-infected mosquitoes.

Descriptors: Recombitek Equine West Nile Virus vaccine, WNV intrathecal challenge model, clinical studies, horses, development of encephalomyelitis, histopathology, antibodies to WNV, mosquito vectors.

Sitati, E.M. and M.S. Diamond (2006). **CD4+ T-cell responses are required for clearance of West Nile virus from the central nervous system.** *Journal of Virology* 80(24): 12060-12069. ISSN: 0022-538X.

Abstract: Although studies have established that innate and adaptive immune responses are important in controlling West Nile virus (WNV) infection, the function of CD4(+) T lymphocytes in modulating viral pathogenesis is less well characterized. Using a mouse model, we examined the role of CD4(+) T cells in coordinating protection against WNV infection. A genetic or acquired deficiency of CD4(+) T cells resulted in a protracted WNV infection in the central nervous system (CNS) that culminated in uniform lethality by 50 days after infection. Mice surviving past day 10 had high-level persistent WNV infection in the CNS compared to wild-type mice, even 45 days following infection. The absence of CD4(+) T-cell help did not affect the kinetics of WNV infection in the spleen and serum, suggesting a role for CD4-independent clearance mechanisms in peripheral tissues. WNV-specific immunoglobulin M (IgM) levels were similar to those of wild-type mice in CD4-deficient mice early during infection but dropped approximately 20-fold at day 15 postinfection, whereas IgG levels in CD4-deficient mice were approximately 100- to 1,000-fold lower than in wild-type mice throughout the course of infection. WNV-specific CD8(+) T-cell activation and trafficking to the CNS were unaffected by the absence of CD4(+) T cells at day 9 postinfection but were markedly compromised at day 15. Our experiments suggest that the dominant protective role of CD4(+) T cells

during primary WNV infection is to provide help for antibody responses and sustain WNV-specific CD8(+) T-cell responses in the CNS that enable viral clearance.
Descriptors: West Nile virus, CNS, clearance, CD4+T cell responses, immune responses, spleen, serum, viral clearance.

Steinman, A., C. Banet Noach, L. Simanov, N. Grinfeld, Z. Aizenberg, O. Levi, D. Lahav, M. Malkinson, S. Perk, and N.Y. Shpigel (2006). **Experimental Infection of Common Garter Snakes (*Thamnophis sirtalis*) with West Nile Virus.** *Vector Borne and Zoonotic Diseases* 6(4): 361-368. ISSN: 1530-3667.
Abstract: The role of various reptilian species in the infectious cycle of several arboviruses is documented, but their role in that of West Nile virus (WNV) is uncertain. Common garter snakes (*Thamnophis sirtalis*) were infected subcutaneously with 10(5) plaque forming units (PFU) WNV-Isr 98, five of nine snakes became viremic, and five exhibited persistent low levels of neutralizing antibodies. Four of the parentally infected snakes died and high titers of virus were found in multiple organ samples. In contrast, orally infected garter snakes did not become viremic, but viral RNA was detected in cloacal swabs. Since oral infection of predator birds by WNV is known, their ingestion of infected snakes may also result in their becoming infected.
Descriptors: reptilian species, West Nile virus, garter snakes, subcutaneous infection, oral infection, viremic, RNA, predator birds.

Styer, L.M., K.A. Bernard, and L.D. Kramer (2006). **Enhanced early West Nile virus infection in young chickens infected by mosquito bite: Effect of viral dose.** *American Journal of Tropical Medicine and Hygiene* 75(2): 337-345. ISSN: 0002-9637.
Abstract: Mosquito transmission of arboviruses potentially affects the course of viral infection in the vertebrate host. Studies were performed to determine if viral infection differed in chickens infected with West Nile virus (WNV) by mosquito bite or needle inoculation. Mosquito-infected chickens exhibited levels of viremia and viral shedding that were up to 1,000 times higher at 6, 12, and 24 hours post-feeding (PF) compared with those inoculated with 10(3) PFU by needle. Follow-up studies were conducted to determine if enhanced early infection was due to a higher viral dose inoculated by mosquitoes. Needle inoculation with successively higher doses of WNV led to higher early viremia and viral shedding; a dose >or= 10(4) PFU by needle was required to attain the high early viremia observed in mosquito-infected chickens. Mosquitoes inoculated WNV at this level as estimated by feeding on a hanging drop of blood (mean: 10(2.5), range: 10(0.7)-10(4.6) PFU). These results indicate that enhanced early infection in mosquito-infected chickens may be explained by higher viral dose delivered by mosquitoes. On the other hand, chickens infected by multiple mosquitoes (N = 3-11) had viremic titers that were 25-50 times higher at 6 and 12 hours PF than in chickens infected by a single mosquito, suggesting that viral dose is not the only factor involved in enhanced early infection. The

likelihood that enhanced early infection in mosquito-infected chickens is due to a higher viral dose inoculated by mosquitoes and/or other factors (saliva, inoculation location, or viral source) is discussed.
Descriptors: chickens, culex mosquitos, insect vectors, poultry diseases, West Nile fever, West Nile virus, viral blood, *Cercopithecus aethiops*, cloaca, subcutaneous injections, insect bites and stings, poultry diseases , specific pathogen free organisms, vero cells.

Teehee, M.L., M.L. Bunning, S. Stevens, and R.A. Bowen (2005). **Experimental infection of pigs with West Nile virus.** *Archives of Virology* 150(6): 1249-12. ISSN: 0304-8608.
Abstract: Young adult and weanling pigs were challenged with the New York 99 strain of West Nile virus through the bite of infected mosquitoes. Each of six adult pigs seroconverted, but virus was isolated from serum of only one pig following challenge. Three of five weanling pigs developed viremia, with peak titers of 10(1.9) and 10(3.1) PFU/mL. Clinical signs attributable to West Nile virus infection were not observed in any of these animals. An additional four pigs were challenged by feeding West Nile virus-infected mice, and none of the four developed a detectable viremia or seroconverted. These results suggest that pigs are unlikely to play a significant role as amplifying hosts of West Nile virus.
Descriptors: West Nile fever transmission, West Nile virus, infected mosquitoes, viral blood antibodies, mice, neutralization tests, swine, viremia.

Temple, J.S., L. Coleman, and M.B. Doughty (2005). **Cloning and expression of West Nile virus RNA polymerase**. *Abstracts of Papers American Chemical Society* 230: U512. ISSN: 0065-7727.
Descriptors: West Nile virus, cloning and expression, RNA polymerase, molecular genetics, polymerase chain reaction techniques.
Notes: Meeting Information: 230th National Meeting of the American Chemical Society, Washington, DC, USA; August 28 -September 1, 2005.

Tesh, R.B., M. Siirin, H. Guzman, A.P. Travassos da Rosa, X. Wu, T. Duan, H. Lei, M.R. Nunes, and S.Y. Xiao (2005). **Persistent West Nile virus infection in the golden hamster: studies on its mechanism and possible implications for other flavivirus infections.** *Journal of Infectious Diseases* 192(2): 287-295.
Abstract: Golden hamsters (*Mesocricetus auratus*) experimentally infected with West Nile virus (WNV) developed chronic renal infection and persistent shedding of virus in urine for up to 8 months, despite initial rapid clearance of virus from blood and the timely appearance of high levels of specific neutralizing antibodies. Infectious WNV could be recovered by direct culture of their urine and by cocultivation of kidney tissue for up to 247 days after initial infection. Only moderate histo-

pathologic changes were observed in the kidneys or brain of the chronically infected hamsters, although WNV antigen was readily detected by immunohistochemistry within epithelium, interstitial cells, and macrophages in the distal renal tubules. Comparison of WNV isolates from serial urine samples from individual hamsters over several months indicated that the virus underwent both genetic and phenotypic changes during persistent infection. These findings are similar to previous reports of persistent infection with tickborne encephalitis and Modoc viruses.
Descriptors: flavivirus infections, West Nile fever, viral blood antibodies, antibody formation, animal disease models, enzyme linked immunosorbent assay, kidney, *Mesocricetus auratus*, golden hamster, viremia immunology, West Nile virus.

Throsby, M., C. Geuijen, J. Goudsmit, A.Q. Bakker, J. Korimbocus, R.A. Kramer, M. Clijsters van der Horst, M. de Jong, M. Jongeneelen, S. Thijsse, R. Smit, T.J. Visser, N. Bijl, W.E. Marissen, M. Loeb, D.J. Kelvin, W. Preiser, J. ter Meulen, and J. de Kruif (2006). **Isolation and characterization of human monoclonal antibodies from individuals infected with West Nile Virus.** *Journal of Virology* 80(14): 6982-6992. ISSN: 0022-538X.
Abstract: Monoclonal antibodies (MAbs) neutralizing West Nile Virus (WNV) have been shown to protect against infection in animal models and have been identified as a correlate of protection in WNV vaccine studies. In the present study, antibody repertoires from three convalescent WNV-infected patients were cloned into an scFv phage library, and 138 human MAbs binding to WNV were identified. One hundred twenty-one MAbs specifically bound to the viral envelope (E) protein and four MAbs to the premembrane (prM) protein. Enzyme-linked immunosorbent assay-based competitive-binding assays with representative E protein-specific MAbs demonstrated that 24/51 (47%) bound to domain II while only 4/51 (8%) targeted domain III. In vitro neutralizing activity was demonstrated for 12 MAbs, and two of these, CR4374 and CR4353, protected mice from lethal WNV challenge at 50% protective doses of 12.9 and 357 mug/kg of body weight, respectively. Our data analyzing three infected individuals suggest that the human anti-WNV repertoire after natural infection is dominated by nonneutralizing or weakly neutralizing MAbs binding to domain II of the E protein, while domain III-binding MAbs able to potently neutralize WNV in vitro and in vivo are rare.
Descriptors: monoclonal antibodies immunology, viral immunology, viral envelope proteins, West Nile fever, West Nile virus, monoclonal genetics, viral genetics, antibody specificity, molecular cloning, mice, protein structure.

Tiawsirisup, S., K.B. Platt, R.B. Evans, and W.A. Rowley (2005). **A comparision of West Nile Virus transmission by *Ochlerotatus trivittatus* (COQ.), *Culex pipiens* (L.), and *Aedes albopictus* (Skuse).** *Vector Borne and Zoonotic Diseases* 5(1): 40-47. ISSN: 1530-3667.

Abstract: Transmission of West Nile virus (WNV) by *Ochlerotatus trivittatus, Culex pipiens*, and *Aedes albopictus* were compared 14 days after taking blood meals from viremic chickens with titers ranging from 10(2.5) to 10(9.5) cell infective dose (50)s (CID50s)/mL serum. Transmission occurred in one of four (25%) *Oc. trivittatus* and one of 25 (4%) *Cx. pipiens* that fed on chickens with titers of 10(5.5) CID50s/mL. No transmission occurred among two of 16 (13%) *Oc. trivittatus* or one of 25 (4%) *Cx. pipiens* that became infected after blood meals with titers of 10(5.0) and 10(4.5) CID50s/mL, the next lowest blood meal titers evaluated. Seventeen of 28 (61%) *Ae. albopictus* transmitted WNV after blood meals with titers of 10(7.0) CID50s/mL, but no infection or transmission was observed among 21 *Ae. albopictus* that fed on chickens with titers of 10(5.0) CID50s/mL, the next lowest titer evaluated. Transmission by all three species increased dramatically after blood meals with WNV titers of > or = 10(5.5) CID50s/mL. No significant differences occurred in dissemination and transmission rates of the three species after taking blood meals with titers of > 10(7.0) CID50s/mL. The cumulative mean +/- SE transmission rates of *Oc. trivittatus, Cx. pipiens* , and *Ae. albopictus* after blood meals with titers of > or = 10(7.0) CID50s/mL were 45.5 +/- 4.1%, 46.8 +/- 4.5%, and 72.4 +/- 5.5%. The cumulative mean dissemination rates of the three species were 78.3 +/- 6.7%, 74.8 +/- 2.6%, and 88.6 +/- 2.1%. The rates of transmission by the three species that developed disseminated infections after blood meals with titers of > or = 10(7.0) CID50s/mL were 58.8 +/- 4.4%, 62.6 +/- 5.8%, and 81.6 +/- 5.4%, respectively. In a previous study, we found that susceptibility of the three species to WNV was essentially the same when fed on chickens with WNV titers of > 10(7.0) CID50s/mL, but *Oc. trivittatus* and *Cx. pipiens* were more susceptible than *Ae. albopictus* to WNV at lower virus titers. The current study strongly suggests that *Ae. albopictus* is a more efficient vector than *Oc. trivittatus* and *Cx. pipiens* when fed blood meals with titers of > 10(7.0) CID50s/mL. However, *Oc. trivittatus* and *Cx. pipiens* might be more efficient as vectors when infected by blood meals with titers of < 10(7.0) CID50s/mL.
Descriptors: insect vectors, West Nile fever transmission, West Nile virus isolation and purification, chickens, culex mosquitoes, disease susceptibility, disease vectors, ochlerotatus, species specificity, viral load.

Tiawsirisup, S., K.B. Platt, F. Fabiosa, and W.A. Rowley (2006). **West Nile virus titers in *Aedes vexans* (Meigen) and *Culex pipiens* (L.) saliva**. *Thai Journal of Veterinary Medicine* 36(1): 87. ISSN: 0125-6491.

Descriptors: West Nile virus titers, mosquito saliva, blood meals, virus isolation, titration plaque assay, *Aedes vexans*, *Culex pipiens*, birds.

Notes: Meeting Information: Proceedings of the Annual Conference of the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand; April 27-28 , 2006.

Tiawsirisup, S., K.B. Platt, B.J. Tucker, and W.A. Rowley (2005). **Eastern cottontail rabbits (*Sylvilagus floridanus*) develop West Nile virus viremias sufficient for infecting select mosquito species.** *Vector Borne and Zoonotic Diseases* 5(4): 342-350. ISSN: 1530-3667.

Abstract: The potential of the eastern cottontail rabbit (CTR; *Sylvilagus floridanus*) to contribute to an enzootic West Nile virus (WNV) cycle was demonstrated by characterizing the WNV viremia profile of 15 CTRs and demonstrating that mosquitoes could become infected by feeding on these CTRs. Eight CTRs were infected with a titer of 10(5.0) cell-infectious dose 50% endpoints (CID50s) of WNV (NY99-Crow) by needle and seven CTRs by bite of one or more WNV-infected mosquitoes. There were no marked differences between the WNV viremia profiles of CTRs infected by either method. West Nile virus was detected in serums of all CTRs by 24 h p.i. The daily mean titers of all 15 CTRs on days 1-4 p.i. were 10(4.1+/-0.4), 10(4.7+/-0.3), 10(4.1+/-0.6), and 10(3.7+/-0.6) respectively, declining to 10(1.2+/-0.1) CID50s/ml of serum by day 6 p.i. No virus was detected in the blood of any CTR on day 7 p.i. The average duration of WNV titers of >or=10(4.3) and <10(5.0) CID50s/mL for all CTRs was 2.2 +/- 0.6 and 1.0 +/- 0.1 days, respectively. The minimum estimated infection rates (MEIRs) of *Culex pipiens* (L.) and *Culex salinarius* (Coq.) that fed on CTRs with titers of >or=10(4.3) and >10(5.0) were 11.5 +/- 5.5 and 21 +/- 6.0%, respectively. These rates increased to 20.5 +/- 6.4% and 25.0 +/- 3.0% when CTR serum titers were >10(5.0) CID50s/mL. Neither *Aedes aegypti* (L.) nor *Aedes albopictus* (Skuse) were infected by feeding on CTRs with titers of <10(5.0) CID50s/mL. The MEIRs of these two species were 11.5 +/- 3.5% and 1.5 +/- 0.5% after feeding on CTRs with titers of >10(5.0) CID50s/ml. None of the CTRs infected by mosquito bite or by needle showed any symptoms of WNV disease.

Descriptors: cottontail rabbit, West Nile virus, viremia, Culex mosquitos, infection rates.

Tilley, P.A., J.D. Fox, G.C. Jayaraman, and J.K. Preiksaitis (2006). **Nucleic acid testing for west nile virus RNA in plasma enhances rapid diagnosis of acute infection in symptomatic patients.** *Journal of Infectious Diseases* 193(10): 1361-1364.

Abstract: Although nucleic acid amplification testing (NAAT) for West Nile virus (WNV) is useful in screening blood donors, such methods have not been studied in symptomatic patients. For diagnosis of WNV infection, 1.0 mL of plasma was tested by NAAT, and WNV-specific immunoglobulin M was assayed. Of 276 WNV cases, 191 were tested by both serology and NAAT. Of these, 86 (45.0%), 111 (58.1%), and 180 (94.2%) were detected by NAAT, serology, and combined NAAT and serology, respectively. NAAT-based screening was most useful within 8 days of the onset of symptoms. Viremia is common in early symptomatic WNV infection, and NAAT enhances diagnostic yield.

Descriptors: nucleic acid amplification techniques, viral RNA analysis, West Nile fever, West Nile virus isolation and purification, Alberta, routine diagnostic tests, immunoglobulin M, predictive value of tests, viral blood RNA, reverse transcriptase polymerase chain reaction, specimen handling, viremia diagnosis.

Tobler, L.H., C. Bianco, S.A. Glynn, G.B. Schreiber, B.J. Dille, H.E. Prince, R.S. Lanciotti, J.M. Linnen, J. Gallarda, V. Shyamala, D. Smith, S.H. Kleinman, and M.P. Busch (2005). **Detection of West Nile virus RNA and antibody in frozen plasma components from a voluntary market withdrawal during the 2002 peak epidemic.** *Transfusion* 45(4): 480-486.

Abstract: BACKGROUND: The US West Nile virus (WNV) epidemic in the summer and fall of 2002 included the first documented cases of transfusion-transmitted WNV infection. In December 2002, the FDA supported a voluntary market withdrawal by the blood banking community of frozen blood components collected in WNV high-activity areas. At the time, the prevalence of viremia and serologic markers for WNV in the blood supply was undefined. STUDY DESIGN AND METHODS: In collaboration with America's Blood Centers, 1468 frozen plasma components (of approx. 60,000 frozen units voluntarily withdrawn from the market) were selectively retrieved from the peak epidemic regions and season (June 23, 2002-September 28, 2002). These units were unlinked, subaliquoted, and tested by WNV enzyme immunoassays (EIAs; Focus Technologies and Abbott Laboratories) and nucleic acid amplification tests (NATs; Gen-Probe Inc. and Roche Molecular Systems). RESULTS: Of the 1468 EIA results from Abbott and Focus, 7 were anti-immunoglobulin M (IgM)- and anti-immunoglobulin G (IgG)-reactive by both assays, 8 and 1 were IgM-only-reactive, and 8 and 23 were IgG-only-reactive, respectively. NAT by Gen-Probe and Roche Molecular Systems yielded one RNA-positive, antibody-negative unit containing approximately 440 RNA copies per mL. An additional 10-fold replicate NAT testing by Gen-Probe on 14 of 15 IgM-reactive specimens yielded 2 additional IgM- and IgG-reactive units with low-level viremia (i.e., 7/10 and 2/10 replicates tested reactive). CONCLUSION: The prevalence of acute (RNA-positive) and recent (IgM-seroreactive) WNV infections indicates that transfusion risk in high-risk areas could have been considerable and that voluntary market withdrawal of frozen components likely averted some WNV transfusion transmissions. The existence of very-low-level viremic units raises concerns, because WNV minipool NAT screening will miss such units and individual NAT may not completely correct this situation.

Descriptors: blood banks, plasma virology, West Nile fever epidemiology, West Nile virus isolation and purification, viral blood antibodies, consumer product safety, disease outbreaks, viral analysis, risk factors, seroepidemiologic studies.

Notes: Comment In: Transfusion. 2005 Apr;45(4):460-2.

Tonry, J.H., S.Y. Xiao, M. Siirin, H. Chen, A.P. da Rosa, and R.B. Tesh (2005). **Persistent shedding of West Nile virus in urine of experimentally infected hamsters.** *American Journal of Tropical Medicine and Hygiene* 72(3): 320-324. ISSN: 0002-9637.
Abstract: Adult hamsters that survived experimental West Nile virus (WNV) infection developed persistent viruria. Infectious WNV could be cultured from their urine for up to 52 days. Immunohistochemical examination of kidneys of viruric animals showed foci of WNV antigen in renal tubular epithelial and vascular endothelial cells. These findings are compatible with virus replication and persistent infection of renal epithelial cells. The potential clinical and virologic significance of these findings as well as their possible epidemiologic importance are discussed.
Descriptors: virus shedding, West Nile fever transmission, antigens, viral analysis, cricetinae, disease models, animal, kidney pathology, liver virology, mesocricetus, West Nile virus isolation and purification.

Torrence, P.F., N. Gupta, C. Whitney, and J.D. Morrey (2006). **Evaluation of synthetic oligonucleotides as inhibitors of West Nile virus replication.** *Antiviral Research* 70(2): 60-65.
Abstract: A series of synthetic oligonucleotide phosphorothioate 15-mers were generated against specific sequences in the West Nile virus RNA genome. These antisense oligonucleotides targeted (1) conserved features of the West Nile virus RNA genome that may be expected to lead to inhibition of virus replication since such features play essential roles in the virus lifecycle; (2) G-quartet oligonucleotides with potential facilitated uptake properties and that also targeted conserved sequences among a range of West Nile virus strains. Several formulations with significant in vitro antiviral activity were found. Among the active oligonucleotides were examples that targeted both C-rich RNA sequences of the West Nile RNA genome as well as recognized conserved sequences key to West Nile virus replication. Since the antiviral activity of the latter oligonucleotides diminished upon 2'-O-methyl substitution, it is likely that their activity involves RNase H-catalyzed RNA degradation. One G-rich oligonucleotide that did not target a West Nile virus RNA sequence also was found. These results suggest the potential of antisense strategies for the control of West Nile virus replication if the attendant problem of oligonucleotide delivery can be adequately addressed.
Descriptors: oligonucleotides, antisense genetics, virus replication, West Nile virus, *Cercopithecus aethiops*, vero cells.

Tyler, K.L., J. Pape, R.J. Goody, M. Corkill, and B.K. Kleinschmidt DeMasters (2006). **CSF findings in 250 patients with serologically confirmed West Nile virus meningitis and encephalitis.** *Neurology* 66(3): 361-365.
Abstract: OBJECTIVE: To provide a large, comprehensive evaluation of the CSF findings in patients with serologically confirmed West Nile virus (WNV), CNS

disease, and their correlation with outcome. METHODS: CSF samples from 334 WNV-infected hospitalized patients were analyzed. Information was available and extracted from the medical records of 250 of these patients, and CSF parameters correlated with clinical and epidemiologic features of disease (e.g., patient age, sex, outcome). RESULTS: Patients with meningitis had a mean of 226 cells/mm3, and those with encephalitis had a mean of 227 cells/mm3. Three percent of meningitis patients and 5% of encephalitis patients had fewer than 5 cells/mm3, and approximately 8% of both groups had more than 500 cells/mm3. Patients with meningitis had a mean of 41% neutrophils, and those with encephalitis had 45%. Forty-five percent of meningitis patients and 37% of encephalitis patients had at least 50% neutrophils in their initial CSF specimen. Neither the mean percent neutrophils nor their distribution differed significantly between groups. Forty-seven percent of encephalitis patients and 16% of meningitis patients had CSF protein of 100 mg/dL or greater ($p < 0.01$). Although specific CSF parameters, including nucleated cell count and protein concentration, correlated significantly with outcome, multivariate analysis suggested that their total predictive value was modest. Age was an additional predictor of outcome independent of CSF variables in all patients. CONCLUSIONS: Serologically confirmed West Nile virus meningitis and encephalitis produce similar degrees of CSF pleocytosis and are frequently associated with substantial CSF neutrophilia. Patients with encephalitis have higher CSF protein concentrations and are more likely to have adverse outcomes, including admission to long-term care facilities or even death after their acute illness. CSF findings were only a modest predictor of disease outcome, with patient age adding important independent prognostic information.

Descriptors: encephalitis, cerebrospinal fluid, viral meningitis, West Nile fever, age factors, cell count, leukocytosis, multivariate analysis, osmolar concentration, predictive value of tests, serologic tests, treatment outcome.

Ujjinamatada, R.K., Y.S. Agasimundin, P. Zhang, R.S. Hosmane, R. Schuessler, P. Borowski, K. Kalicharran, and A. Fattom (2005). **A novel imidazole nucleoside containing a diaminodihydro-S-triazine as a substituent: Inhibitory activity against the West Nile virus NTPase/helicase.** *Nucleosides, Nucleotides and Nucleic Acids* 24(10-12): 1775-1788.

Abstract: The attempted synthesis of a ring-expanded guanosine (1) containing the imidazo[4,5-e][1,3]diazepine ring system by condensation of 1-(2'-deoxy-beta-D-erythropentofuranosyl)-4-ethoxycarbonylimidazole-5-carbaldehyde (2) with guanidine resulted in the formation of an unexpected product, 1-(2'-deoxy-beta-D-erythropentofuranosyl)-5-(2, 4-diamino-3, 6-dihydro-1,3, 5-triazin-6-yl) imidazole-4-carboxamide (7). The structure as well as the pathway of formation of 7 was corroborated by isolation of the intermediate, followed by its conversion to the

product. Nucleoside 7 showed promising in vitro anti-helicase activity against the West Nile virus NTPase/helicase with an IC50 of 3-10 microg/mL.
Descriptors: antiviral agents, imidazoles, RNA helicases, viral proteins, West Nile virus, dose-response relationship, guanosine, nucleoside triphosphatases.

Vaidyanathan, R. and T.W. Scott (2006). **Apoptosis in mosquito midgut epithelia associated with West Nile virus infection.** *Apoptosis* 11(9): 1643-1651. ISSN: 1360-8185.
Abstract: The mosquito *Culex pipiens pipiens* is a documented vector of West Nile virus (WNV, Flaviviridae, Flavivirus). Our laboratory colony of *C. p. pipiens*, however, was repeatedly refractory to experimental transmission of WNV. Our goal was to identify if a cellular process was inhibiting virus infection of the midgut. We examined midguts of mosquitoes fed control and WNV-infected blood meals. Three days after feeding, epithelial cells from abdominal midguts of mosquitoes fed on WNV fluoresced under an FITC filter following Acridine Orange staining, indicating apoptosis in this region. Epithelial cells from experimental samples examined by TEM exhibited ultrastructural changes consistent with apoptosis, including shrinkage and detachment from neighbors, heterochromatin condensation, nuclear degranulation, and engulfment of apoptotic bodies by adjacent cells. Virions were present in cytoplasm and within cytoplasmic vacuoles of apoptotic cells. No apoptosis was detected by TEM in control samples. In parallel, we used Vero cell plaque assays to quantify infection after 7 and 10 day extrinsic incubation periods and found that none of the mosquitoes (0/55 and 0/10) which imbibed infective blood were infected. We propose that programmed cell death limits the number of WNV-infected epithelial cells and inhibits disseminated viral infections from the mosquito midgut.
Descriptors: apoptosis, culex mosquitoes, intestinal mucosa, West Nile virus, *Cercopithecus aethiops*, insect vectors, electron transmission microscopy.

Venter, M., T.G. Myers, M.A. Wilson, T.J. Kindt, J.T. Paweska, F.J. Burt, P.A. Leman, and R. Swanepoel (2005). **Gene expression in mice infected with West Nile virus strains of different neurovirulence.** *Virology* 342(1): 119-140. ISSN: 0042-6822.
Abstract: West Nile virus causes febrile illness in humans with a proportion of cases progressing to meningoencephalitis, encephalitis, hepatitis, and death. Isolates of the virus fall into two genetic lineages, with differences in neuroinvasiveness for mice occurring between strains within both lineages. We used DNA microarrays to compare gene expression in mice infected peripherally with seven lineage 1 and 2 strains confirmed to be of either high or low neuroinvasiveness in mice and associated with severe or benign infection in humans and birds. The 4 strains with highest neuroinvasiveness induced increased expression of 47 genes in the brain, 111 genes in the liver, and 70 genes in the spleen, relative to the 3 least neuroinvasive strains. Genes involved in interferon signaling pathways, protein degradation, T-cell recruitment,

MHC class I and II antigen presentation, and apoptosis were identified that may have both pathogenic and protective effects, but increased expression of certain acute proteins, central nervous system specific proteins and proteins associated with T-cell hepatitis, implicate mechanisms related to exalted virulence.
Descriptors: gene expression, West Nile fever genetics, West Nile virus, brain metabolism, gene expression profiling, liver metabolism, mice, oligonucleotide array sequence analysis, reverse transcriptase polymerase chain reaction, spleen metabolism.

Vijayasri, S. and S. Agrawal (2005). **Domain-based homology modeling and mapping of the conformational epitopes of envelope glycoprotein of West Nile virus.** *Journal of Molecular Modeling Online* 11(3): 248-255.
Abstract: Knowledge-based modeling has proved significantly accurate for generating the quality models for proteins whose sequence identity with the structurally known targets is greater than or equal to 40%. On the other hand, models obtained for low sequence identities are not reliable. Hence, a reliable and alternative strategy that uses knowledge of domains in the protein can be used to improve the quality of the model generated by the homology method. Here, we report a method for developing a 3D-model for the envelope glycoprotein (Egp) of west nile virus (WNV), using knowledge of structurally conserved functional domains amongst the target sequence (Egp of WNV) and its homologous templates belonging to the same protein family, flaviviridae. This strategy is found to be highly effective in reducing the root mean square deviation (RMSD) value at the Calpha positions of the target and its experimental homologues. The 3D structure of a protein is a prerequisite for structure-based drug design as well as for identifying the conformational epitopes that are essential for the designing vaccines. The conformational epitopes are mapped from the 3D structure of Egp of WNV modeled using the concept of an antigenic domain. A total of five such epitope regions/sites have been identified. They have been found distributed in the loop regions (surface) of the whole protein model composed of dimerization, central and immunological domains. These sites are proposed as the binding sites for HLA proteins/B-cell receptors. Binding is required to activate the immune response against WNV.
Descriptors: epitope mapping, gene products, 3D models, envelope glycoprotein (Egp), West Nile virus .

Vinayagamoorthy, T., K. Mulatz, M. Drebot, and R. Hodkinson (2005). **Molecular typing of West Nile Virus, Dengue, and St. Louis encephalitis using multiplex sequencing.** *Journal of Molecular Diagnostics* 7(2): 152-159.
Abstract: We report the development of an assay to simultaneously identify three of the clinically important flaviviruses (West Nile Virus, Dengue, and St. Louis encephalitis). This assay is based on the nucleotide sequence variations within a 266-bp region of the non-structural protein 5. Further, based on the nucleotide varia-

tions in the same region of the non-structural protein 5, four of the present Dengue serotypes were identified. To identify some of the subtypes of WNV we have developed a second assay using multiplex sequencing technology. The format of the result of this assay is an electropherogram of two genomic segments of the WNV genome: a 48-nucleotide sequence from the anchored core protein C and a 45-nucleotide sequence coding for the non-structural proteins (proteinase and putative helicase genes).

Descriptors: dengue virus, St. Louis encephalitis virus, viral genome, DNA sequence analysis, West Nile virus, DNA base sequence, viral genetics, molecular sequence data, variation genetics, West Nile virus classification.

Wang, T., Y. Gao, E. Scully, C.T. Davis, J.F. Anderson, T. Welte, M. Ledizet, R. Koski, J.A. Madri, A. Barrett, Z. Yin, J. Craft, and E. Fikrig (2006). **Gamma delta T cells facilitate adaptive immunity against West Nile virus infection in mice.** *Journal of Immunology* 177(3): 1825-1832.

Abstract: West Nile (WN) virus causes fatal meningoencephalitis in laboratory mice, and gammadelta T cells are involved in the protective immune response against viral challenge. We have now examined whether gammadelta T cells contribute to the development of adaptive immune responses that help control WN virus infection. Approximately 15% of TCRdelta(-/-) mice survived primary infection with WN virus compared with 80-85% of the wild-type mice. These mice were more susceptible to secondary challenge with WN virus than the wild-type mice that survived primary challenge with the virus. Depletion of gammadelta T cells in wild-type mice that survived the primary infection, however, does not affect host susceptibility during secondary challenge with WN virus. Furthermore, gammadelta T cells do not influence the development of Ab responses during primary and at the early stages of secondary infection with WN virus. Adoptive transfer of CD8(+) T cells from wild-type mice that survived primary infection with WN virus to naive mice afforded partial protection from lethal infection. In contrast, transfer of CD8(+) T cells from TCRdelta(-/-) mice that survived primary challenge with WN virus failed to alter infection in naive mice. This difference in survival correlated with the numeric and functional reduction of CD8 memory T cells in these mice. These data demonstrate that gammadelta T cells directly link innate and adaptive immunity during WN virus infection.

Descriptors: gamma delta T cells, T lymphocyte subsets, West Nile fever, West Nile virus, adoptive transfer, CD8 positive T lymphocytes, genetic predisposition to disease genetics, cellular genetics, immunoglobulin G biosynthesis, immunoglobulin M biosynthesis, immunologic memory genetics, lymphocyte depletion, knockout inbred c57bl, mice, receptors.

Ward, M.P. (2006). **Spread of equine West Nile virus encephalomyelitis during the 2002 Texas epidemic.** *American Journal of Tropical Medicine and Hygiene* 74(6): 1090-1095. ISSN: 0002-9637.

Abstract: Using reports of clinical West Nile virus (WNV) encephalomyelitis in Texas equids during 2002, the distribution of disease was analyzed using cluster statistics and spatial modeling to develop hypotheses of disease spread during the first year of its detection. Significant ($P < 0.05$) clusters of cases reported early during the outbreak were identified in east, northcentral, and north Texas, and significant ($P < 0.05$) clusters late during the outbreak were detected in central, south, and west Texas. Two counties on the south Texas coast first reported disease significantly ($P < 0.05$) earlier than their 10 nearest neighboring counties. The estimated incidence of disease was greatest in the high plains of north Texas and in northcentral Texas. Higher rates were also estimated in eastern and southern areas of the Gulf Coast. The spatial and temporal distribution observed indicates that the equine WNV epidemic began in two parts of Texas and spread elsewhere throughout the state. The mechanism of introduction and spread remains speculative.

Descriptors: disease outbreaks, horse diseases, West Nile fever, cluster analysis, geography, statistical models, Texas, zoonoses.

Ward, M.P. (2005). **Epidemic West Nile virus encephalomyelitis: A temperature-dependent, spatial model of disease dynamics.** *Preventive Veterinary Medicine* 71(3-4): 253-264.

Abstract: Since first being detected in New York in 1999, West Nile virus (WNV) has spread throughout the United States and more than 20,000 cases of equine WNV encephalomyelitis have been reported. A spatial model of disease occurrence was developed, using data from an outbreak of serologically confirmed disease in an unvaccinated population of horses at 108 locations in northern Indiana between 3 August and 17 October 2002. Daily maximum temperature data were recorded at meteorological stations surrounding the study area. The distribution of the total number of degree-days elapsing between July 4 and the date of diagnosis of each case was best described by a normal distribution (mean=5243 degrees F, S.D.=1047). The days on which the average risk was >25, >50 and >75% were predicted (versus observed) to occur on August 23 (August 9), August 31 (September 2) and September 9 (September 9). The epidemic was predicted to occur 3 days earlier, or 4 days later, than observed if temperatures in the study area were uniformly increased, or decreased, by 5 degrees F, respectively. Maps indicated that WNV encephalomyelitis risk always remained greater in the northwest quadrant of the study area. Since WNV might exist at a hypoendemic level of infection, and occasionally re-emerge as a cause of epidemics in equine populations, by identifying factors that contributed to this epidemic, the potential impact of future epidemics can be reduced. Such studies rely

on a GIS framework, availability of meteorological and possibly remotely sensed data and information on host and landscape factors. An early-warning system for WNV transmission in equine populations could be developed.
Descriptors: animal disease outbreaks, horse diseases, West Nile fever, horses, Indiana, statistical models, space-time clustering, West Nile virus.

Warner, R.D., R.C. Kimbrough, J.L. Alexander, J.J. Rush Pierce, T. Ward, and L.P. Martinelli (2006). **Human west nile virus neuroinvasive disease in Texas, 2003 epidemic: Regional differences.** *Annals of Epidemiology* 16(10): 749-755. ISSN: 1047-2797.
Abstract: PURPOSE: Arboviral diseases, such as West Nile virus (WNV) epizootics, tend to be geographically unique because of the biomes that support the vector(s) and reservoir host(s). Understanding such details aids in preventive efforts. We studied the 2003 epidemic of human West Nile neuroinvasive disease (WNND) in Texas because it initially appeared that incidence was not uniform across regions of the state. METHODS: The epidemic was described by age, sex, and region of residence. These variables were used to compare age-specific incidence, standardized cumulative incidence, and age-adjusted relative risk (RR). We verified case data and used routine software, with population estimates from the US Census Bureau. RESULTS: Regardless of sex, risk increased with age. Males had the greater risk (RR, 1.69); however, males aged 5 to 17 years had the greatest RR. Of the five regions compared, two posed more (RRs, 7.98 and 2.14) and one posed less (RR, 0.40) risk than the remainder of the state. Proportions of Culex vector species differed significantly between regions. CONCLUSIONS: During 2003, the risk for WNND varied considerably across Texas. This suggests that various risks for WNV infection deserve additional research for preventive interventions to be regionally appropriate and effective.
Descriptors: West Nile virus, epizootics, reservoir hosts, preventive efforts, epidemic, variables, risk, Culex vector, Texas.

Wicker, J.A., M.C. Whiteman, D.W. Beasley, C.T. Davis, S. Zhang, B.S. Schneider, S. Higgs, R.M. Kinney, and A.D. Barrett (2006). **A single amino acid substitution in the central portion of the West Nile virus NS4B protein confers a highly attenuated phenotype in mice.** *Virology* 349(2): 245-253. ISSN: 0042-6822.
Abstract: West Nile virus (WNV) NS4B is a small hydrophobic nonstructural protein that is hypothesized to participate both in viral replication and evasion of host innate immune defenses. The protein has four cysteine residues (residues 102, 120, 227, and 237). Since cysteines are often critical for the function of proteins, each of the four cysteine residues found in WNV NS4B was mutated to serine by site-directed mutagenesis. While three of these substitutions had little effect on replication or mouse virulence phenotypes, the C102S mutation was associated with a

temperature-sensitive phenotype at 41 degrees C as well as attenuation of the neuroinvasive and neurovirulence phenotypes in mice.
Descriptors: amino acid substitution, viral nonstructural proteins, West Nile virus, amino acid sequence, *Cercopithecus aethiops*, cysteine genetics, animal disease models, heat, lethal dose (LD) 50, mice, West Nile fever, cysteine residues, C102S mutation.

Wolf, R.F., J.F. Papin, R. Hines Boykin, M. Chavez Suarez, G.L. White, M. Sakalian, and D.P. Dittmer (2006). **Baboon model for West Nile virus infection and vaccine evaluation.** *Virology* 355(1): 44-51. ISSN: 0042-6822.
Abstract: Animal models that closely mimic the human condition are of paramount significance to study pathogenic mechanisms, vaccine and therapy scenarios. This is particularly true for investigations that involve emerging infectious diseases. Non-human primate species represent an alternative to the more intensively investigated rodent animal models and in a number of instances have been shown to represent a more reliable predictor of the human response to infection. West Nile virus (WNV) has emerged as a new pathogen in the Americas. It has a 5% fatality rate, predominantly in the elderly and immune compromised. Typically, infections are cleared by neutralizing antibodies, which suggests that a vaccine would be efficacious. Previously, only macaques had been evaluated as a primate model for WNV vaccine design. The macaques did not develop WNV disease nor express the full complement of IgG subclasses that is found in humans. We therefore explored baboons, which exhibit the similar four IgG subclasses observed in humans as a new model for WNV infection and vaccine evaluation. In this present report, we describe the experimental infection of baboons with WNV and test the efficacy of an inactivated WNV vaccination strategy. All experimentally infected animals developed transient viremia and subsequent neutralizing antibodies. Anti-WNV IgM antibodies peaked at 20 days post-infection. Anti-WNV IgG antibodies appeared later and persisted past 60 days. Prior vaccination with chemically inactivated virus induced neutralizing titers and a fast, high titer IgG recall response, which resulted in lower viremia upon challenge. This report is the first to describe the development of the baboon model for WNV experimental infection and the utility of this model to characterize the immunologic response against WNV and a candidate WNV vaccine.
Descriptors: animal disease models, Papio, West Nile fever, West Nile virus vaccines, immunoglobulin G, immunoglobulin M, neutralization tests, viremia.

Yiannakoulias, N.W., D.P. Schopflocher, and L.W. Svenson (2006). **Modelling geographic variations in West Nile virus.** *Canadian Journal of Public Health* 97(5): 374-378.
Abstract: BACKGROUND: This paper applies a method for modelling the spatial variation of West Nile virus (WNv) in humans using bird, environmental and human testing data. METHODS: We used data collected from 503 Alberta municipalities. In order to manage the effects of residual spatial autocorrelation, we used general-

ized linear mixed models (GLMM) to model the incidence of infection. RESULTS: There were 275 confirmed cases of WNv in the 2003 calendar year in Alberta. Our spatial model indicates that living in the grasslands natural region and levels of human testing are significant positive predictors of WNv; living in an urban area is a significant negative predictor. CONCLUSION: Infected bird data contribute little to our model. The variability of West Nile virus incidence in Alberta may be partly confounded by the variations in the rate of testing in different parts of the province. However, variation in infection is also associated with known environmental risk factors. Our findings are consistent with existing knowledge of WNv in North America.
Descriptors: ecology, linear models, rural health, urban health, West Nile fever, Alberta, birds, seroepidemiologic studies.

Yuan, F., Z. Lou, X. Li, Y.W. Chen, J.I. Bell, Z. Rao, and G.F. Gao (2005). **Refolding, crystallization and preliminary X-ray structural studies of the West Nile virus envelope (E) protein domain III.** *Acta Crystallographica. Section F, Structural Biology and Crystallization Communications* 61(Pt 4): 421-423. ISSN: 1744-3091.
Abstract: Domain III of the West Nile virus envelope protein, the putative receptor-binding domain, is a major virion-surface determinant for virulence. This protein was reported to be intrinsically unstable and has defied previous crystallization attempts. It has now been purified from inclusion bodies by protein refolding and was crystallized using the hanging-drop vapour-diffusion method at 291 K. The crystals belong to space group P222(1), with unit-cell parameters a = 52.6, b = 59.7, c = 95.0 A. A complete data set was collected to 2.8 A at 100 K with Cu Kalpha X-rays from a rotating-anode generator.
Descriptors: viral envelope proteins, West Nile virus, binding sites, crystallization methods, inclusion bodies, viral protein folding, x ray diffraction.

Zhang, J.S., P.H. Zhang, B.Y. Si, H. Yang, and W.C. Cao (2005). **[Comparison and discrimination of the biological characteristics between West Nile virus and Japanese encephalitis virus]**. *Chinese Journal of Experimental and Clinical Virology* 19(4): 340-343.
Abstract: BACKGROUND: To compare the biological characteristics of West Nile virus (WNV) and Japanese encephalitis virus (JEV), including cells sensitivity, pathogenicity, viral morphology, as well as the results of immunological and molecular biological detection. METHODS: Cytopathic effect (CPE) and pathogenicity were observed in C6/36 cells and in suckling mice inoculated intracerebrally with the WNV or JEV, respectively. The sliced tissue samples for electron microscopic examination were prepared for the morphologic observation of the viruses. Serum antibody to WNV or JEV was detected using indirect immunofluorescence assay (IFA), and the viral RNA was analyzed by RT-PCR method. RESULTS: WNV or JEV-caused

CPE was characterized by cell fusion and cell shedding, respectively. There was no significant difference in the pathogenicity to suckling mice between WNV and JEV. The morphologic observation showed that the shape and size of the two virions were similar. WNV and JEV were found to have antigenic cross-reactivity. The viral RNA could be detected from both WNV and JEV samples with universal primer set, but only nucleoside fragments of corresponding virus could be amplified when specific primers were used. CONCLUSION: CPE in C6/36 cell and detection of the viral RNA should be useful in discrimination of WNV and JEV, and simultaneously examining the titers of serum antibodies against WNV and JEV may be helpful to diagnosis of infection with these agents.
Descriptors: West Nile virus, Japanese encephalitis virus, biological characteristics, pathogenicity, morphology, viral RNA, diagnosis.
Language of Text: Chinese.

Zhang, S., L. Li, S.E. Woodson, C.Y. Huang, R.M. Kinney, A.D. Barrett, and D.W. Beasley (2006). **A mutation in the envelope protein fusion loop attenuates mouse neuroinvasiveness of the NY99 strain of West Nile virus.** *Virology* 353(1): 35-40. ISSN: 0042-6822.
Abstract: Substitutions were engineered individually and in combinations at the fusion loop, receptor-binding domain and a stem-helix structure of the envelope protein of a West Nile virus strain, NY99, and their effects on mouse virulence and presentation of epitopes recognized by monoclonal antibodies (MAbs) were assessed. A single substitution within the fusion loop (L107F) attenuated mouse neuroinvasiveness of NY99. No substitutions attenuated NY99 neurovirulence. The L107F mutation also abolished binding of a non-neutralizing MAb, 3D9, whose epitope had not been previously identified. MAb 3D9 was subsequently shown to be broadly cross-reactive with other flaviviruses, consistent with binding near the highly conserved fusion loop.
Descriptors: mutation, viral envelope proteins, West Nile fever, West Nile virus, monoclonal antibodies, mice , neutralization tests, virulence.

Zhou, H., N.J. Singh, and K.S. Kim (2006). **Homology modeling and molecular dynamics study of West Nile virus NS3 protease: A molecular basis for the catalytic activity increased by the NS2B cofactor.** *Proteins* 65(3): 692-701.
Abstract: The West Nile virus (WNV) NS3 serine protease, which plays an important role in assembly of infective virion, is an attractive target for anti-WNV drug development. Cofactors NS2B and NS4A increase the catalytic activity of NS3 in dengue virus and Hepatitis C virus, respectively. Recent studies on the WNV-NS3 characterize the catalytically active form of NS3 by tethering the 40-residue cofactor NS2B. It is suggested that NS2B is essential for the NS3 activity in WNV, while there is no information of the WNV-NS3-related crystal structure. To understand

the role of NS2B/substrate in the NS3 catalytic activity, we built a series of models: WNV-NS3 and WNV-NS3-NS2B and WNV-NS3-NS2B-substrate using homology modeling and molecular modeling techniques. Molecular dynamics (MD) simulations were performed for 2.75 ns on each model, to investigate the structural stabilization and catalytic triad motion of the WNV NS3 protease with and without NS2B/substrate. The simulations show that the NS3 rearrangement occurs upon the NS2B binding, resulting in the stable D75-OD1...H51-NH hydrogen bonding. After the substrate binds to the NS3-NS2B active site, the NS3 protease becomes more stable, and the catalytic triad is formed. These results provide a structural basis for the activation and stabilization of the enzyme by its cofactor and substrate.
Descriptors: West Nile virus, serine protease, infective virion, drug development, dengue virus, Hepatitis C, modeling techniques.

Surveillance Programs

Anon (2006). **West nile virus activity--United States, January 1-November 7, 2006.** *MMWR. Morbidity and Mortality Weekly Report* 55(44): 1204-1205.

Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Standard Time, November 7, 2006. A total of 41 states and the District of Columbia had reported 3,830 cases of human WNV illness to CDC.

Descriptors: West Nile fever, bird diseases, culicidae, horse diseases, rodent diseases, sciuridae, United States, West Nile virus isolation and purification.

Anon (2006). **West Nile virus activity--United States, January 1-October 10, 2006.** *MMWR. Morbidity and Mortality Weekly Report* 55(40): 1097-1098.

Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Daylight Time, October 10, 2006. A total of 41 states and the District of Columbia had reported 3,135 cases of human WNV illness to CDC. A total of 1,717 (55%) cases for which such data were available occurred in males; median age of patients was 50 years (range: 3 months-99 years). Dates of illness onset ranged from January 6 to September 25; a total of 97 cases were fatal.

Descriptors: West Nile fever epidemiology, West Nile virus isolation and purification, bird diseases, culicidae, horse diseases, rodent diseases, sciuridae, United States.

Anon (2006). **West Nile virus activity--United States, January 1-September 12, 2006.** *MMWR. Morbidity and Mortality Weekly Report* 55(36): 996.

Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Daylight Time, September 12, 2006. A total of 36 states and the District of Columbia had reported 1,634 cases of human WNV illness to CDC. A total of 921 (57%) cases for which such data were available occurred in males; median age of patients was 51 years (range: 3 months-95 years). Dates of illness onset ranged from January 6 to September 10; a total of 52 cases were fatal.

Descriptors: West Nile fever, humans, birds, humans, United States, fatality reports.

Anon (2006). **West Nile virus activity--United States, January 1-August 15, 2006.** *Morbidity and Mortality Weekly Report* 55(32): 879-880.

Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Daylight Time, August 15, 2006. A total of 26 states had reported 388 cases of human WNV illness to CDC. A total

of 214 (56%) cases for which such data were available occurred in males; median age of patients was 49 years (range: 2-91 years). Dates of illness onset ranged from January 6 to August 10; a total of 13 cases were fatal. A total of 68 presumptive West Nile viremic blood donors (PVDs) have been reported to ArboNET during 2006. Of these, 20 were reported from Nebraska; 18 were reported from Texas; five were reported from California; four were reported from Utah; three each were reported from Oklahoma and South Dakota; two each were reported from Idaho, Iowa, Kentucky, and Mississippi; and one each was reported from Arizona, Colorado, Minnesota, Nevada, North Dakota, Wisconsin, and Wyoming. Of the 68 PVDs, 10 persons (median age: 43 years [range: 18-59 years]) subsequently had West Nile fever.
Descriptors: West Nile fever epidemiology, humans, bird diseases, culicidae, horse diseases, rodent diseases, United StatesWest Nile virus isolation and purification.

Anon (2006). **Assessing capacity for surveillance, prevention, and control of West Nile virus infection--United States, 1999 and 2004.** *Morbidity and Mortality Weekly Report* 55(6): 150-153.
Abstract: Indigenous human disease caused by West Nile virus (WNV) was first identified in the United States in August 1999 in the greater New York City area. By the end of 2004, human WNV disease had been reported in all states except Washington, Hawaii, and Alaska, and WNV transmission to humans had been documented by five routes: mosquito bites (principally from Culex spp.), blood transfusions, organ transplantation, transplacental transfer, and breastfeeding. During 1999-2005, a total of 19,525 cases of WNV disease in humans and 771 deaths were reported in the United States. In 2000, CDC first published guidelines for WNV surveillance, prevention, and control and created ArboNET, an electronic surveillance and reporting system. Beginning in 1999, WNV surveillance and prevention activities had been initiated in selected states and large cities through the CDC Epidemiology and Laboratory Capacity (ELC) cooperative agreements for emerging infectious diseases and subsequently expanded to all 50 states, six large cities/counties, and Puerto Rico. In 2005, to assess the capacity of state and large-city/county health departments to conduct WNV surveillance, prevention, and control activities, the Council of State and Territorial Epidemiologists (CSTE), with assistance from the Association of Public Health Laboratories (APHL) and CDC, surveyed WNV programs in the 50 states and six large-city/county health departments. This report describes the results of that assessment, which indicated that all participating states and cities had well-developed surveillance and control programs for human, avian, equine, or mosquito WNV.
Descriptors: public health practice, West Nile fever epidemiology, prevention and control, population surveillance, United States, West Nile virus.

Anon (2005). **West Nile virus activity--United States, January 1-December 1, 2005.** *Morbidity and Mortality Weekly Report* 54(49): 1253-1256.
Abstract: West Nile virus (WNV) is the leading cause of arboviral encephalitis in the United States. Originally discovered in Africa in 1937, WNV was first detected in the western hemisphere in 1999 in New York City. Since then it has caused seasonal epidemics of febrile illness and severe neurologic disease. During January 1-December 1, 2005, a total of 2,744 cases of WNV disease in humans were reported in the United States, an increase from 2,359 during the same period in 2004. A total of 1,165 cases were WNV neuroinvasive disease (WNND). WNV infections in humans, birds, mosquitoes, and nonhuman mammals are reported to CDC through ArboNET, an Internet-based arbovirus surveillance system managed by state health departments and CDC. During 2005, WNV transmission to humans or animals expanded into 21 counties that had not previously reported transmission and recurred in 1,196 counties where transmission had been reported in previous years. This report summarizes provisional WNV surveillance data through December 1, 2005, and highlights the need for ongoing surveillance, mosquito control, promotion of personal protection from mosquito bites, and research into additional prevention strategies.
Descriptors: West Nile fever epidemiology, birds, culicidae, dogs, horses, population surveillance, sciuridae, United States, West Nile virus isolation and purification.

Anon (2005). **Update: West Nile virus activity--United States, 2005.** *Morbidity and Mortality Weekly Report* 54(43): 1105-1106.
Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Standard Time, November 1, 2005.
Descriptors: West Nile fever epidemiology, birds, culicidae, dogs, horses, sciuridae, United States, West Nile virus isolation and purification.

Bell, J.A., C.M. Brewer, N.J. Mickelson, G.W. Garman, and J.A. Vaughan (2006). **West Nile virus epizootiology, central Red River Valley, North Dakota and Minnesota, 2002-2005.** *Emerging Infectious Diseases* 12(8): 1245-1247.
Abstract: West Nile virus (WNV) epizootiology was monitored from 2002 through 2005 in the area surrounding Grand Forks, North Dakota. Mosquitoes were tested for infection, and birds were surveyed for antibodies. In 2003, WNV was epidemic; in 2004, cool temperatures precluded WNV amplification; and in 2005, immunity in passerines decreased, but did not preclude, WNV amplification.
Descriptors: bird diseases, West Nile virus, insect vectors, Minnesota, North Dakota, zoonoses.

Beveroth, T.A., M.P. Ward, R.L. Lampman, A.M. Ringia, and R.J. Novak (2006). **Changes in seroprevalence of West Nile virus across Illinois in free-ranging birds from 2001 through 2004.** *American Journal of Tropical Medicine and Hygiene* 74(1): 174-179. ISSN: 0002-9637.

Abstract: Of the 5,236 birds sampled for antibodies to West Nile virus (WNV) in Illinois from 2001 through 2004, 348 (6.6%) birds were seropositive. Our multiple year surveillance identified several avian species that had particularly high percentages of seropositive individuals. The importance of these species in the enzootic and/or epizootic transmission of WNV is discussed relative to their regional abundance and literature on host competency. The species with the highest exposure rates to WNV differed both temporally and regionally. In general, birds that bred or were born in Illinois were more likely to have antibodies than transient birds. There was also a significant difference in the seroprevalence between adults (12.1%) and juveniles (5.5%), indicating that the acquired antibody response from previous years is a critical concern when interpreting seroprevalence rates in wild-caught birds. The most common hosts for St. Louis encephalitis virus were also the most common hosts for WNV, which strongly supports the role of similar vectors for both flaviviruses. Avian species with high WNV seroprevalence rates tended to be those that bred throughout the year, have open cup nests, and live in close proximity to humans.

Descriptors: aves, age, viral disease prevalence relations, viral diseases, West Nile virus, host records, prevalence, annual changes and age relations, Illinois, viral disease prevalence and host records.

Bradford, C.M., M.A. Nascarella, T.H. Burns, J.R. Montford, E.J. Marsland, C.B. Pepper, and S.M. Presley (2005). **First report of West Nile virus in mosquitoes from Lubbock County, Texas.** *Journal of the American Mosquito Control Association* 21(1): 102-105.

Abstract: Since July 2002, ongoing surveillance efforts have been conducted to determine potential vectors of West Nile virus (WNV) and Saint Louis encephalitis virus (SLEV) in the mosquito population occurring in Lubbock County, Texas. Adult mosquitoes collected in Lubbock County during 2002 and 2003 represented 7 genera, with *Culex tarsalis* and *Ochlerotatus sollicitans* being the predominant species collected. Mosquitoes were initially screened for WNV and SLEV by using the VecTest antigen panel assay. Positive VecTest results were confirmed by reverse transcriptase-polymerase chain reaction. West Nile virus-positive pools of mosquitoes were detected in 2002 and 2003, with the majority of the positive pools consisting of *Cx. tarsalis*. None of the mosquito pools tested positive for SLEV.

Descriptors: culicidae, West Nile virus, St. Louis encephalitis virus, ochlerotatus, reverse transcriptase polymerase chain reaction, Texas.

Bunde, J.M., E.J. Heske, N.E. Mateus Pinilla, J.E. Hofmann, and R.J. Novak (2006). **A survey for West Nile virus in bats from Illinois.** *Journal of Wildlife Diseases* 42(2): 455-458.

Abstract: A blocking enzyme-linked immunosorbent assay was used to test 97 serum samples from big brown bats (*Eptesicus fuscus*) captured in six counties in Illinois between May 2002 and February 2004 for West Nile virus (WNV) antibodies. One female big brown bat tested positive for WNV antibodies. Samples of kidney, liver, and heart tissue were collected from 312 bats of seven species that were submitted to the Illinois (USA) Department of Public Health or the Illinois Department of Agriculture diagnostic laboratories between January 2001 and December 2003. Tissue samples were tested for WNV using TaqMan reverse transcriptase polymerase chain reaction and all were negative. Prevalence of WNV antibodies in the bats (1%) was lower than previously reported for other flaviviruses, but similar to the prevalence (2%) of WNV antibodies reported in bats from New Jersey and New York, USA. Additional research is needed to determine potential impact of WNV infections on bats and to determine whether they play a role in the WNV transmission cycle.

Descriptors: viral blood antibodies, big brown bats, West Nile fever, West Nile virus, enzyme linked immunosorbent assay methods, myocardium, reverse transcriptase polymerase chain reaction methods.

Busch, M.P., D.J. Wright, B. Custer, L.H. Tobler, S.L. Stramer, S.H. Kleinman, H.E. Prince, C. Bianco, G. Foster, L.R. Petersen, G. Nemo, and S.A. Glynn (2006). **West Nile virus infections projected from blood donor screening data, United States, 2003.** *Emerging Infectious Diseases* 12(3): 395-402.

Abstract: National blood donor screening for West Nile virus (WNV) RNA using minipool nucleic acid amplification testing (MP-NAT) was implemented in the United States in July 2003. We compiled national NAT yield data and performed WNV immunoglobulin M (IgM) testing in 1 WNV-epidemic region (North Dakota). State-specific MP-NAT yield, antibody seroprevalence, and the average time RNA is detectable by MP-NAT were used to estimate incident infections in 2003. WNV donor screening yielded 944 confirmed viremic donors. MP-NAT yield peaked in August with >0.5% of donations positive for WNV RNA in 4 states. Peak IgM seroprevalence for North Dakota was 5.2% in late September. The average time viremia is detectable by MP-NAT was 6.9 days (95% confidence interval [CI] 3.0-10.7). An estimated 735,000 (95% CI 322,000-1,147,000) infections occurred in 2003, with 256 (95% CI 112-401) infections per neuroinvasive case. In addition to preventing transfusion-transmitted WNV infection, donor screening can serve as a tool to monitor seasonal incidence in the general population.

Descriptors: blood donors, West Nile fever, RNA, viral blood, seasons, sensitivity and specificity, time factors, United State, West Nile virus.

Chadalavada, S. (2005). ***A procedure to identify West Nile virus potential risk areas for seven counties in Nebraska using GIS and remote sensing.*** Dissertation, University of Nebraska. Lincoln:
Descriptors: West Nile Virus, potential risk areas, identify, procedure, GIS, remote sensing, Nebraska.
Notes: Thesis (M.A.).

Chevalier, V., R. Lancelot, A. Diaite, B. Mondet, B. Sall, and X. DE Lamballerie (2006). **Serological assessment of west nile Fever virus activity in the pastoral system of ferlo, senegal.** *Annals of the New York Academy of Sciences* 1081: 216-25.
Abstract: The Ferlo area (north-central Senegal) is characterized by a system of temporary ponds favorable to arboviruses among which West Nile fever (WNF) was already identified. During the rainy season in 2003, a serological study was undertaken on horses to assess the activity of the WNF virus (WNFV) in Barkedji (Ferlo). The observed serological prevalence rate was 78.3% for neutralizing antibodies, with a 95% confidence interval (CI) of [64.0, 92.7]. This prevalence rate significantly increased with age (P = 10(-5)). This study confirmed that WNF was endemic in the Ferlo. The transmission risks depended on the introduction of the WNFV in the ecosystem-probably with migrating birds, on its amplification in hosts and on the vector-population dynamic. Further studies are needed to investigate how the cycle is initiated in Barkedji at the beginning of the rainy season and the impact of climatic variations on the risk of transmission of WNF. A surveillance system should be implemented: (a) to assess the clinical impact of the WNF on human and equine populations, (b) to provide an early detection of virulent strains, and (c) to assess the risk of WNF transmission to disease-free ecosystems via migrating birds.
Descriptors: West Nile virus, serological assessment, virus activity, ponds, horses, antibodies, transmission, migrating birds.

Cooke, W.H.3., K. Grala, and R.C. Wallis (2006). **Avian GIS models signal human risk for West Nile virus in Mississippi.** *International Journal of Health Geographics* 5: 36.
Abstract: BACKGROUND: West Nile virus (WNV) poses a significant health risk for residents of Mississippi. Physicians and state health officials are interested in new and efficient methods for monitoring disease spread and predicting future outbreaks. Geographic Information Systems (GIS) models have the potential to support these efforts. Environmental conditions favorable for mosquito habitat were modeled using GIS to derive WNV risk maps for Mississippi. Variables important to WNV dissemination were selected and classified as static and dynamic. The static variables included road density, stream density, slope, and vegetation. The dynamic variable represented seasonal water budget and was calculated using precipitation and evaporation estimates. Significance tests provided deterministic evidence of variable importance to the models. RESULTS: Several models were developed to estimate

WNV risk including a landscape-base model and seasonal climatic sub-models. P-values from t-tests guided variable importance ranking. Variables were ranked and weights assigned as follows: road density (0.4), stream density (0.3), slope (0.2) and vegetation (0.1). This landscape-base model was modified by climatic conditions to assess the importance of climate to WNV risk. Human case data at the zip code level were used to validate modeling results. All models were summarized by zip codes for interpretation and model validation. For all models, estimated risk was higher for zip codes with at least one human case than for zip codes where no human cases were recorded. Overall median measure of risk by zip code indicated that 67% of human cases occurred in the high-risk category. CONCLUSION: Modeling results indicated that dead bird occurrences are correlated with human WNV risk and can facilitate the assessment of environmental variables that contribute to that risk. Each variable's importance in GIS-based risk predictions was assigned deterministically. Our models indicated non-uniform distribution of risk across the state and showed elevated risk in urban and as well as rural areas. Model limitations include resolution of human data, zip code aggregation issues, and quality/availability of vegetation and stream density layers. Our approach verified that WNV risk can be modeled at the state level and can be modified for risk predictions of other vector-borne diseases in varied ecological regions.

Descriptors: disease outbreaks, environment, geographic information systems, models, theoretical, West Nile fever epidemiology, West Nile virus isolation and purification, birds, mississippi epidemiology, risk factors, West Nile virus.

Corrigan, R.L., C. Waldner, T. Epp, J. Wright, S.M. Whitehead, H. Bangura, E. Young, and H.G. Townsend (2006). **Prediction of human cases of West Nile virus by equine cases, Saskatchewan, Canada, 2003.** *Preventive Veterinary Medicine* 76(3-4): 263-72.

Abstract: In 2003, an outbreak of West Nile virus (WNV) occurred in Saskatchewan, Canada from July to September. One-hundred thirty-three horse cases and 947 human cases were recorded and data were analyzed retrospectively for evidence of clustering to determine if clinical infection in the horse population could be used to estimate human risk of infection with WNV. Kulldorff's scan statistic was used to identify spatial-temporal clusters in both the human and horse cases. In most areas, human clusters were not preceded by horse clusters. In one area, a significant cluster of horse cases preceded human cases by 1 week; however, 1 week does not provide sufficient time for human-health authorities to act and provide advance warning for the public.

Descriptors: disease outbreaks, horse diseases epidemiology, diseases transmission, West Nile fever epidemiology, zoonoses, cluster analysis, horse diseases prevention and control, predictive value of tests, retrospective studies, Saskatchewan epidemiol-

ogy, space time clustering, time factors, West Nile fever, prevention and control, West Nile fever transmission, West Nile virus isolation and purification.

Deegan, C.S., J.E. Burns, M. Huguenin, E.Y. Steinhaus, N.A. Panella, S. Beckett, and N. Komar (2005). **Sentinel pigeon surveillance for West Nile virus by using lard-can traps at differing elevations and canopy cover classes.** *Journal of Medical Entomology* 42(6): 1039-1044.

Abstract: Sentinel pigeons, *Columba livia*, were installed in lard-can traps at heights of 1.5 m and 7.6-9.1 m within differing canopy cover classes in New York City. Adult mosquitoes were collected weekly from July to October 2002, as were serum samples from each pigeon. *Culex pipiens L.* and *Culex restuans Theobald* comprised 97% of mosquitoes collected and were most numerous in canopy-level, forested traps. The West Nile virus (family Flaviviridae, genus Flavivirus, WNV) seroconversion rate was significantly greater for pigeons in canopy-level traps, although seroconversions occurred concurrently with human cases in the city and were of little prognostic value to public health agencies. Our results indicate that sentinel pigeons were most effective for monitoring enzootic transmission of WNV when placed in single-sentinel caging 7.6-9.1 m above ground level.

Descriptors: bird diseases, insect vectors, sentinel surveillance, West Nile fever, New York City, polymerase chain reaction methods, species specificity, trees.

DiMenna, M.A., R.J. Bueno, R.R. Parmenter, D.E. Norris, J.M. Sheyka, J.L. Molina, E.M. LaBeau, E.S. Hatton, and G.E. Glass (2006). **Comparison of mosquito trapping method efficacy for West Nile virus surveillance in New Mexico.** *Journal of the American Mosquito Control Association* 22(2): 246-253.

Abstract: As part of the West Nile virus surveillance program for the state of New Mexico, 13 sites along the Rio Grande River were sampled for mosquitoes during spring and summer 2003. We evaluated 3 different trapping procedures for their effectiveness at capturing selected species of mosquitoes. The 3 methods used were a dry ice-baited Centers for Disease Control and Prevention (CDC) light trap set 1.5 m above the ground (standard method), a CDC light trap suspended within the forest canopy, and a gravid trap set on the ground. Thirteen sites were sampled for 10 1-night periods biweekly from May through September. The relative numbers of captured *Culex tarsalis, Cx. salinarius, Cx. quinquefasciatus*, and *Aedes vexans* as well as the numbers of total recorded captures of all species were compared for each trapping method. Significant differences were observed for each species by location and by trapping method. *Culex tarsalis* was most commonly caught in canopy or standard CDC traps, especially in cottonwood bosque. *Culex salinarius* was found most frequently in association with marshy water, and was most often caught in gravid or standard light traps. *Culex quinquefasciatus* was captured almost exclusively in gravid traps within urban areas. *Aedes vexans* was primarily sampled in standard CDC light

traps and found most frequently in wooded areas near floodplains. With the exception of *Cx. Quinquefasciatus*, no species was collected significantly more frequently in gravid or canopy traps than in the standard CDC light trap. Our findings do not support altering the methods currently used in New Mexico, namely, the use of 1.5-m CDC light traps and gravid traps. An increased use of gravid traps seems to be warranted in monitoring urban vector populations (specifically *Cx. quinquefasciatus* and *Cx. salinarius*) that may be involved in human transmission.
Descriptors: West Nile virus, ecosystem, New Mexico, population surveillance, mosquitoes.

DiMenna, M.A., R.J. Bueno, R.R. Parmenter, D.E. Norris, J.M. Sheyka, J.L. Molina, E.M. LaBeau, E.S. Hatton, and G.E. Glass (2006). **Emergence of West Nile virus in mosquito (Diptera: Culicidae) communities of the New Mexico Rio Grande Valley.** *Journal of Medical Entomology* 43(3): 594-599.
Abstract: The first appearances of West Nile virus (family Flaviviridae, genus Flavivirus, WNV) in New Mexico were reported in late summer to early fall 2002. Several dead birds tested positive for WNV, and 78 equine cases were confirmed. All mosquito pools tested (n = 268) were negative. A statewide surveillance program was launched in May 2003 to study the emergence and spread of this new arbovirus in mosquitoes from the Rio Grande valley. Mosquitoes were trapped at 32 sites along a 750-km stretch of the Rio Grande valley. Sites were trapped for one night either weekly or biweekly, by using CO2-baited CDC light traps and gravid traps. Pools of captured mosquitoes were tested for WNV by reverse transcription-polymerase chain reaction. By mid-July 2003, WNV levels in the mosquito population had reached levels that were detectable by the surveillance program. Positive pools of mosquitoes were found in the Rio Grande valley from mid-July through late September. In total, 75 positive pools were found, from sites throughout the study area. The predominant species infected with WNV in this region were *Culex tarsalis* (Coquillett) in rural areas, and *Culex salinarius* (Coquillett) and *Culex pipiens quinquefasciatus* (Say) in urban areas. There were 202 human cases and 438 equine cases of WNV in New Mexico in 2003, which corresponded well in time with the positive mosquitoes. Our results seemed to be consistent with introduction of WNV in late summer 2002, followed by a period of transmission and amplification cycles between local avian hosts and mosquito vectors.
Descriptors: West Nile virus, horse diseases, insect vectors, New Mexico, seasons, West Nile fever transmission.

Durand, B., G. Dauphin, H. Zeller, J. Labie, I. Schuffenecker, S. Murri, F. Moutou, and S. Zientara (2005). **Serosurvey for West Nile virus in horses in southern France.** *Veterinary Record: Journal of the British Veterinary Association.* 157(22): 711-713. ISSN:

0042-4900.
Descriptors: horses, West Nile virus, seroprevalence, surveillance programs, France.

Dusek, R.J., D. Richardson, K.F. Egstad, and D.M. Heisey (2006). **Evaluating red-cockaded woodpeckers for exposure to West Nile virus and blood parasites**. *Southeastern Naturalist* 5(3): 561-565. ISSN: 1528-7092.
Descriptors: red cockaded woodpeckers, parasitology, West Nile virus exposure.

Eidson, M., K. Schmit, Y. Hagiwara, M. Anand, P.B. Backenson, I. Gotham, and L. Kramer (2005). **Dead crow density and West Nile virus monitoring, New York.** *Emerging Infectious Diseases* 11(9): 1370-1375. ISSN: 1080-6040.
Abstract: New York State used the health commerce system to monitor the number of West Nile virus (WNV) human disease cases and the density of dead crows. In each year from 2001 to 2003 and for the 3 years combined, persons living in New York counties (excluding New York City) with elevated weekly dead crow densities (above a threshold value of 0.1 dead crows per square mile) had higher risk (2.0-8.6 times) for disease caused by WNV within the next 2 weeks than residents of counties reporting fewer dead crows per square mile. This type of index can offer a realtime, relatively inexpensive window into viral activity in time for prevention and control. Changes in reporting, bird populations, and immunity may require that thresholds other than 0.1 be used in later years or in other areas.
Descriptors: corvus, viral diseases, West Nile virus, mortality rate as index for use in human disease monitoring, evaluation , transmission of viruses, mortality, New York.

Elizondo Quiroga, D., C.T. Davis, I. Fernandez Salas, R. Escobar Lopez, D.V. Olmos, L.C.S. Gastalum, M.A. Acosta, A. Elizondo Quiroga, J.I. Gonzalez Rojas, J.F.C. Cordero, H. Guzman, A.T. da Rosa, B.J. Blitvich, A.D. Barrett, B.J. Beaty, and R.B. Tesh (2005). **West Nile virus isolation in human and mosquitoes, Mexico.** *Emerging Infectious Diseases* 11(9): 1449-1452. ISSN: 1080-6040.
Abstract: West Nile virus has been isolated for the first time in Mexico, from a sick person and from mosquitoes (*Culex quinquefasciatus*). Partial sequencing and analysis of the 2 isolates indicate that they are genetically similar to other recent isolates from northern Mexico and the western United States.
Descriptors: *Culex quinquefasciatus*, human hosts, viral diseases, West Nile virus, new host record and pathogen characterization, Mexico, Nuevo Leon, Pesqueria Municipality, Ejido Francisco Villa.

Farfan Ale, J.A., B.J. Blitvich, N.L. Marlenee, M.A. Lorono Pino, F. Puerto Manzano, J.E. Garcia Rejon, E.P. Rosado Paredes, L.F. Flores Flores, A. Ortega Salazar, J. Chavez Medina, J.C. Cremieux Grimaldi, F. Correa Morales, G. Hernandez Gaona, J.F. Mendez Galvan, and B.J. Beaty (2006). **Antibodies to West Nile virus**

in asymptomatic mammals, birds, and reptiles in the Yucatan Peninsula of Mexico. *American Journal of Tropical Medicine and Hygiene* 74(5): 908-914. ISSN: 0002-9637.

Abstract: Surveillance for evidence of West Nile virus (WNV) infection in taxonomically diverse vertebrates was conducted in the Yucatan Peninsula of Mexico in 2003 and 2004. Sera from 144 horses on Cozumel Island, Quintana Roo State, 415 vertebrates (257 birds, 52 mammals, and 106 reptiles) belonging to 61 species from the Merida Zoo, Yucatan State, and 7 farmed crocodiles in Ciudad del Carmen, Campeche State were assayed for antibodies to flaviviruses. Ninety (62%) horses on Cozumel Island had epitope-blocking enzyme-linked immunosorbent assay (ELISA) antibodies to flaviviruses, of which 75 (52%) were seropositive for WNV by plaque reduction neutralization test (PRNT). Blocking ELISA antibodies to flaviviruses also were detected in 13 (3%) animals in the Merida Zoo, including 7 birds and 2 mammals (a jaguar and coyote) seropositive for WNV by PRNT. Six (86%) crocodiles in Campeche State had PRNT-confirmed WNV infections. All animals were healthy at the time of serum collections and none had a history of WNV-like illness.

Descriptors: vertebrata, viral diseases, West Nile virus, prevalence of antibodies in asymptomatic individuals, immune response, antibodies, against viral disease, prevalence in asymptomatic individuals, Mexico , Yucatan Peninsula, prevalence of antibodies against viral disease in asymptomatic individuals.

Gibbs, S.E., A.E. Ellis, D.G. Mead, A.B. Allison, J.K. Moulton, E.W. Howerth, and D.E. Stallknecht (2005). **West Nile virus detection in the organs of naturally infected blue jays (*Cyanocitta cristata*).** *Journal of Wildlife Diseases* 41(2): 354-362.

Abstract: Blue jays (*Cyanocitta cristata*) are an effective indicator species for West Nile virus (WNV) and may be regionally important in surveillance efforts. The sites of WNV replication and sensitivity of virus detection techniques are undefined for blue jays. The objectives of this study were to describe the gross and microscopic pathology associated with natural WNV infection in blue jays, as well as determine the most appropriate tissues to be used for virus isolation, reverse transcription-nested polymerase chain reaction, and immunohistochemistry (IHC) techniques. Blue jays were collected in Georgia, USA, between May and September 2001. Initial screening by virus isolation indicated that 36 of 59 blue jays chosen for evaluation were WNV positive. From this group, 20 positive and five negative birds were chosen to compare virus detection techniques. Six positive and five negative birds were selected for histopathology examination. Splenomegaly and poor body condition were the most consistent gross findings among positive birds. The most consistent histopathologic findings in the tissues of WNV-positive blue jays were mononuclear leukocytosis and epicarditis/myocarditis. Brain, heart, and lung had the highest viral titers, and WNV antigen was most often detected by IHC in heart, kidney, liver, and lung. Reverse

transcription-nested polymerase chain reaction proved to be the most sensitive diagnostic test applied in this study irrespective of the tissue type. Brain tissue could be used effectively for both virus isolation and RT-nPCR, and this tissue is simple to remove and process. The success of IHC is highly dependent on tissue selection, and the use of multiple tissues including heart, kidney, liver, or lung is recommended.
Descriptors: blue jays, *Cyanocitta cristata*, WNV indicator species, disease surveillance, virus detection techniques, immunohistochemistry techniques (IHC), tissue selection, heart, kidney, lung, liver.

Gibbs, S.E., D.M. Hoffman, L.M. Stark, N.L. Marlenee, B.J. Blitvich, B.J. Beaty, and D.E. Stallknecht (2005). **Persistence of antibodies to West Nile virus in naturally infected rock pigeons (*Columba livia*).** *Clinical and Diagnostic Laboratory Immunology* 12(5): 665-667.
Abstract: Wild caught rock pigeons (*Columba livia*) with antibodies to West Nile virus were monitored for 15 months to determine antibody persistence and compare results of three serologic techniques. Antibodies persisted for the entire study as detected by epitope-blocking enzyme-linked immunosorbent assay and plaque reduction neutralization test. Maternal antibodies in squabs derived from seropositive birds persisted for an average of 27 days.
Descriptors: viral blood antibodies, West Nile fever, West Nile virus transmission, wild animals, disease transmission, enzyme-linked immunosorbent assay, plaque assay.

Gibbs, S.E., A.B. Allison, M.J. Yabsley, D.G. Mead, B.R. Wilcox, and D.E. Stallknecht (2006). **West Nile virus antibodies in avian species of Georgia, USA: 2000-2004.** *Vector Borne and Zoonotic Diseases* 6(1): 57-72. ISSN: 1530-3667.
Abstract: West Nile virus (WNV) was first isolated in the state of Georgia in the summer of 2001. As amplifying hosts of WNV, avian species play an important role in the distribution and epidemiology of the virus. The objective of this study was to identify avian species that are locally involved as potential amplifying hosts of WNV and can serve as indicators of WNV transmission over the physiographic and land use variation present in the southeastem United States. Avian serum samples (n = 14,077) from 83 species of birds captured throughout Georgia during the summers of 2000-2004 were tested by a plaque reduction neutralization test for antibodies to WNV and St. Louis encephalitis virus. Over the 5-year period, WNV-neutralizing antibodies were detected in 869 (6.2%) samples. The WNV seroprevalence increased significantly throughout the study and was species dependent. The highest antibody prevalence rates were detected in rock pigeons (*Columba livia*), northern cardinals (*Cardinalis cardinalis*), common ground doves (*Columbina passerina*), grey catbirds (*Deumetella carolinensis*), and northern mockingbirds (*Mimus polyglottos*). Northem cardinals, in addition to having high geometric mean antibody titers and seropreva-

lence rates, were commonly found in all land use types and physiographic regions. Rock pigeons, common ground doves, grey catbirds, and northern mockingbirds, although also having high seroprevalence rates and high antibody titers against WNV, were more restricted in their distribution and therefore may be of more utility when attempting to assess exposure rates in specific habitat types. Of all species tested, northern cardinals represent the best potential avian indicator species for widespread serologic-based studies of WNV throughout Georgia due to their extensive range, ease of capture, and high antibody rates and titers. Due to the large geographic area covered by this species, their utility as a WNV sentinel species may include most of the eastern United States.

Descriptors: aves, viral diseases, West Nile virus, host records and prevalence, Georgia, USA, viral disease.

Godhardt, J.A., K. Beheler, M.J. O'Connor, T.J. Whyte, E.S. Reisdorf, S.J. Ubl, P.N. Bochsler, and K.L. Toohey Kurth (2006). **Evaluation of antigen-capture ELISA and immunohistochemical methods for avian surveillance of West Nile virus.** *Journal of Veterinary Diagnostic Investigation* 18(1): 85-89.

Abstract: Accurate detection of West Nile virus (WNV) in corvids is essential for monitoring the spread of virus during the mosquito season. Viremia in corvids is very high, with titers approaching 10(8) viral particles/ml. In the presence of such marked viremia, the sensitivity of real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis is unnecessary, and more cost-effective methods should be assessed. To this end, antigen-capture ELISA (ACE) and immunohistochemical (IHC) assays were evaluated. Skin, cloacal swab specimens, and feathers from corvids were tested by use of ACE, and results were compared with results obtained from use of real-time RT-PCR analysis. Of the 3 sample types, skin gave the best sensitivity (98%) and specificity (100%). Skin, brain, kidney, and spleen from corvids were analyzed by IHC, and results were compared with real-time RT-PCR results. Kidney and spleen were more often positive by use of IHC than were brain and skin tissue; however, IHC did not perform as well as ACE in the identification of virus-positive birds. Results of this study support the use of a skin sample in an ACE format as an effective surveillance method for corvids.

Descriptors: West Nile virus, ELISA, avian surveillance, antigen capture, evaluation, immunohistochemical methods, accurate detection.

Gu, W., R. Lampman, N. Krasavin, R. Berry, and R. Novak (2006). **Spatio-temporal analyses of West Nile virus transmission in culex mosquitoes in northern Illinois, USA, 2004.** *Vector Borne and Zoonotic Diseases* 6(1): 91-98 . ISSN: 1530-3667.

Abstract: After a severe outbreak of West Nile virus (WNV) in Cook County, Illinois, in 2002, detections of WNV in mosquitoes were frequent across the state in the following years despite small numbers of human cases. We conducted a spatio-

temporal analysis of Culex (subgenus Culex) mosquitoes collected in 2004 in three mosquito abatement districts (MAD) in Cook County by calculating monthly estimates of mosquito density, prevalence of infected mosquitoes, and exposure intensity, which in turn is a product of mosquito density and infection rates. Mosquito infections were detected early at three sites in late May and were widely detected throughout the three MADs in the summer with infection rates as high as 13 per 1000 in August. Exposure intensities were higher at sites adjacent to the Des Plaines River, especially in August and September. The aggregated pattern of WNV transmission along the river might be related to the existence of substantial forest preserves and wetlands that might produce ecological conditions favorable for mosquito proliferation and interactions between mosquitoes and birds.
Descriptors: West Nile virus, culex mosquitoes, infection rate, wetlands, birds, prevalence, ecological conditions.

Hull, J., A. Hull, W. Reisen, Y. Fang, and H. Ernest (2006). **Variation of West Nile virus antibody prevalence in migrating and wintering hawks in central California.** *Condor* 108(2): 435-439. ISSN: 0010-5422.
Abstract: To assess the extent of West Nile virus (WNV) exposure of migrating (Marin Headlands) and wintering (Central Valley) hawks in California, plasma from 271 Red-tailed Hawks (*Buteo jamaicensis*), 19 Red-shouldered Hawks (*B. lineatus*), and 30 Cooper's Hawks (*Accipiter cooperii*) was tested for WNV antibodies during the winter of 2004-2005. WNV antibodies were found in 5% of migrating and 15% of wintering Red-tailed Hawks, 20% of migrating and 58% of wintering Red-shouldered Hawks, and 13% of migrating Cooper's Hawks. No individuals demonstrated visible signs of WNV illness. Red-tailed Hawks that tested positive for WNV antibodies displayed no difference from Red-tailed Hawks without WNV antibodies in weight to wing chord ratio or white blood cell counts. In the Central Valley, WNV antibodies were significantly more prevalent in Red-shouldered Hawks than in Red-tailed Hawks. Significantly more Red-tailed Hawks sampled on wintering grounds tested positive for WNV antibodies than Red-tailed Hawks sampled during migration.
Descriptors: *Accipiter cooperii*, *Buteo jamaicensis*, *Buteo lineatus*, migration, viral diseases, West Nile virus, antibody prevalence, temporal variation, migrating and wintering individuals, immune response, viral disease antibody prevalence, California, central.
Language of Text: English, Spanish.

Johnson, G.D., M. Eidson, K. Schmit, A. Ellis, and M. Kulldorff (2006). **Geographic prediction of human onset of West Nile virus using dead crow clusters: An evaluation of year 2002 data in New York State.** *American Journal of Epidemiology* 163(2): 171-180.

Abstract: The risk of becoming a West Nile virus case in New York State, excluding New York City, was evaluated for persons whose town of residence was proximal to spatial clusters of dead American crows (*Corvus brachyrhynchos*). Weekly clusters were delineated for June-October 2002 by using both the binomial spatial scan statistic and kernel density smoothing. The relative risk of a human case was estimated for different spatial-temporal exposure definitions after adjusting for population density and age distribution using Poisson regression, adjusting for week and geographic region, and conducting Cox proportional hazards modeling, where the week that a human case was identified was treated as the failure time and baseline hazard was stratified by region. The risk of becoming a West Nile virus case was positively associated with living in towns proximal to dead crow clusters. The highest risk was consistently for towns associated with a cluster in the current or prior 1-2 weeks. Weaker, but positive associations were found for towns associated with a cluster in just the 1-2 prior weeks, indicating an ability to predict onset in a timely fashion.
Descriptors: bird diseases, epidemiology, crows, West Nile fever, cluster analysis, disease reservoirs, New York, population surveillance, proportional hazards models, West Nile virus isolation and purification.

Jozan, M., R. Evans, J. Webb, R. Cummings, S. Bennett, R. Hall, J. Nevarez, J. Weir, and K. De Collibus (2005). **Monitoring of wild bird populations for arbovirus surveillance and new issued [issues] raised by the recent introduction of west Nile virus in Southern Carolina.** *Arbovirus Research in Australia* 9: 169-176. ISSN: 0725-4989.
Descriptors: aves, viral diseases, arboviruses including West Nile virus, monitoring to detect presence and prevalence, California, south, monitoring to detect presence and prevalence of arboviruses including West Nile virus.

Kile, J.C., N.A. Panella, N. Komar, C.C. Chow, A. MacNeil, B. Robbins, and M.L. Bunning (2005). **Serologic survey of cats and dogs during an epidemic of West Nile virus infection in humans.** *Journal of the American Veterinary Medical Association* 226(8): 1349-1353.
Abstract: OBJECTIVE: To estimate West Nile virus (WNV) infection rates, assess environmental variables that correlated with seropositivity in dogs and cats, and assess whether pets should be considered as possible sentinels for WNV and therefore of potential human exposure. DESIGN: Cross-sectional serosurvey. ANIMALS: 442 dogs and 138 cats. PROCEDURE: Serum samples were screened for seropositivity against WNV by use of the plaque reduction neutralization test. RESULTS: 116 (26%) dogs and 13 (9%) cats yielded positive results. The odds of seropositivity against WNV for outdoor-only family dogs were almost 19 times as great as those for indoor-only family dogs and almost twice as great for stray dogs as for family dogs. Family dogs not receiving heartworm medication were 2.5 times as likely to yield positive results for antibodies against WNV as family dogs receiving heartworm

medication. CONCLUSIONS AND CLINICAL RELEVANCE: Seropositivity was greater for outdoor family dogs than for indoor family dogs. Further investigation of the potential use of stray dogs as sentinel indicators for WNV infection and the potential risk of human exposure is warranted.
Descriptors: infection rates, seropositivity, dogs, cats, sentinel animals, West Nile virus, serosurveys, potential risk, human exposure, transmission of disease.

Komar, O., M.B. Robbins, G.G. Contreras, B.W. Benz, K. Klenk, B.J. Blitvich, N.L. Marlenee, K.L. Burkhalter, S. Beckett, G. Gonzalvez, C.J. Pena, A.T. Peterson, and N. Komar (2005). **West Nile virus survey of birds and mosquitoes in the Dominican Republic.** *Vector Borne and Zoonotic Diseases* 5(2): 120-126. ISSN: 1530-3667.
Abstract: We report West Nile virus (WNV) activity from a new area on Hispaniola, in the vicinity of Monte Cristi National Park in northwest Dominican Republic. Specific anti-WNV antibodies were detected in 12 of 58 (21%) resident birds sampled in March 2003, representing six species in the orders Cuculiformes (cuckoos), Strigiformes (owls), and Passeriformes (song birds). This seroprevalence is the highest reported from any site in the Caribbean Basin. Virus was not detected in any mosquitoes or tissues from bird specimens. Testing of 20 sick or dead birds was negative for WNV. Undetermined flavivirus antibodies were detected in four resident birds at Monte Cristi, as well as in five resident birds at Sierra de Baoruco National Park in southwest Dominican Republic. These data suggest that an unidentified flavivirus, as well as WNV, is active in the Dominican Republic.
Descriptors: bird diseases , songbirds, West Nile fever, West Nile virus, viral analysis, disease reservoirs, Dominican Republic, enzyme linked immunosorbent assay, flavivirus, seroepidemiologic studies.

Korves, C.T., S.J. Goldie, and M.B. Murray (2006). **Blood screening for west nile virus: the cost-effectiveness of a real-time, trigger-based strategy.** *Clinical Infectious Diseases* 43(4): 490-493.
Abstract: Previous studies have demonstrated that universal blood screening for West Nile virus is not cost-effective. A newly proposed, real-time, trigger-based screening strategy was analyzed and was also shown to be not cost-effective. These results were highly sensitive to pricing of screening assays.
Descriptors: mass screening economics, West Nile fever diagnosis, West Nile virus isolation and purification, cost-benefit analysis.

Kronenwetter Koepel, T.A., J.K. Meece, C.A. Miller, and K.D. Reed (2005). **Surveillance of above- and below-ground mosquito breeding habitats in a rural midwestern community: baseline data for larvicidal control measures against West Nile Virus vectors.** *Clinical Medicine and Research* 3(1): 3-12.
Abstract: BACKGROUND: Mosquitoes in the genus Culex are thought to play a

major role as vectors in the transmission cycle of West Nile virus (WNV) and other arboviruses in the United States. Effective control of mosquitoes through larviciding and adulticiding is expensive for communities and should be guided by reliable surveillance data on the distribution of mosquito breeding habitats. However, few small to medium sized cities in rural areas of the midwestern United States have this type of baseline information available. OBJECTIVE: During the summer of 2004, we investigated the characteristics of Culex and other mosquito-breeding habitats in a rural central Wisconsin community with a population of approximately 19,000. Such baseline information will aid in the development of rational strategies to control mosquito populations and prevent human exposure to WNV and other mosquito-transmitted viruses. METHODS: Mosquito larvae were collected and identified weekly from 14 below-ground storm water catch basins and 10 above-ground standing water sites distributed throughout the community. Collection began June 4, 2004 and continued through September 24, 2004. For each collection site the primary and adjacent land use patterns were determined. RESULTS: Over the study period, 1,244 larvae were collected from catch basins; 94% were Culex species. Breeding activity was first detected in early July. Peak breeding was observed during a period of several weeks when average daily temperatures were at the maximum observed and rainfall had declined. Organically enriched catch basins in low intensity urban sites adjacent to forests and wetlands were found to be more productive breeding habitats compared to catch basins having little organic debris located in isolated high intensity urban sites. Above-ground standing water sites produced 1,504 larvae; 66% of which were Culex species. Flood control ditches and permanent wetlands with stagnant water were most productive, while ditches with moving water were least productive habitats. Larvae were produced earlier in the season by above-ground sites than were produced by catch basins. However, larvae production was more variable in above-ground sites since half the sites became dry at some point during the study period. CONCLUSION: The observed differences in Culex larvae production based on the variables of habitat-type, temperature, and precipitation support the need for ongoing surveillance in communities to guide public health officials in planning for and prioritizing mosquito control efforts.
Descriptors: Culex, insect vectors, mosquito control, West Nile Virus, breeding environment, insect control methods, rain, rural population, seasons, temperature, time factors, water, weather, West Nile fever prevention and control, Wisconsin.

Lee, B.Y. and B.J. Biggerstaff (2006). **Screening the United States blood supply for West Nile Virus: A question of blood, dollars, and sense.** *PLoS Medicine* 3(2): E99.
Online: http://medicine.plosjournals.org/perlserv/?request=get-document&doi=10.1371/journal.pmed.0030099
Descriptors: mass screening, West Nile fever, blood bank standards, blood donors,

blood transfusion, cost of illness, cost-benefit analysis, health policy, seasons, specimen handling.
Notes: Comment On: PLoS Med. 2006 Feb;3(2):E21.

Lefrancois, T., B.J. Blitvich, J. Pradel, S. Molia, N. Vachiery, and D. Martinez (2006). **West nile virus in guadeloupe: introduction, spread, and decrease in circulation level: 2002-2005.** *Annals of the New York Academy of Sciences* 1081: 206-215. ISSN: 0077-8923.
Abstract: In July 2002, a surveillance system was implemented on Guadeloupe to detect for the potential introduction and monitor the spread of West Nile virus (WNV). From 2002 to 2004, equines and chickens were serologically assayed for antibodies to WNV by IgG and IgM enzyme-linked immunosorbent assay (ELISA), epitope-blocking ELISA, and plaque reduction neutralization tests. After introduction, probably through migratory birds at the end of 2001, many seroconversions occurred between July and October 2002 resulting in a high seroprevalence (19.3%) in equines in 2003. WNV circulation levels decreased dramatically in 2003 and 2004 as assessed by the absence of seroconversion in equine and the very low prevalence in chickens. This decrease coincided with a 7-month drought that presumably caused a decrease in vector populations. In 2005, a sentinel survey was implemented in equines and chickens placed in areas at high risk and the very low rate of seroconversion (1 equine out of 106, no chicken) demonstrated that WNV circulation is now occurring at a very low level.
Descriptors: West Nile virus, equines, chickens, antibodies, serological assay, ELISA, birds, vector populations.

Liu, R., J. Shuai, J. Wu, and H. Zhu (2006). **Modeling spatial spread of West Nile virus and impact of directional dispersal of birds**. *Mathematical Biosciences and Engineering* 3(1): 145-160. ISSN: 1547-1063.
Descriptors: West Nile virus spread, modeling, bird dispersal, disease transmission, spatial spread model, long range dispersal patterns.

Lukacik, G., M. Anand, E.J. Shusas, J.J. Howard, J. Oliver, H. Chen, P.B. Backenson, E.B. Kauffman, K.A. Bernard, L.D. Kramer, and D.J. White (2006). **West Nile virus surveillance in mosquitoes in New York State, 2000-2004.** *Journal of the American Mosquito Control Association* 22(2): 264-271.
Abstract: A West Nile virus (WNV) surveillance system was created and implemented in New York State (NYS) in 2000 and described previously (White et al. 2001). We examine and evaluate the results of mosquito and virus surveillance for 2000 through 2004 exclusive of New York City. Forty-nine counties submitted 1,095,426 mosquitoes in 35,280 pools for WNV assay. Specimens of 47 species were tested, with Culex species accounting for 47.6% of all pools tested. WNV was

detected in 814 pools from 10 species, with combined *Culex pipiens/Culex restuans* pools accounting for 90.8% of all detections. Pools submitted from gravid traps were 5.7 times more likely to be positive than submissions from carbon dioxide-baited light traps. Most human WNV cases resided in counties that conducted mosquito surveillance. Local health departments' use of mosquito surveillance information often led to an enhanced disease prevention response. In NYS, *Cx. pipiens/Cx. restuans* groups are most likely vectors of WNV. Future efforts to improve system efficacy are discussed.
Descriptors: Culicidae virology, West Nile virus, Aedes virology, *Culex sp*., New York, Ochlerotatus, population surveillance, seasons.

Morgan, D. (2006). **Control of arbovirus infections by a coordinated response: West Nile Virus in England and Wales.** *FEMS Immunology and Medical Microbiology* 48(3): 305-312.
Abstract: Although there is no recognized transmission of human arboviral infections in the UK, concerns about the possible spread of West Nile virus (WNV) have precipitated coordinated activities around both surveillance and response. The Department of Health has chaired a UK WNV task force since the end of 2000. This is a multidisciplinary group of senior representatives from Agencies and Government Departments involved in human and animal health, entomology and academic departments. Activities include surveillance for WNV infections in humans, and in dead birds, mosquitoes and horses. All have been negative for WNV. A WNV contingency plan was produced in 2004, and this could be used as a generic plan for an effective and coordinated response in the event of the emergence of a new vector-borne zoonotic infection.
Descriptors: West Nile virus surveillance, task force, dead birds, horses, humans, mosquitoes, contingency plan.

Murphy, T.D., J. Grandpre, S.L. Novick, S.A. Seys, R.W. Harris, and K. Musgrave (2005). **West Nile virus infection among health-fair participants, Wyoming 2003: Assessment of symptoms and risk factors.** *Vector Borne and Zoonotic Diseases* 5(3): 246-251. ISSN: 1530-3667.
Abstract: Wyoming experienced heavy West Nile virus (WNV) activity for the first time in 2003 and the area hardest hit was Goshen County. Little was known about the epidemiology of WNV in this region. This study describes the symptomatology of WNV and the association between certain behaviors and infection in Goshen County. Study participants were recruited from attendees of a health-fair sponsored by a local hospital, held October 1-3, 2003. A blood sample for WNV testing was obtained from each participant, and participants completed a questionnaire seeking information about the presence of specified symptoms consistent with WNV infection and risk factors possibly associated with infection. The samples were tested for

anti-WNV IgM and IgG at the Wyoming Public Health Laboratory. Eight-hundred sixty-nine residents of Goshen County participated, and 122 (14.0%) were seropositive for anti-WNV IgM or IgG. Sixty (59.4%) of 101 persons seropositive for anti-WNV IgM experienced at least one symptom in the previous 4 months consistent with WNV infection, compared with 323 (43.2%) of 747 seronegative persons, resulting in an attributable risk of WNV seropositivity of 16.2%. Of the many symptoms queried, muscle aches (OR 2.63, 95% CI 1.69-4.09), skin rash (OR 6.35, 95% CI 3.74-10.80), fever (OR 2.56, 95% Cl 1.50-4.36), and muscle weakness (OR 2.33, 95% CI 1.34-4.02) were significantly associated with seropositivity on univariate analysis. By multivariate analysis, only skin rash remained significant. Risk factor analysis showed those spending > or =3 hours outside per day were more likely to be seropositive than those spending less time outside per day ($p < 0.05$). This study corroborates the belief that a minority of persons infected with WNV develop symptoms attributable to WNV, and also demonstrates that some symptoms are more significantly associated with infection than others.
Descriptors: viral blood antibodies, West Nile fever, exanthema, health surveys, blood immunoglobulin G, blood immunoglobulin M, muscle weakness, risk factors, seroepidemiologic studies, West Nile virus, Wyoming.

Murtagh, B., Y. Wadia, G. Messner, P. Allison, Y. Harati, and R. Delgado (2005). **West Nile virus infection after cardiac transplantation.** *Journal of Heart and Lung Transplantation* 24(6): 774-776.
Abstract: West Nile virus is a mosquito-borne RNA Flavivirus infection transmitted to humans and other vertebrates, mainly by the Culex species of mosquito. Since the mid-1990s, the frequency and apparent clinical severity of West Nile virus outbreaks have increased. We report the case of a patient who developed West Nile virus encephalitis shortly after undergoing cardiac transplantation. Clinicians should be aware of the possibility of West Nile virus infection in transplant recipients and in patients receiving blood transfusions.
Descriptors: blood transfusion, adverse effects, heart transplantation, West Nile fever etiology, middle aged humans.

Naugle, D., C. Aldridge, B. Walker, K. Doherty, M. Matchett, J. McIntosh, T. Cornish, and M. Boyce (2005). **West Nile virus and sage-grouse: What more have we learned?** *Wildlife Society Bulletin.* 33(2): 616-623. ISSN: 0091-7648.
Descriptors: *Centrocercus urophasianus*, grouse, West Nile virus, mortality, surveys, Wyoming, Montana, Alberta, Colorado, California.

Ohajuruka, O.A., R.L. Berry, S. Grimes, and S. Farkas (2005). **West Nile Virus detection in kidney, cloacal, and nasopharyngeal specimens.** *Emerging Infectious Diseases* 11(9): 1437-1439. ISSN: 1080-6040 .

Abstract: We compared kidney tissue samples and cloacal and nasopharyngeal swab samples from field-collected dead crows and blue jays for West Nile virus surveillance. Compared to tissue samples, 35% more swab samples were false negative. Swab samples were usually positive only when the corresponding tissue sample was strongly positive.
Descriptors: cloaca, crow, kidney, nasopharynx, West Nile virus isolation and purification, false negative reactions, linear models, Ohio , population surveillance methods, reverse transcriptase polymerase chain reaction.

Orme Zavaleta, J., J. Jorgensen, B. D'Ambrosio, E. Altendorf, and P.A. Rossignol (2006). **Discovering spatio-temporal models of the spread of West Nile virus.** *Risk Analysis* 26(2): 413-422.

Abstract: Emerging infectious diseases are characterized by complex interactions among disease agents, vectors, wildlife, humans, and the environment. Since the appearance of West Nile virus (WNV) in New York City in 1999, it has infected over 8,000 people in the United States, resulting in several hundred deaths in 46 contiguous states. The virus is transmitted by mosquitoes and maintained in various bird reservoir hosts. Its unexpected introduction, high morbidity, and rapid spread have left public health agencies facing severe time constraints in a theory-poor environment, dependent largely on observational data collected by independent survey efforts and much uncertainty. Current knowledge may be expressed as a priori constraints on models learned from data. Accordingly, we applied a Bayesian probabilistic relational approach to generate spatially and temporally linked models from heterogeneous data sources. Using data collected from multiple independent sources in Maryland, we discovered the integrated context in which infected birds are plausible indicators for positive mosquito pools and human cases for 2001 and 2002.
Descriptors: biological models, West Nile fever transmission, Bayesian theorem, birds, disease outbreaks, disease reservoirs, Maryland epidemiology, risk, West Nile virus isolation and purification.

Orton, S.L., S.L. Stramer, and R.Y. Dodd (2006). **Self-reported symptoms associated with West Nile virus infection in RNA-positive blood donors.** *Transfusion* 46(2): 272-277.

Abstract: BACKGROUND: In 2003, West Nile virus (WNV) nucleic acid amplification testing (NAT) was implemented to detect potentially infected donors. Of more than 5.3 million donations screened prospectively by the American Red Cross during the epidemic periods of 2003 and 2004, 974 were NAT-reactive and 519 confirmed-positive. A subset of both the confirmed-positive and the false-positive groups was assessed for demographic characteristics, symptoms, and symptom reporting relative to date of donation. STUDY DESIGN AND METHODS: All donors with initial WNV NAT-reactive results were invited to participate in a study that included

a demographic, symptom, and date-of-symptom questionnaire. WNV confirmed-positive cases were compared to false-positive controls for comparison of frequency of symptom reporting before, on the day of, and after donation. RESULTS: Enrolled cases and controls were similar in all characteristics except cases were more likely to live in rural areas. Symptoms were reported by 61 percent of cases versus 20 percent of controls, with 74 percent of symptoms reported by cases within the 14 days after donation. The frequency of headache and fever reported together in the 7 days before donation was not significantly different between cases and controls; only the individual frequencies of headache, eye pain, and new rash during this time were significantly different. The most commonly reported symptoms, after adjustment for symptom reporting by controls, were headache, new rash, and generalized weakness; these symptoms were reported by 25 percent of cases. CONCLUSIONS: The demographic characteristics of infected donors reflected the rural nature of the 2003 to 2004 WNV epidemics. This study suggests that asking donors about predonation headache and fever had no detectable contribution to blood safety.
Descriptors: blood donors, West Nile fever diagnosis, West Nile virus, fever, headache, logistic models, mass screening, middle aged humans, multivariate analysis, viral RNA.

Ozkul, A., Y. Yildirim, D. Pinar, A. Akcali, V. Yilmaz, and D. Colak (2006). **Serological evidence of West Nile Virus (WNV) in mammalian species in Turkey.** *Epidemiology and Infection* 134(4): 826-829.
Abstract: In this study, the sera collected from a variety of mammalian species (ass-mules, cat, cattle, dog, horse, human and sheep) in 10 representative provinces of Turkey, were surveyed for the presence of neutralizing antibodies to West Nile virus (WNV). Overall, 1 of 40 (2.5%) ass-mules, 4 of 100 (4%) cattle, 43 of 114 (37.7%) dogs, 35 of 259 (13.5%) horses, 18 of 88 (20.4%) humans and 1 of 100 (1%) sheep, tested positive for WNV-neutralizing antibodies. The results indicate that a wide range of mammals are exposed to a West Nile-related virus and this could contribute to the long-term survival of this virus in the absence of overt disease.
Descriptors: animals, viral blood antibodies, West Nile virus isolation and purification, cats, cattle, disease reservoirs, dogs, equidae, seroepidemiologic studies, sheep, Turkey.

Rappole, J.H., B.W. Compton, P. Leimgruber, J. Robertson, D.I. King, and S.C. Renner (2006). **Modeling movement of West Nile virus in the Western hemisphere.** *Vector Borne and Zoonotic Diseases* 6(2): 128-139. ISSN: 1530-3667.
Abstract: We modeled West Nile virus (WNV) movement rates and patterns based on a migratory bird agent (the Swainson's Thrush) and a resident bird agent (the House Sparrow), and compared the results of these models with actual movement data to investigate the likelihood that the pattern of WNV outbreaks observed in the

New World was consistent with migrant bird-mediated spread, as reported from the Old World. We found that, contrary to Old World patterns, WNV activity in the Western Hemisphere does not seem consistent with movement by infected migrant birds. Instead WNV spread appears best explained by a non-directional movement, perhaps that of dispersing resident birds.
Descriptors: bird disease transmission, West Nile fever, disease outbreaks, biological models , North America, West Nile virus.

Reisen, W.K., C.M. Barker, R. Carney, H.D. Lothrop, S.S. Wheeler, J.L. Wilson, M.B. Madon, R. Takahashi, B. Carroll, S. Garcia, Y. Fang, M. Shafii, N. Kahl, S. Ashtari , V. Kramer, C. Glaser, and C. Jean (2006). **Role of corvids in epidemiology of west Nile virus in southern California.** *Journal of Medical Entomology* 43(2): 356-367. ISSN: 0022-2585.
Abstract: The invasion of different southern California landscapes by West Nile virus (WNV) and its subsequent amplification to epidemic levels during 2004 enabled us to study the impact of differing corvid populations in three biomes: the hot Colorado desert with few corvids (Coachella Valley), the southern San Joaquin Valley (Kern County) with large western scrub-jay but small American crow populations, and the cool maritime coast (Los Angeles) with a large clustered American crow population. Similar surveillance programs in all three areas monitored infection rates in mosquitoes, seroconversion rates in sentinel chickens, seroprevalence in wild birds, numbers of dead birds reported by the public, and the occurrence of human cases. Infection rates in *Culex tarsalis Coquillett* and sentinel chicken seroconversion rates were statistically similar among all three areas, indicating that highly competent mosquito hosts were capable of maintaining enzootic WNV transmission among less competent and widely distributed avian hosts, most likely house sparrows and house finches. In contrast, infection rates in *Culex pipiens quinquefasciatus* Say were statistically higher in Kern and Los Angeles counties with elevated corvid populations than in Coachella Valley with few corvids. Spatial analyses of dead corvids showed significant clusters near known American crow roosts in Los Angeles that were congruent with clusters of human cases. In this area, the incidence of human and *Cx. p. quinquefasciatus* infection was significantly greater within corvid clusters than without, indicating their importance in virus amplification and as a risk factor for human infection. In contrast the uniform dispersion by territorial western scrub-jays resulted in a high, but evenly distributed, incidence of human disease in Kern County.
Descriptors: crows, culex mosquitoes, insect vectors, West Nile fever epidemiology, West Nile virus, California, chickens, incidence, population density, population surveillance, sentinel surveillance, zoonoses transmission.

Reisen, W.K., Y. Fang, H.D. Lothrop, V.M. Martinez, J. Wilson, P. O'Connor, R. Carney, B. Cahoon Young, M. Shafii, and A.C. Brault (2006). **Overwintering of West Nile virus in southern California.** *Journal of Medical Entomology* 43(2): 344-355. ISSN: 0022-2585.

Abstract: West Nile virus (family Flaviviridae, genus Flavivirus, WNV) invaded southern California dining 2003, successfully overwintered, amplified to epidemic levels, and then dispersed to every county in the state. Although surveillance programs successfully tracked and measured these events, mechanisms that allowed the efficient overwintering and subsequent amplification of WNV have not been elucidated. Our current research provided evidence for three mechanisms whereby WNV may have persisted in southern California during the winters of 2003-2004 and 2004-2005: 1) continued enzootic transmission, 2) vertical transmission by Culex mosquitoes, and 3) chronic infection in birds. WNV was detected in 140 dead birds comprising 32 species, including 60 dead American crows, thereby verifying transmission during the November-March winter period. Dead American crows provide evidence of recent transmission because this species always succumbs rapidly after infection. However, WNV RNA was not detected concurrently in 43,043 reproductively active female mosquitoes comprising 11 species and tested in 1,258 pools or antibody in sera from 190 sentinel chickens maintained in 19 flocks. Although efficient vertical transmission by WNV was demonstrated experimentally for Culex tarsalis Coquillett infected per os, 369 females collected diapausing in Kern County and tested in 32 pools were negative for WNV. Vertical transmission was detected in Culex pipiens quinquefinciatus Say adults reared from field-collected immatures collected from Kern County and Los Angeles during the summer transmission period. Chronic infection was detected by finding WNV RNA in 34 of 82 birds that were inoculated with WNV experimentally, held for >6 wk after infection, and then necropsied. Frequent detection of WNV RNA in kidney tissue in experimentally infected birds >6 wk postinfection may explain, in part, the repeated detection of WNV RNA in dead birds recovered during winter, especially in species such as mourning doves that typically do not die after experimental infection. In summary, our study provides limited evidence to support multiple modes of WNV persistence in southern California. Continued transmission and vertical transmission by *Culex p. quinquefasciatus* Say seem likely candidates for further study.

Descriptors: aves, viral diseases, West Nile virus, overwintering, transmission of viruses, California, south, viral disease overwintering.

Reisen, W., S. Wheeler, S. Yamamoto, Y. Fang, and S. Garcia (2005). **Nesting ardeid colonies are not a focus of elevated West Nile virus activity in southern California.** *Vector Borne and Zoonotic Diseases* 5(3): 258-266. ISSN: 1530-3667.

Abstract: A large nesting colony of Ardeid birds at the Finney-Ramer Wildlife

Refuge in Imperial County, California, did not appear to be a focus of West Nile virus (WNV) amplification during the summer of 2004. Blood samples taken during June and July from 155 nestlings of four species of Ardeid birds (cattle egrets, black-crowned night herons, great egrets, and snowy egrets) and five nestling double-crested cormorants yielded a single WNV isolation from a 3-week-old cattle egret. Antibody was detected by enzyme immunoassay from 20 nestlings (13%), 14 (70%) of which were confirmed as positive by plaque reduction neutralization test (PRNT). However, titration end points against WNV and St. Louis encephalitis virus (SLEV) were similar precluding viral identification. The grouping of positives within few nests, highest PRNT titers in youngest birds (<1 weeks of age), the decline of titer with nestling age, and the lack of antibody specificity indicated that antibody may have been acquired maternally and did not represent new infections. Infection rates in *Culex tarsalis* mosquitoes collected near the Ardeid colony at Ramer Lake (3.1 per 1,000) were statistically similar to rates estimated at the nearby Wister Unit wetlands (5.3 per 1,000) that lacked an Ardeid nesting colony. Black-crowned night heron nestlings experimentally infected with the NY99 strain of WNV produced viremias >5 log10 plaque forming units (PFU)/mL and were considered moderately competent hosts, whereas cattle egret nestlings had viremias that remained <5 log10 PFU/mL and were incompetent hosts.

Descriptors: *Ardea alba*, *Bubulcus ibis*, *Egretta thula*, *Nycticorax nycticorax*, *Phalacrocorax auritus*, viral diseases, West Nile virus, nestling infection rate and host competence survey, California, , Imperial County, Finney Ramer Wildlife Refuge.

Rios, J., C.S. Hacker, C.A. Hailey, and R.E. Parsons (2006). **Demographic and spatial analysis of West Nile virus and St. Louis encephalitis in Houston, Texas.** *Journal of the American Mosquito Control Association* 22(2): 254-263.

Abstract: This descriptive prevalence study describes the relationships between mosquito density and the presence of arboviruses (in mosquitoes and humans) with various socioeconomic and environmental factors present near the time of teh arbovirus outbreak in Harris County, Texas, in 2002. This study suggests that mosquito density increased if the trap was located in an area with a large number of containers that may inadvertently retain rainwater ($P = 0.056$). When considering only virus-positive mosquitoes, significant relationships were observed if the trap was located near waste materials ($P < 0.001$) or near containers that may inadvertently retain rainwater ($P = 0.037$). Furthermore, the presence of arbovirus activity (in mosquitoes or humans) in a geographic area tended to be associated with the socioeconomic status of the local community. Although the results of socioeconomic comparisons were not significant, they were suggestive, demonstrating an interesting trend. Compared with communities where virus activity was not observed, the socioeconomic status of the arbovirus-positive community was consistently lower. Specifically,

results showed that the populations residing in virus-positive census tracts attained less education, earned less income per household, and were more likely to be below the poverty level. In addition, this study found that virus-positive mosquitoes were randomly distributed throughout the study area, whereas severe human infection cases were clustered. Based on the results of this study, we conclude that the health outcome of a local community as it relates to West Nile virus and St. Louis encephalitis is dependent on many factors, including eh socioeconomic and environmental characteristics of the community.

Descriptors: Culicidae virology, St. Louisencephalitis epidemiology, West Nile virus, disease outbreaks, ecosystem, educational status, encephalitis transmission, population surveillance, poverty, socioeconomic factors, Texas.

Roberts, R.S. and I.M. Foppa (2006). **Prediction of equine risk of West Nile Virus infection based on dead bird surveillance.** *Vector Borne and Zoonotic Diseases* 6(1): 1-6. ISSN: 1530-3667.

Abstract: Since the introduction of West Nile Virus (WNV) to the United States in 1999, the efficacy of dead bird surveillance for the prediction of human and veterinary WNV infection has been an issue of debate. We utilized South Carolina's Department of Health and Environmental Control surveillance data from 2003 to determine whether dead bird surveillance accurately predicts equine WNV infection on a county level. We adjusted for human population density as a potential confounder of an association between WNV-positive dead bird counts and mammalian WNV risk. We found a strong positive association between avian risk of WNV death and subsequent equine mortality due to WNV in South Carolina even after adjusting for human population density. Sensitivity of dead bird surveillance as a predictor of future equine WNV risk was far superior to mosquito surveillance (95% vs. 9.5%, respectively). A Poisson regression model of the equine WNV rate as a function of WNV-positive dead bird rate, adjusting for population density and taking into account effect modification by population density shows a good fit with the data. Unlike most previous studies, we control for potential confounding of the dead, WNV-positive bird-equine WNV infection association by human population density. Yet, the positive association between dead bird surveillance and equine WNV risk remains strong and statistically significant, indicating that dead bird surveillance remains a valuable tool of WNV surveillance.

Descriptors: aves, pathological techniques, viral disease effect on mortality, dead host surveillance use in disease risk prediction in mammals, viral diseases, West Nile virus, mortality effects, mortality, viral disease effects, South Carolina.

Rockx, B., L. van Asten, C. van den Wijngaard, G.J. Godeke, L. Goehring, H. Vennema, H. van der Avoort, W. van Pelt, and M. Koopmans (2006). **Syndromic surveillance in the Netherlands for the early detection of West Nile virus epidemics.** *Vector Borne*

and Zoonotic Diseases 6(2): 161-169. ISSN: 1530-3667.
Abstract: West Nile virus (WNV) is an arthropod-borne flavivirus that is endemic in Africa, Europe, and Eastern Asia. The recent introduction and rapid dissemination of the virus in the United States as well as an increase in WNV outbreaks in Europe, has raised concerns for its spread in Europe. A surveillance system was developed to allow timely detection of an introduction of WNV infections in The Netherlands. This program focuses on cases presenting with neurological disease and includes the monitoring of hospital discharge diagnoses, trends in cerebrospinal fluid (CSF) diagnostic requests, laboratory testing of CSF, and monitoring of neurological disease in horses. Retrospective data from the hospital discharge records showed yearly peaks of unexplained meningitis and (meningo)encephalitis in the summer. A total of 781 CSF samples from humans and 71 serum and/or CSF samples from horses presenting with neurological disease of suspected viral etiology tested negative for the presence of specific antibodies to WNV. With a coverage rate of 59% in 2003, the probability that a cluster of five WNV cases presenting with neurological symptoms would have been detected was 99%. We conclude that, from 1999 to 2004, no evidence of WNV infection could be found in either humans or horses in The Netherlands.
Descriptors: cerebrospinal fluid, horse diseases, hospitals statistics, numerical data, sentinel surveillance, West Nile fever, Netherlands, retrospective studies, seasons, West Nile virus isolation and purification.

Sabio, I.J., A.J. Mackay, A. Roy, and L.D. Foil (2006). **Detection of West Nile virus RNA in pools of three species of ceratopogonids (Diptera: Ceratopogonidae) collected in Louisiana.** *Journal of Medical Entomology* 43(5): 1020-1022. ISSN: 0022-2585.
Abstract: Light traps were used to collect ceratopogonids in East Baton Rouge parish, Louisiana. In total, 46,496 ceratopogonids were sorted from 4,968 light trap collections from 20 November 2002 through 25 November 2004. Two hundred and nine pools containing specimens of 18 species of *Culicoides Latreille*, seven pools containing specimens of *Atrichopogon Kieffer*, and five pools containing specimens of *Forcipomyia Meigen* were tested for West Nile virus (family Flaviviridae, genus Flavivirus, WNV) RNA using real-time reverse transcriptase polymerase chain reaction. Five out of the 209 pools of Culicoides specimens were positive for WNV RNA.
Descriptors: West Nile virus, detection, trap collection, Culicoides, RNA, reverse transcriptase polymerase chain reaction, Louisiana.

Santaella, J., R. McLean, J.S. Hall, J.S. Gill, R.A. Bowen, H.H. Hadow, and L. Clark (2005). **West Nile Virus serosurveillance in Iowa white-tailed deer (1999-2003).** *American Journal of Tropical Medicine and Hygiene* 73(6): 1038-1042. ISSN: 0002-9637.
Abstract: Sera from white-tailed deer (*Odocoileus virginianus*) were collected in Iowa during the winter months (1999-2003), 2 years before and after West Nile virus (WNV) was first reported in Iowa (2001), and were analyzed for antibodies to WNV.

Samples from 1999 to 2001 were antibody negative by blocking enzyme-linked immunosorbent assay (bELISA) and plaque reduction neutralization test (PRNT90). Prevalence derived from bELISA (2002, 12.7%; 2003,11.2%) and WNV PRNT90 (2002, 7.9%; 2003, 8.5%) assays were similar. All samples were negative for antibodies against St. Louis encephalitis virus as determined by PRNT90. Antibodies to flaviviruses were detected by indirect enzyme-linked immunosorbent assay (iELISA) prior to the first WNV cases reported in Iowa (1999-2001) with prevalence ranging from 2.2% to 3.2%, suggesting the circulation of an additional undescribed flavivirus prior to the introduction of WNV into the area. Flavivirus prevalence as determined by iELISA increased in 2002 and 2003 (23.3% and 31.9%, respectively). The increase in prevalence exceeded estimates of WNV prevalence, suggesting that conditions favored general flavivirus transmission (including WNV) during the 2002-2003 epizootic. These data indicate that serologic analysis of deer sera collected from hunter harvests may prove useful for surveillance and evidence of local transmission of WNV and other pathogens and identify white-tailed deer as a species for further studies for host competency.

Descriptors: *Odocoileus virginianus*, viral diseases, West Nile virus, serosurveillance before and after epizootic, Iowa.

Scheidler, L.C., M.M. Dunphy Daly, B.J. White, D.R. Andrew, N.Z. Mans, and M.C. Garvin (2006). **Survey of *Aedes triseriatus* (Diptera: Culicidae) for Lacrosse encephalitis virus and West Nile virus in Lorain County, Ohio.** *Journal of Medical Entomology* 43(3): 589-593. ISSN: 0022-2585.

Abstract: From June through September 2003, we conducted a survey of female *Aedes triseriatus* (Say) for infection with La Crosse encephalitis virus (family Bunyaviridae, genus Orthobunyavirus, LACV) and West Nile virus (family Flaviviridae, genus Flavivirus, WNV) at three locations in Lorain County, Ohio. To determine infection rate and seasonal variation of both viruses in the *Ae. triseriatus* population, *Ae. triseriatus* were collected weekly by using gravid traps and CO2-baited CDC light traps and tested for virus by using reverse transcriptase polymerase chain reaction. In total, 170 pools comprised of 2,143 females were tested for LACV, of which seven were positive; the maximum likelihood estimate of infection rate combined throughout the season was 3.22/1,000. None of 170 pools comprised of 2,158 females tested for WNV were positive. LACV-positive pools were detected between late July and early September.

Descriptors: West Nile virus isolation and purification, insect vectors, la crosse virus genetics, Ohio, viral RNA analysis, reverse transcriptase polymerase chain reaction, seasons.

Schellenberg, T.L., M.E. Anderson, M.A. Drebot, M.T. Vooght, A.R. Findlater, P.S. Curry, C.A. Campbell, and W.D. Osei (2006). **Seroprevalence of West Nile virus in Saskatchewan's Five Hills Health Region, 2003.** *Canadian Journal of Public Health* 97(5): 369-373.

Abstract: BACKGROUND: The Five Hills Health Region of Saskatchewan reported the highest West Nile virus (WNV) case rates in the 2003 outbreak. A serologic and telephone survey was undertaken to assess the seroprevalence of the virus and the knowledge, attitudes and behaviours of the residents. METHODS: Respondents had to be at least 18 years of age, and residents of the Five Hills Health Region between July 1st and September 15th, 2003. Blood samples of respondents were tested at the National Microbiology Laboratory for flavivirus immunoglobulin using a WNV IgG ELISA and plaque reduction neutralization test. Descriptive analyses performed related to respondents' demographics, knowledge, attitudes, behaviours, and seropositivity. WNV infection risk was assessed using odds ratio. RESULTS: There were 619 questionnaire respondents, of whom 501 donated a blood sample. The seroprevalence of WNV in the Five Hills Health Region was 9.98% (95% CI 7.37-12.59%). Seropositivity of rural areas was 16.8% and urban was 3.2%. Most (97%) of participants thought WNV was an important health issue. Forty-eight percent of the participants used insect repellents containing DEET most of the time. There was good knowledge regarding WNV transmission and prevention of the spread of WNV. Rural compared to urban residents were six times more likely to be positive for WNV (OR=6.13, 95% CI 2.82-13.34). INTERPRETATION: This is the highest seroprevalence rate of West Nile virus recorded in North America thus far. Many factors could have influenced this outbreak, such as eco-region, early prolonged hot weather, level of mosquito control programs, urban and rural community differences, and personal protective behaviours.

Descriptors: disease outbreaks, West Nile fever, human attitudes to health, questionnaires, rural health, Saskatchewan, urban health, West Nile virus, blood.

Schweitzer, B.K., W.L. Kramer, A.R. Sambol, J.L. Meza, S.H. Hinrichs, and P.C. Iwen (2006). **Geographic factors contributing to a high seroprevalence of West Nile virus-specific antibodies in humans following an epidemic.** *Clinical and Vaccine Immunology* 13(3): 314-318. ISSN: 1556-6811.

Abstract: Sera of 624 blood donors were evaluated to determine seroprevalence of West Nile virus (WNV) antibodies following the 2003 WNV epidemic in Nebraska. Geographic factors contributing to differences in WNV seropositivity were evaluated. The overall prevalence of WNV in Nebraska was higher than reported previously in other U.S. locations (9.5% WNV immunoglobulin G seroprevalence rate), with the highest prevalence identified in the western part of the state (19.7%), followed by the central (13.8%) and the eastern (4.2%) parts. Regions of the state with the highest

WNV-positive mosquito rates correlated with the highest human WNV seroprevalence rates. The results showed that both the western and central parts of the state, where mosquito positivity rates were highest, had significantly higher seroprevalence rates than the eastern region. Additional studies are needed to determine whether the high prevalence rates in Nebraska will be reflected in other states and what impact environmental and geographical factors may have on future outbreaks of WNV infection.

Descriptors: viral blood antibodies, disease outbreaks, West Nile fever, West Nile virus, Nebraska, seroepidemiologic studies.

Shuai, J., P. Buck, P. Sockett, J. Aramini, and F. Pollari (2006). **A GIS-driven integrated real-time surveillance pilot system for national West Nile virus dead bird surveillance in Canada.** *International Journal of Health Geographics* 5: 17.

Abstract: BACKGROUND: An extensive West Nile virus surveillance program of dead birds, mosquitoes, horses, and human infection has been launched as a result of West Nile virus first being reported in Canada in 2001. Some desktop and web GIS have been applied to West Nile virus dead bird surveillance. There have been urgent needs for a comprehensive GIS services and real-time surveillance. RESULTS: A pilot system was developed to integrate real-time surveillance, real-time GIS, and Open GIS technology in order to enhance West Nile virus dead bird surveillance in Canada.Driven and linked by the newly developed real-time web GIS technology, this integrated real-time surveillance system includes conventional real-time web-based surveillance components, integrated real-time GIS components, and integrated Open GIS components. The pilot system identified the major GIS functions and capacities that may be important to public health surveillance. The six web GIS clients provide a wide range of GIS tools for public health surveillance. The pilot system has been serving Canadian national West Nile virus dead bird surveillance since 2005 and is adaptable to serve other disease surveillance. CONCLUSION: This pilot system has streamlined, enriched and enhanced national West Nile virus dead bird surveillance in Canada, improved productivity, and reduced operation cost. Its real-time GIS technology, static map technology, WMS integration, and its integration with non-GIS real-time surveillance system made this pilot system unique in surveillance and public health GIS.

Descriptors: bird diseases and associated mortality, sentinel surveillance, West Nile fever, automatic data processing methods, Canada, cluster analysis, pilot projects.

Sirigireddy, K.R., G.A. Kennedy, A. Broce, L. Zurek, and R.R. Ganta (2006). **High prevalence of west nile virus: a continuing risk in acquiring infection from a mosquito bite.** *Vector Borne and Zoonotic Diseases* 6(4): 351-360. ISSN: 1530-3667.

Abstract: The prevalence of West Nile Virus (WNV) was evaluated by diplex real-time RT-PCR assay for the years 2001-2005 in Culex species of mosquitoes, several

species of dead birds, and clinically suspected mammals collected in Kansas. The analysis was performed using a TaqMan-based diplex real-time RT-PCR assay targeted against two regions of the WNV genome, envelope glycoprotein gene and 3' untranslated region. The assay aided in the accurate detection of WNV in mosquitoes at high prevalence for the years 2002-2005. Similarly, high incidence of birds that tested positive for WNV was detected in 2002-2004. WNV positives in mammals by the diplex real rime RT-PCR assay included horses, squirrels, mules, sheep and a mountain goat. Majority of the equine WNV positives were detected only in the year 2002. Sequence analysis of a segment of the envelope glycoprotein gene from 31 randomly selected WNV positive samples revealed variations in six samples at one or two nucleotide positions. The identity of high levels of WNV positives in Kansas parallels the recent reports on the widespread distribution of the virus in the United States. The continued detection of WNV in the mosquitoes is of significant public health concern and calls for continued surveillance and public health activities.
Descriptors: West Nile virus, prevalnce, risk, evaluated, dead birds, mosquitoes, surveillance, public health, distribution.

Stein, K.J. and D.M. Claborn (2005). **Telephonic survey of surveillance and control procedures for the mosquito vectors of West Nile virus near naval installations in the Eastern United States**. *Military Medicine* 170(8): 658-662. ISSN: 0026-4075.
Online: http://www.ingentaconnect.com/content/amsus/zmm/2005/00000170/00000008/art00008
Descriptors: West Nile virus, telephone surveillance, control procedures, viral vectors, United States, dead birds, mosquitoes.

Stephen, C., N. Plamondon, and P. Belton (2006). **Notes on the distribution of mosquito species that could potentially transmit West Nile virus on Vancouver Island, British Columbia.** *Journal of the American Mosquito Control Association* 22(3): 553-556.
Abstract: We record the spatial and temporal distribution of 8 potential vectors of West Nile virus (WNV) on Vancouver Island in 2003 and 2004. Five species were widely distributed, but the other 3 were restricted to specific larval habitats. Adults were trapped from early April to September. The findings indicate a potential for WNV transmission if the virus arrives on the island. The results extend the published range of 5 mosquito species.
Descriptors: Culicidae virology, West Nile virus, Aedes, Anopheles, British Columbia, insect vectors, larva, population surveillance, West Nile fever transmission.

Stramer, S.L., B. Custer, M.P. Busch, and R.Y. Dodd (2006). **Strategies for testing blood donors for West Nile virus.** *Transfusion* 46(12): 2036-2037.
Descriptors: blood donors, West Nile virus, surveillance, strategies, detection, testing.

Sullivan, H., G. Linz, L. Clark, and M. Salman (2006). **West Nile virus antibody prevalence in red-winged blackbirds (*Agelaius phoeniceus*) from North Dakota, USA (2003-2004).** *Vector Borne and Zoonotic Diseases* 6(3): 305-309. ISSN: 1530-3667.
Abstract: This study was designed to explore the role that red-winged blackbirds (*Agelaius phoeniceus*) may have played in disseminating West Nile virus (WNV) across the United States. Using enzyme-linked immunosorbent assays designed to detect WNV antibodies in avian species we were able to determine the WNV antibody prevalence in a cohort of red-winged blackbirds in central North Dakota in 2003 and 2004. The peak WNV antibody prevalence was 22.0% in August of 2003 and 18.3% in July of 2004. The results of this study suggest that red-winged blackbird migratory populations may be an important viral dispersal mechanism with the ability to spread arboviruses such as WNV across the United States.
Descriptors: West Nile virus, red winged black birds, antibody prevalence, migratory populations, disseminating, disperal, spread.

Tachiiri, K., B. Klinkenberg, S. Mak, and J. Kazmi (2006). **Predicting outbreaks: A spatial risk assessment of West Nile virus in British Columbia.** *International Journal of Health Geographics* 5: 21.
Abstract: BACKGROUND: West Nile virus (WNv) has recently emerged as a health threat to the North American population. After the initial disease outbreak in New York City in 1999, WNv has spread widely and quickly across North America to every contiguous American state and Canadian province, with the exceptions of British Columbia (BC), Prince Edward Island and Newfoundland. In this study we develop models of mosquito population dynamics for *Culex tarsalis* and *C. pipiens*, and create a spatial risk assessment of WNv prior to its arrival in BC by creating a raster-based mosquito abundance model using basic geographic and temperature data. Among the parameters included in the model are spatial factors determined from the locations of BC Centre for Disease Control mosquito traps (e.g., distance of the trap from the closest wetland or lake), while other parameters were obtained from the literature. Factors not considered in the current assessment but which could influence the results are also discussed. RESULTS: Since the model performs much better for *C. tarsalis* than for *C. pipiens*, the risk assessment is carried out using the output of *C. tarsalis* model. The result of the spatially-explicit mosquito abundance model indicates that the Okanagan Valley, the Thompson Region, Greater Vancouver, the Fraser Valley and southeastern Vancouver Island have the highest potential abundance of the mosquitoes. After including human population data, Greater Vancouver,

due to its high population density, increases in significance relative to the other areas. CONCLUSION: Creating a raster-based mosquito abundance map enabled us to quantitatively evaluate WNv risk throughout BC and to identify the areas of greatest potential risk, prior to WNv introduction. In producing the map important gaps in our knowledge related to mosquito ecology in BC were identified, as well, it became evident that increased efforts in bird and mosquito surveillance are required if more accurate models and maps are to be produced. Access to real time climatic data is the key for developing a real time early warning system for forecasting vector borne disease outbreaks, while including social factors is important when producing a detailed assessment in urban areas.

Descriptors: *Culex sp*., forecasting methods, West Nile virus, British Columbia, geographic information systems (GIS), theoretical models, population density, population dynamics, risk, temperature.

Tilley, P.A., G.A. Zachary, R. Walle, and P.F. Schnee (2005). **West Nile virus detection and commercial assays.** *Emerging Infectious Diseases* 11(7): 1154-1155. ISSN: 1080-6040.

Descriptors: viral blood antibodies, blood immunoglobulin M, diagnostic reagent kits, West Nile fever diagnosis, West Nile virus isolation and purification, blood immunoglobulin G, time factors.

Tilley, P.A.G., R. Walle, A. Chow, G.C. Jayaraman, K. Fonseca, M.A. Drebot, J. Preiksaitis, and J. Fox (2005). **Clinical Utility of Commercial Enzyme Immunoassays during the Inaugural Season of West Nile Virus Activity, Alberta, Canada.** *Journal of Clinical Microbiology.* 43(9): 4691-4695. ISSN: 0095-1137.

Abstract: West Nile virus (WNV) has spread rapidly across North America, creating a need for rapid and accurate laboratory diagnosis on a large scale. Immunoglobulin M (IgM) capture enzyme immunoassays (EIA) became commercially available in the summer of 2003, but limited data are available on their clinical performance. Consolidated human WNV diagnostic testing for the province of Alberta, Canada, at the public health laboratory permitted a large-scale evaluation of the assays, covering a wide clinical spectrum. Two thousand nine hundred sixty-nine sera were tested, from 2,553 Alberta residents, and 266 cases were identified. Sensitivities of the Focus assay and first-generation Panbio IgM capture EIA were 79 and 80%, respectively. During the first week of illness only 53 to 58% of cases were positive, but sensitivity was 96 to 97% after day 8. Sensitivity for neurological cases was 92% overall. Specificity was high for the Focus kit at 98.9%, but only 82.9% for the first Panbio kit. A positive Focus WNV IgG result with a twofold rise in IgG index was a reliable indicator of acute flavivirus infection (67/67 WNV). Agreement between the IgG test and hemagglutinin inhibition titers in paired sera was at least 82%. Commercial IgM and IgG EIA proved useful for WNV diagnosis, provided follow-up sera were collected

after 8 days of illness.
Descriptors: West Nile virus, detection, surveillance, diagnosis, immunoglobulin M, enzyme assays, EIA, Canada.

Vamvakas, E.C., S. Kleinman, H. Hume, and G.D. Sher (2006). **The development of West Nile virus safety policies by Canadian blood services: Guiding principles and a comparison between Canada and the United States.** *Transfusion Medicine Reviews* 20(2): 97-109.
Abstract: To address the emerging threat of West Nile virus (WNV) to the blood supply, Canadian Blood Services (CBS) made a series of policy decisions that were either similar to those adopted in the United States or more stringent than policies formulated in the United States at the same time. More stringent Canadian policies included the development of an in-house WNV RNA assay, the stockpiling of frozen plasma components in the winter for transfusion in WNV-affected areas in the summer, a special recruitment campaign for red blood cell collections before the start of the 2003 WNV season, and an inventory exchange (ie, WNV-tested for untested red blood cells) initiated 2 weeks after the onset of WNV screening, as well as the implementation of targeted individual-donation WNV testing on August 2, 2004, in the absence of any positive donors or clinical cases of WNV infection in Canada. The general principles that guided CBS decision making with regard to WNV safety included application of the precautionary principle, harmonization with policies in the United States, a consideration of logistic issues, compliance with Health Canada requests, responsiveness to public expectations about transfusion safety, and transparency in decision making with timely communication to stakeholders. Before implementing WNV blood safety policies, CBS assessed their impact on blood availability. When policies were implemented, data were obtained quickly to ensure that the prior impact assessments were accurate. This review discusses the guiding principles affecting CBS policy development and compares CBS WNV safety policies to policies adopted in the United States.
Descriptors: blood bank standards, blood transfusion safety, West Nile fever prevention and control, West Nile virus, blood specimen collection standards, blood transfusion, Canada, health policy, United States.

Ward, M.P. (2006). **Spread of equine West Nile virus encephalomyelitis during the 2002 Texas epidemic.** *American Journal of Tropical Medicine and Hygiene* 74(6): 1090-1095. ISSN: 0002-9637.
Abstract: Using reports of clinical West Nile virus (WNV) encephalomyelitis in Texas equids during 2002, the distribution of disease was analyzed using cluster statistics and spatial modeling to develop hypotheses of disease spread during the first year of its detection. Significant ($P < 0.05$) clusters of cases reported early during the outbreak were identified in east, northcentral, and north Texas, and significant (P

< 0.05) clusters late during the outbreak were detected in central, south, and west Texas. Two counties on the south Texas coast first reported disease significantly (P < 0.05) earlier than their 10 nearest neighboring counties. The estimated incidence of disease was greatest in the high plains of north Texas and in northcentral Texas. Higher rates were also estimated in eastern and southern areas of the Gulf Coast. The spatial and temporal distribution observed indicates that the equine WNV epidemic began in two parts of Texas and spread elsewhere throughout the state. The mechanism of introduction and spread remains speculative.
Descriptors: disease outbreaks, horse diseases, West Nile fever, cluster analysis, geography, statistical models, Texas, zoonoses.

Ward, M.P. (2005). **Epidemic West Nile virus encephalomyelitis: A temperature-dependent, spatial model of disease dynamics.** *Preventive Veterinary Medicine* 71(3-4): 253-264.
Abstract: Since first being detected in New York in 1999, West Nile virus (WNV) has spread throughout the United States and more than 20,000 cases of equine WNV encephalomyelitis have been reported. A spatial model of disease occurrence was developed, using data from an outbreak of serologically confirmed disease in an unvaccinated population of horses at 108 locations in northern Indiana between 3 August and 17 October 2002. Daily maximum temperature data were recorded at meteorological stations surrounding the study area. The distribution of the total number of degree-days elapsing between July 4 and the date of diagnosis of each case was best described by a normal distribution (mean=5243 degrees F, S.D.=1047). The days on which the average risk was >25, >50 and >75% were predicted (versus observed) to occur on August 23 (August 9), August 31 (September 2) and September 9 (September 9). The epidemic was predicted to occur 3 days earlier, or 4 days later, than observed if temperatures in the study area were uniformly increased, or decreased, by 5 degrees F, respectively. Maps indicated that WNV encephalomyelitis risk always remained greater in the northwest quadrant of the study area. Since WNV might exist at a hypoendemic level of infection, and occasionally re-emerge as a cause of epidemics in equine populations, by identifying factors that contributed to this epidemic, the potential impact of future epidemics can be reduced. Such studies rely on a GIS framework, availability of meteorological and possibly remotely sensed data and information on host and landscape factors. An early-warning system for WNV transmission in equine populations could be developed.
Descriptors: animal disease outbreaks, horse diseases, West Nile fever, horses, Indiana, statistical models, space-time clustering, West Nile virus.

Ward, M.R., D.E. Stallknecht, J. Willis, M.J. Conroy, and W.R. Davidson (2006). **Wild bird mortality and West Nile virus surveillance: biases associated with detection, reporting, and carcass persistence.** *Journal of Wildlife Diseases* 42(1): 92-106. ISSN:

0090-3558.

Abstract: Surveillance targeting dead wild birds, in particular American crows (*Corvus brachyrhynchos*), plays a critical role in West Nile virus (WNV) surveillance in the United States. Using crow decoy surrogates, detection and reporting of crow carcasses within urban and rural environments of DeKalb County, Georgia were assessed for potential biases that might occur in the county's WNV surveillance program. In each of two replicated trials, during July and September 2003, 400 decoys were labeled with reporting instructions and distributed along randomly chosen routes throughout designated urban and rural areas within DeKalb County. Information-theoretic methods were used to compare alternative models incorporating the effects of area and trial on probabilities of detection and reporting. The model with the best empirical support included the effects of area on both detection and reporting of decoys. The proportion of decoys detected in the urban area (0.605, SE = 0.024) was approximately twice that of the rural area (0.293, SE = 0.023), and the proportion of decoys reported in the urban area (0.273, SE = 0.023) was approximately three times that of the rural area (0.103, SE = 0.028). These results suggest that human density and associated factors can substantially influence dead crow detection and reporting and, thus, the perceived distribution of WNV. In a second and separate study, the persistence and fate of American crow and house sparrow (*Passer domesticus*) carcasses were assessed in urban and rural environments in Athens-Clarke, Madison, and Oconee counties, Georgia. Two replicated trials using 96 carcasses of each species were conducted during July and September 2004. For a portion of the carcasses, motion sensitive cameras were used to monitor scavenging species visits. Most carcasses (82%) disappeared or were decayed by the end of the 6-day study. Carcass persistence averaged 1.6 days in rural areas and 2.1 days in urban areas. We analyzed carcass persistence rates using a known-fate model framework in program MARK. Model selection based on Akaike's Information Criteria (AIC) indicated that the best model explaining carcass persistence rates included species and number of days of exposure; however, the model including area and number of days of exposure received approximately equal support. Model-averaged carcass persistence rates were higher for urban areas and for crow carcasses. Six mammalian and one avian species were documented scavenging upon carcasses. Dead wild birds could represent potential sources of oral WNV exposure to these scavenging species. Species composition of the scavenger assemblage was similar in urban and rural areas but "scavenging pressure" was greater in rural areas.

Descriptors: aves, ecological techniques, wild bird mortality monitoring and application in West Nile virus surveillance, biases, pathological techniques, viral diseases, West Nile virus, wild bird mortality monitoring application in disease surveillance, mortality, Georgia USA.

Ward, M., D. Stallknecht, J. Willis, M. Conroy, and W. Davidson (2006). **Wild bird mortality and West Nile virus surveillance: biases associated with detection, reporting, and carcass persistence.** *Journal of Wildlife Diseases.* 42(1): 92-106. ISSN: 0090-3558.

Descriptors: disease surveillance, *Corvus brachyrhynchos*, dead animals, *Passer domesticus*, urban areas, rural areas, disease transmission, mathematical models, Georgia, scavenging.

White, B.J., D.R. Andrew, N.Z. Mans, O.A. Ohajuruka, and M.C. Garvin (2006). **West Nile virus in mosquitoes of northern Ohio, 2003.** *American Journal of Tropical Medicine and Hygiene* 75(2): 346-349. ISSN: subcutaneous.

Abstract: From June 19, 2003 to August 18, 2003, we surveyed the mosquitoes of Oberlin, OH, for West Nile Virus (WNV) infection using reverse transcriptase-polymerase chain reaction. A total of 12,055 mosquitoes, representing 17 species or species groups and 4 genera, were collected in gravid traps at seven sites throughout the city, with *Culex pipiens/restuans* being the most abundant and showing the highest minimum infection rate (MIR) of 0.78. This represents a decrease in WNV enzootic activity from the previous year. Both *Cx. pipiens/restuans* abundance and MIR increased significantly with date. However, we found no correlation between *Cx. pipiens/restuans* abundance and MIR.

Descriptors: culex mosquitos, insect vectors, West Nile virus isolation and purification, culicidae classification, Ohio, population density, population surveillance methods, reverse transcriptase polymerase chain reaction methods, time factors, West Nile fever transmission.

Vaccines and Immunology

Brien, J.D. and J. Nikolich Zugich (2006). **Immunological basis of age-related vulnerability to West Nile virus**. *Journal of Immunology* 176(Suppl. S): S121. ISSN: 0022-1767.
Descriptors: immune system, West Nile fever, nervous system disease, viral disease, immune response, age related vulnerability.
Notes: Meeting Information: Annual Meeting of the American Association of Immunologists, Boston, Massachusettes, USA; May 12 -16, 2006.

Chiang, Y., C. Jensen, T. Holt, C. Waldbillig, D. Hathaway, N. Jennings, T. Ng, and H. Chu (2005). **Demonstration of efficacy of a West Nile virus DNA vaccine in foals.** *Proceedings of the Annual Convention of the American Association of Equine Practioners* 51(51): 183-190 . ISSN: 0065-7182.
Descriptors: mosquito borne diseases, vaccine development, maternal immunity, antibody formation, plasmids, immunoglobulin G, viremia, foals, West Nile virus, DNA.
Notes: Meeting Information: December 3-7, 2005 in Seattle, Washington.

Davidson, A.H., J.L. Traub Dargatz, R.M. Rodeheaver, E.N. Ostlund, D.D. Pedersen, R.G. Moorhead, J.B. Stricklin, R.D. Dewell, S.D. Roach, R.E. Long, S.J. Albers, R.J. Callan, and M.D. Salman (2005). **Immunologic responses to West Nile virus in vaccinated and clinically affected horses.** *Journal of the American Veterinary Medical Association* 226(2): 240-245.
Abstract: OBJECTIVE: To compare neutralizing antibody response between horses vaccinated against West Nile virus (WNV) and horses that survived naturally occurring infection. DESIGN: Cross-sectional observational study. ANIMALS: 187 horses vaccinated with a killed WNV vaccine and 37 horses with confirmed clinical WNV infection. PROCEDURE: Serum was collected from vaccinated horses prior to and 4 to 6 weeks after completion of an initial vaccination series (2 doses) and 5 to 7 months later. Serum was collected from affected horses 4 to 6 weeks after laboratory diagnosis of infection and 5 to 7 months after the first sample was obtained. The IgM capture ELISA, plaque reduction neutralization test (PRNT), and microtiter virus neutralization test were used. RESULTS: All affected horses had PRNT titers > or = 1:100 at 4 to 6 weeks after onset of disease, and 90% (18/20) maintained this titer for 5 to 7 months. After the second vaccination, 67% of vaccinated horses had PRNT titers > or = 1:100 and 14% had titers < 1:10. Five to 7 months later, 33% (28/84) of vaccinated horses had PRNT titers > or = 1:100, whereas 29% (24/84)

had titers < 1:10. Vaccinated and clinically affected horses' end point titers had decreased by 5 to 7 months after vaccination. CONCLUSIONS AND CLINICAL RELEVANCE: A portion of horses vaccinated against WNV may respond poorly. Vaccination every 6 months may be indicated in certain horses and in areas of high vector activity. Other preventative methods such as mosquito control are warranted to prevent WNV infection in horses.
Descriptors: horse diseases, viral vaccines, West Nile fever, West Nile virus, enzyme linked immunosorbent assay, mosquito control.

Despres, P., C. Combredet, M.P. Frenkiel, C. Lorin, M. Brahic, and F. Tangy (2005). **Live measles vaccine expressing the secreted form of the West Nile virus envelope glycoprotein protects against West Nile virus encephalitis.** *Journal of Infectious Diseases* 191(2): 207-214.
Abstract: The Schwarz strain of measles virus (MV), a live attenuated RNA virus, is one of the safest and most effective human vaccines available. Immunization with MV vaccine expressing heterologous antigen is an attractive strategy to prevent emerging viral diseases. West Nile virus (WNV), which recently emerged in North America, is an important mosquito-borne flavivirus that causes numerous cases of human encephalitis, thus urging the development of a vaccine. To evaluate the efficacy of recombinant MV for the prevention of WNV encephalitis, we constructed a live attenuated Schwarz MV (MVSchw-sE(WNV)) expressing the secreted form of the envelope glycoprotein from the virulent IS-98-ST1 strain of WNV. Inoculation of MV-susceptible mice with MVSchw-sE(WNV) induced both high levels of specific anti-WNV neutralizing antibodies and protection from a lethal challenge with WNV. Passive administration with antisera to MVSchw-sE(WNV) prevented WNV encephalitis in BALB/c mice challenged with a high dose of WNV. The present study is the first to report that a recombinant live attenuated vector based on an approved and widely used MV vaccine can protect against a heterologous, medically important pathogen.
Descriptors: encephalitis, measles vaccine, viral envelope proteins, West Nile virus, *Cercopithecus aethiops*, molecular cloning, inbred BalbC mice.

Eisenstein, M. (2005). **Antibody neutralizes West Nile virus.** *Lab Animal* 34(6): 10.
Descriptors: viral antibodies immunology, West Nile fever, West Nile virus cell line, mice, neutralization tests.

Epp, T., C. Waldner, K. West, F. Leighton, and H. Townsend (2005). **Efficacy of vaccination for West Nile virus in Saskatchewan horses.** *Proceedings of the Annual Convention of the American Association of Equine Practioners* 51(51): 180-182. ISSN: 0065-7182.
Descriptors: horses, mosquito borne diseases, inactivated vaccines, mosquito

control, disease incidence, West Nile virus, Saskatchewan.
Notes: Meeting Information: December 3-7, 2005 in Seattle, Washington.

Gould, L.H., J. Sui, H. Foellmer, T. Oliphant, T. Wang, M. Ledizet, A. Murakami, K. Noonan, C. Lambeth, K. Kar, J.F. Anderson, A.M. de Silva, M.S. Diamond, R.A. Koski, W.A. Marasco, and E. Fikrig (2005). **Protective and therapeutic capacity of human single-chain Fv-Fc fusion proteins against West Nile virus.** *Journal of Virology* 79(23): 14606-14613. ISSN: 0022-538X.
Abstract: West Nile virus has spread rapidly across the United States, and there is currently no approved human vaccine or therapy to prevent or treat disease. Passive immunization with antibodies against the envelope protein represents a promising means to provide short-term prophylaxis and treatment for West Nile virus infection. In this study, we identified a panel of 11 unique human single-chain variable region antibody fragments (scFvs) that bind the envelope protein of West Nile virus. Selected scFvs were converted to Fc fusion proteins (scFv-Fcs) and were tested in mice for their ability to prevent lethal West Nile virus infection. Five of these scFv-Fcs, 11, 15, 71, 85, and 95, protected 100% of mice from death when given prior to infection with virus. Two of them, 11 and 15, protected 80% of mice when given at days 1 and 4 after infection. In addition, four of the scFv-Fcs cross-neutralized dengue virus, serotype 2. Binding assays using yeast surface display demonstrated that all of our scFvs bind to sites within domains I and II of West Nile virus envelope protein. These recombinant human scFvs are potential candidates for immunoprophylaxis and therapy of flavivirus infections.
Descriptors: viral antibodies, viral vaccines , West Nile fever prevention and control, West Nile virus, antibody specificity, immunoglobulin fragments, recombinant fusion proteins.

Iglesias, M.C., M.P. Frenkiel, K. Mollier, P. Souque, P. Despres, and P. Charneau (2006). **A single immunization with a minute dose of a lentiviral vector-based vaccine is highly effective at eliciting protective humoral immunity against West Nile virus.** *Journal of Gene Medicine* 8(3): 265-274.
Abstract: BACKGROUND: Lentiviral vectors, due to their capacity to transduce non-dividing cells, have become precious and worldwide used gene transfer systems. Their ability to efficiently and stably transduce dendritic cells (DCs) has led to their successful use as vaccination vectors for eliciting strong, specific and protective cellular immune responses mostly in anti-tumoral but also in anti-viral applications. However, the ability of lentiviral vectors to elicit an antibody-based protective immunity has, to date, not been evaluated. In the present study, we evaluated the potential of a lentiviral vector-based vaccine to elicit humoral immunity against West Nile virus (WNV). WNV is a mosquito-borne flavivirus that emerged in North America and causes encephalitis in humans, birds and horses. Neutralizing anti-WNV anti-

bodies have been shown to be crucial for protection against WNV encephalitis. METHODS: The ability of lentiviral vector TRIP/sE(WNV), expressing the secreted soluble form of the envelope E-glycoprotein (sE(WNV)) from the highly virulent IS-98-ST1 strain of WNV, to induce a specific humoral response and protection against WNV infection was assessed in a mouse model of WNV encephalitis. RESULTS: Remarkably, a single immunization with a minute dose of TRIP/sE(WNV) was efficient at eliciting a long-lasting, protective and sterilizing humoral immunity, only 1 week after priming. CONCLUSIONS: This study broadens the applicability of lentiviral vectors as efficient non-replicating vaccines against pathogens for which a neutralizing humoral response is one active arm of the protective immunity. The TRIP/sE(WNV) lentiviral vector appears to be a promising tool for veterinary vaccination against zoonotic WNV.

Descriptors: antibody formation, lentivirus, viral vaccines, West Nile fever, West Nile virus, genetic vectors, immunization schedule, mice, transduction, viral envelope proteins.

Johnson, B.W., O. Kosoy, D.A. Martin, A.J. Noga, B.J. Russell, A.A. Johnson, and L.R. Petersen (2005). **West Nile virus infection and serologic response among persons previously vaccinated against yellow fever and Japanese encephalitis viruses.** *Vector Borne and Zoonotic Diseases* 5(2): 137-145. ISSN: 1530-3667.

Abstract: It is hypothesized that previous heterologous flaviviral exposure may modulate clinical illness among persons infected with West Nile virus (WNV). Little is known about the serological response in such persons. In summer 2003, a WNV outbreak occurred in Colorado, the location of the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases (DVBID). DVBID employees, most previously vaccinated with yellow fever virus (YFV) or Japanese encephalitis virus (JEV) vaccines, were studied to determine whether previous vaccination affected symptom development among those subsequently infected with WNV during the outbreak, as well as their serological response. Serum samples collected in December 2003 and previously banked samples were tested using the plaque reduction neutralization test (PRNT) against WNV, Saint Louis encephalitis virus, dengue- 4 virus, JEV, and YFV. Specimens shown to have WNV antibody by PRNT were tested by IgM and IgG enzymelinked immunosorbent assays (ELISAs). Ten (9%) of 113 serosurvey participants had WNV neutralizing antibody titers in December 2003. PRNT titers from previous specimens showed that one of the ten had seroconverted to WNV before 2003. Of the remaining nine participants, seven reported illness in the summer of 2003, two of which were unvaccinated and five previously vaccinated. In the December 2003 specimens, five persons previously unvaccinated or vaccinated only against YFV had a fourfold or greater neutralizing titer with WNV than with other flaviviruses, whereas no persons previously vaccinated against JEV or JEV and

YFV showed a similar difference in neutralizing titers. Eight of nine persons infected in 2003 had negative or indeterminate WNV MAC-ELISA results in the December 2003 sample; the ninth person was vaccinated against YFV one month previously, and was also YFV positive by MAC-ELISA. We conclude that previous flaviviral vaccination does not markedly affect the development of WNV fever and that the IgM antibody response in patients without neuroinvasive WNV disease is transient.
Descriptors: Japanese encephalitis vaccines, adverse effects, West Nile fever immunology, West Nile virus, yellow fever vaccine, viral biosynthesis, Colorado, enzyme linked immunosorbent assay.

Johnson, S. (2005). **Avian titer development against West Nile virus after extralabel use of an equine vaccine.** *Journal of Zoo and Wildlife Medicine* 36(2): 257-264. ISSN: 1042-7260.
Abstract: West Nile virus affects many animals, but the highest prevalence of morbidity and mortality is observed in birds, horses, and humans. The purpose of this study was to determine a protocol in birds of prey and corvids, using a vaccine developed for horses. The birds were assigned to five groups. Groups 1-4 received 0.25 ml, 0.5 ml, 0.75 ml, and 1.0 ml, respectively, and group 5 served as a control group. The greatest percentage of seroconversion (58.3%) was observed in the vaccine group that received a dose of 1.0 ml administered thrice, 3 wk apart. This report demonstrates that a vaccine developed for equines against West Nile virus can be administered to birds.
Descriptors: corvidae, falconiformes, strigiformes, treatment techniques, vaccination, protocol development for West Nile virus using equine vaccine, viral diseases, West Nile virus, immune response, antibodies.

Julander, J.G., Q.A. Winger, A.L. Olsen, C.W. Day, R.W. Sidwell, and J.D. Morrey (2005). **Treatment of West Nile virus-infected mice with reactive immunoglobulin reduces fetal titers and increases dam survival.** *Antiviral Research* 65(2): 79-85. ISSN: 0166-3542.
Abstract: The objectives of this study were to determine if injection of West Nile virus (WNV) into timed-pregnant mice would result in fetal infection and if administration of WNV-reactive immunoglobulin would increase dam survival and reduce fetal viral titers. Dams injected on 7.5 days post-coitus (dpc) had detectable viral titers in the placenta 10.5dpc with a mean titer of 10(4.9) 50% cell-culture infectious doses per gram of tissue (CCID(50)/g tissue). The mean placental titer increased to 10(8.6)CCID(50)/g tissue at 12.5dpc. Infectious virus was detectable 12.5dpc in 10 of 10 fetuses with a mean titer of 10(7.5)CCID(50)/g tissue. Treatment of dams (challenged with WNV on 7.5dpc) with WNV-reactive human immunoglobulin (Ig) on 8.5 and 9.5dpc resulted in a significant reduction of virus in fetuses as compared with non-reactive human Ig-treated females on 12.5dpc (P< or =0.001). Treatment

also resulted in survival of dams to term. Treatment of dams with WNV-reactive human Ig on 12.5 and 13.5dpc also resulted in reduction of viral titer on 14.5dpc, indicating that later treatment may also be efficacious. This suggests that Ig treatment may be useful in treating fetal WNV infection in women.
Descriptors: viral antibodies, immunoglobulins, pregnancy complications, West Nile fever , West Nile virus, disease transmission, mice, infectious immunology.

Karaca, K., R. Bowen, L.E. Austgen, M. Teehee, L. Siger, D. Grosenbaugh, L. Loosemore, J.C. Audonnet, R. Nordgren, and J.M. Minke (2005). **Recombinant canarypox vectored West Nile virus (WNV) vaccine protects dogs and cats against a mosquito WNV challenge.** *Vaccine* 23(29): 3808-3813.
Abstract: The safety and efficacy of a canarypox vector expressing PrM and E genes of West Nile virus (WNV) (ALVAC-WNV) was evaluated in dogs and cats. One group of 17 dogs (vaccinated with 10(5.6) TCID(50)) and two groups of cats (groups 1 [n=14] vaccinated with 10(7.5) TCID(50) and 2 [n=8] 10(5.6) TCID(50)) were vaccinated twice at 28-day intervals. Fifteen dogs and eleven cats served as negative controls. The cats and dogs were challenged 120 and 135 days after the second immunization, respectively via the bites of Aedes albopictus mosquitoes infected with WNV. The first dose of vaccine induced a detectable antibody response in four dogs and five cats (one immunized with low and four with high doses). After the second dose, all the vaccinated dogs and all of the cats, immunized with high dose had detectable antibody titers, whereas only four of eight cats in the low dose group were seropositive. None of the vaccinated dogs and one vaccinated cat developed viremia following the WNV mosquito-challenge. In contrast, 14 of the 15 control dogs and 9 of the 11 control cats developed viremia. The experimental vaccine described in this study may be of value in the prevention of WNV infection in dogs and cats.
Descriptors: canarypox vector, cat diseases , dog diseases, viral vaccines, West Nile fever, West Nile virus, viral blood antibodies, synthetic genetics, viral envelope proteins, viral vaccines, West Nile fever prevention and control.

Ledizet, M., K. Kar, H.G. Foellmer, T. Wang, S.L. Bushmich, J.F. Anderson, E. Fikrig, and R.A. Koski (2005). **A recombinant envelope protein vaccine against West Nile virus.** *Vaccine* 23(30): 3915-3924.
Abstract: West Nile (WN) virus is a flavivirus that first appeared in North America in 1999. Since then, more than 600 human deaths and 22,000 equine infections have been attributed to the virus in the United States. We expressed a truncated form of WN virus envelope (E) protein in Drosophila S2 cells. This soluble recombinant E protein was recognized by antibodies from naturally infected horses, indicating that it contains native epitopes. Mice and horses produced high-titer antibodies when immunized with recombinant E protein combined with aluminum hydroxide. Immunized mice were resistant to challenge with a lethal viral dose. Sera from immu-

nized horses, administered to naive mice, conferred resistance against a lethal WN viral challenge. In addition, sera of immunized horses neutralized West Nile virus in vitro, as demonstrated by plaque reduction assays. This recombinant form of E protein, combined with aluminum hydroxide, is a candidate vaccine that may protect humans and horses against WN virus infections.
Descriptors: viral envelope proteins, viral vaccines, West Nile fever, West Nile virus, immunologic adjuvants, aluminum hydroxide, cultured cells, drosophila, horses, passive immunization, immunoglobulin G biosynthesis, inbred C57BL mice, plaque assay, protein conformation, synthetic vaccines, viral envelope.

Long, M., E. Gibbs, K. Seino, M. Mellencamp, S. Zhang, S. Beachboard, and P. Humphrey (2005). **Safety and efficacy of a live attenuated West Nile virus chimera vaccine in horses with experimentally induced West Nile virus clinical disease.** *Proceedings of the Annual Convention of the American Association of Equine Practioners* 51(51): 177-179. ISSN: 0065-7182.
Descriptors: live vaccines, antibody formation, histopathology, immunity, West Nile virus, safety, efficacy, horses.
Notes: Meeting Information: December 3-7, 2005 in Seattle, Washington.

Mateo, R., S.Y. Xiao, H. Guzman, H. Lei, A.P. Da Rosa, and R.B. Tesh (2006). **Effects of immunosuppression on West Nile virus infection in hamsters.** *American Journal of Tropical Medicine and Hygiene* 75(2): 356-362. ISSN: 0002-9637.
Abstract: A research study, comparing the pathogenesis of experimental West Nile virus (WNV) infection in immunocompetent and immunosuppressed golden hamsters, is described. Cyclophosphamide was used to immunosuppress the animals. The immunosuppressed hamsters had a prolonged period of viremia, depressed humoral immune response, more extensive and severe pathology, and higher fatality rate than the untreated immunocompetent animals. Histopathological and immunohistochemical studies of tissues from the two groups showed that pathologic changes in the untreated infected animals were confined to the brain and spinal cord, whereas the histopathological changes and WNV antigen distribution in the immunosuppressed animals were much more extensive and diffuse, involving the adrenal, kidney, heart and lung, and brain and spinal cord. Results of this study in the hamster model provide insight into the increased severity of WNV infection observed in immunosuppressed people.
Descriptors: central nervous system, West Nile fever, West Nile virus, antibody formation, viral isolation and purification, *Cercopithecus aethiops*, cyclophosphamide, disease models, animal immunocompetence, immunocompromised host, immunosuppressive agents.

Mehlhop, E. and M.S. Diamond (2006). **Protective immune responses against West Nile virus are primed by distinct complement activation pathways.** *Journal of Experimental Medicine* 203(5): 1371-1381.

Abstract: West Nile virus (WNV) causes a severe infection of the central nervous system in several vertebrate animals including humans. Prior studies have shown that complement plays a critical role in controlling WNV infection in complement (C) 3(-/-) and complement receptor 1/2(-/-) mice. Here, we dissect the contributions of the individual complement activation pathways to the protection from WNV disease. Genetic deficiencies in C1q, C4, factor B, or factor D all resulted in increased mortality in mice, suggesting that all activation pathways function together to limit WNV spread. In the absence of alternative pathway complement activation, WNV disseminated into the central nervous system at earlier times and was associated with reduced CD8+ T cell responses yet near normal anti-WNV antibody profiles. Animals lacking the classical and lectin pathways had deficits in both B and T cell responses to WNV. Finally, and somewhat surprisingly, C1q was required for productive infection in the spleen but not for development of adaptive immune responses after WNV infection. Our results suggest that individual pathways of complement activation control WNV infection by priming adaptive immune responses through distinct mechanisms.

Descriptors: CD8 positive T lymphocytes, West Nile fever, West Nile virus antibodies, B lymphocytes immunology, central nervous system, knockout mice, spleen.

Mehlhop, E., K. Whitby, T. Oliphant, A. Marri, M. Engle, and M.S. Diamond (2005). **Complement activation is required for induction of a protective antibody response against West Nile virus infection.** *Journal of Virology* 79(12): 7466-7477. ISSN: 0022-538X.

Abstract: Infection with West Nile virus (WNV) causes a severe infection of the central nervous system (CNS) with higher levels of morbidity and mortality in the elderly and the immunocompromised. Experiments with mice have begun to define how the innate and adaptive immune responses function to limit infection. Here, we demonstrate that the complement system, a major component of innate immunity, controls WNV infection in vitro primarily in an antibody-dependent manner by neutralizing virus particles in solution and lysing WNV-infected cells. More decisively, mice that genetically lack the third component of complement or complement receptor 1 (CR1) and CR2 developed increased CNS virus burdens and were vulnerable to lethal infection at a low dose of WNV. Both C3-deficient and CR1- and CR2-deficient mice also had significant deficits in their humoral responses after infection with markedly reduced levels of specific anti-WNV immunoglobulin M (IgM) and IgG. Overall, these results suggest that complement controls WNV infection, in part through its ability to induce a protective antibody response.

Descriptors: viral blood antibodies, complement activation, West Nile fever, West Nile virus, complement c3 genetics, cricetinae, mice, neutralization tests, complement genetics, West Nile fever.

Monath, T.P., J. Liu, N. Kanesa Thasan, G.A. Myers, R. Nichols, A. Deary, K. McCarthy, C. Johnson, T. Ermak, S. Shin, J. Arroyo, F. Guirakhoo, J.S. Kennedy, F.A. Ennis, S. Green, and P. Bedford (2006). **A live, attenuated recombinant West Nile virus vaccine.** *Proceedings of the National Academy of Sciences of the United States of America* 103(17): 6694-6699.

Abstract: West Nile (WN) virus is an important cause of febrile exanthem and encephalitis. Since it invaded the U.S. in 1999, >19,000 human cases have been reported. The threat of continued epidemics has spurred efforts to develop vaccines. ChimeriVax-WN02 is a live, attenuated recombinant vaccine constructed from an infectious clone of yellow fever (YF) 17D virus in which the premembrane and envelope genes of 17D have been replaced by the corresponding genes of WN virus. Preclinical tests in monkeys defined sites of vaccine virus replication in vivo. ChimeriVax-WN02 and YF 17D had similar biodistribution but different multiplication kinetics. Prominent sites of replication were skin and lymphoid tissues, generally sparing vital organs. Viruses were cleared from blood by day 7 and from tissues around day 14. In a clinical study, healthy adults were inoculated with 5.0 log(10) plaque-forming units (PFU) (n = 30) or 3.0 log10 PFU (n = 15) of ChimeriVax-WN02, commercial YF vaccine (YF-VAX, n = 5), or placebo (n = 30). The incidence of adverse events in subjects receiving the vaccine was similar to that in the placebo group. Transient viremia was detected in 42 of 45 (93%) of ChimeriVax-WN02 subjects, and four of five (80%) of YF-VAX subjects. All subjects developed neutralizing antibodies to WN or YF, respectively, and the majority developed specific T cell responses. ChimeriVax-WN02 rapidly elicits strong immune responses after a single dose, and is a promising candidate warranting further evaluation for prevention of WN disease.

Descriptors: viral vaccines, West Nile virus, double blind method, *Macaca fascicularis*, molecular sequence data, neutralization tests, safety, T lymphocytes, attenuated recombinant vaccines, infectious clone of yellow fever, preclinical tests, ChimeriVax-WN02, prevention of WN fever.

Oliphant, T., M. Engle, G.E. Nybakken, C. Doane, S. Johnson, L. Huang, S. Gorlatov, E. Mehlhop, A. Marri, K.M. Chung, G.D. Ebel, L.D. Kramer, D.H. Fremont, and M.S. Diamond (2005). **Development of a humanized monoclonal antibody with therapeutic potential against West Nile virus.** *Nature Medicine* 11(5): 522-530.

Abstract: Neutralization of West Nile virus (WNV) in vivo correlates with the development of an antibody response against the viral envelope (E) protein. Using random mutagenesis and yeast surface display, we defined individual contact residues of 14

newly generated monoclonal antibodies against domain III of the WNV E protein. Monoclonal antibodies that strongly neutralized WNV localized to a surface patch on the lateral face of domain III. Convalescent antibodies from individuals who had recovered from WNV infection also detected this epitope. One monoclonal antibody, E16, neutralized 10 different strains in vitro, and showed therapeutic efficacy in mice, even when administered as a single dose 5 d after infection. A humanized version of E16 was generated that retained antigen specificity, avidity and neutralizing activity. In postexposure therapeutic trials in mice, a single dose of humanized E16 protected mice against WNV-induced mortality, and may therefore be a viable treatment option against WNV infection in humans.
Descriptors: monoclonal antibodies, viral envelope (E) protein, neutralization of West Nile virus in vivo, mice, treatment option.

Pletnev, A.G., D.E. Swayne, J. Speicher, A.A. Rumyantsev, and B.R. Murphy (2006). **Chimeric West Nile/dengue virus vaccine candidate: Preclinical evaluation in mice, geese and monkeys for safety and immunogenicity.** *Vaccine* 24(40-41): 6392-6404.
Abstract: A live attenuated virus vaccine is being developed to protect against West Nile virus (WN) disease in humans. Previously, it was found that chimeric West Nile/dengue viruses (WN/DEN4 and WN/DEN4Delta30) bearing the membrane precursor and envelope protein genes of WN on a backbone of dengue type 4 virus (DEN4) with or without a deletion of 30 nucleotides (Delta30) in the 3' noncoding region of the DEN4 part of the chimeric genome were attenuated and efficacious in mice and monkeys against WN challenge. Here, we report the generation of a clinical lot of WN/DEN4Delta30 virus and its further preclinical evaluation for safety and immunogenicity in mice, geese and monkeys. The vaccine candidate had lost neuro-invasiveness in highly sensitive immunodeficient mice inoculated intraperitoneally and had greatly reduced neurovirulence in suckling mice inoculated intracerebrally (IC). Compared to the wild-type WN parent, the chimeric virus was highly restricted in replication in both murine and human neuroblastoma cells as well as in brains of suckling mice. The WN/DEN4Delta30 virus failed to infect geese, indicating that chimerization of WN with DEN4 completely attenuated WN for this avian host. This observation suggests that the WN/DEN4 chimeric viruses would be restricted in their ability to be transmitted from vaccinees to domestic or wild birds. In monkeys, the WN/DEN4Delta30 vaccine candidate was highly immunogenic despite its low level of replication with undetectable viremia. Furthermore, the WN/DEN4Delta30 vaccine virus was safe and readily induced neutralizing antibodies against WN in monkeys immune to each of the four serotypes of dengue virus. These studies confirm the attenuation of WN/DEN4Delta30 for non-human primates, including dengue-immune monkeys, and demonstrate both a highly restricted replication

(>10(8)-fold decrease) in the brain of mice inoculated IC and an absence of infectivity for birds, findings that indicate this vaccine should be safe for both the recipient and the environment.
Descriptors: West Nile virus, live attenuated vaccine, mice, monkeys, preclinical evaluation, geese, induced neutralizing antibodies.

Redig, P., M. Saggese, T. Tully, A. Roy, B. Ritchie, and A. Allison (2005). **Update on West Nile virus disease and vaccine**. In: *Small animal and exotics: Proceedings of the North American Veterinary Conference, January 8, 2005-January 12, 2005, Orlando, Florida, USA,* Eastern States Veterinary Association: Gainesville, USA, Vol. 19, p. 1197-1198.
Online: http://www.navc.org
Descriptors: West Nile virus, virus disease, vaccines, updates.

Samina, I., Y. Khinich, M. Simanov, and M. Malkinson (2005). **An inactivated West Nile virus vaccine for domestic geese-efficacy study and a summary of 4 years of field application.** *Vaccine* 23(41): 4955-4958.
Abstract: Following the isolation in 1997 of West Nile virus from the brains of geese with an acute neuroparalytic disease in Israel, which reappeared in the following years, an inactivated vaccine was prepared from suckling mouse brains. The brain homogenate was inactivated with formaldehyde and blended with mineral oil adjuvant. In 2000, the first flocks were vaccinated according to a schedule of two subcutaneous doses, commencing at the age of 2 weeks and given with a 2-weeks interval. In efficacy trials, the challenge virus was injected at 7 weeks by the intracranial route, and over 85% protection was recorded in vaccinated geese. In extensive field trials conducted in 2001--2003, the vaccine was demonstrated to be safe and efficacious, and over 3 million doses were manufactured in 2000--2003.
Descriptors: bird diseases, geese, Israel, inactivate vaccine for West Nile virus, vaccination of birds, challenge virus, 85% protection rate, field trials of vaccines.

Samuel, M.A. and M.S. Diamond (2006). **Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion.** *Journal of Virology* 80(19): 9349-9360. ISSN: 0022-538X.
Descriptors: immunity, natural immunology, West Nile fever, West Nile virus, central nervous system, disease susceptibility.

Samuel, M.A. and M.S. Diamond (2005). **Alpha/beta interferon protects against lethal West Nile virus infection by restricting cellular tropism and enhancing neuronal survival.** *Journal of Virology* 79(21): 13350-13361. ISSN: 0022-538X.
Abstract: West Nile virus (WNV) is a mosquito-borne flavivirus that is neurotropic in humans, birds, and other animals. While adaptive immunity plays an important role in preventing WNV spread to the central nervous system (CNS), little is known

about how alpha/beta interferon (IFN-alpha/beta) protects against peripheral and CNS infection. In this study, we examine the virulence and tropism of WNV in IFN-alpha/beta receptor-deficient (IFN- alpha/betaR-/-) mice and primary neuronal cultures. IFN-alpha/betaR-/- mice were acutely susceptible to WNV infection through subcutaneous inoculation, with 100% mortality and a mean time to death (MTD) of 4.6 +/- 0.7 and 3.8+/- 0.5 days after infection with 10(0) and 10(2) PFU, respectively. In contrast, congenic wild-type 129Sv/Ev mice infected with 10(2) PFU showed 62% mortality and a MTD of 11.9 +/- 1.9 days. IFN-alpha/betaR-/- mice developed high viral loads by day 3 after infection in nearly all tissues assayed, including many that were not infected in wild-type mice. IFN-alpha/betaR-/- mice also demonstrated altered cellular tropism, with increased infection in macrophages, B cells, and T cells in the spleen. Additionally, treatment of primary wild-type neurons in vitro with IFN-beta either before or after infection increased neuronal survival independent of its effect on WNV replication. Collectively, our data suggest that IFN-alpha/beta controls WNV infection by restricting tropism and viral burden and by preventing death of infected neurons.

Descriptors: interferon alpha analysis, interferon beta analysis, West Nile fever , West Nile virus, antiviral agents, brain immunology, cultured cells, cultured, inbred mice, skeletal muscle, organ specificity, messenger RNA genetics, reverse transcriptase polymerase chain reaction, spinal cord , spleen, virus replication.

Samuel, M.A., K. Whitby, B.C. Keller, A. Marri, W. Barchet, B.R. Williams, R.H. Silverman, M.J. Gale, and M.S. Diamond (2006). **PKR and RNase L contribute to protection against lethal West Nile Virus infection by controlling early viral spread in the periphery and replication in neurons.** *Journal of Virology* 80(14): 7009-7019. ISSN: 0022-538X.

Abstract: West Nile virus (WNV) is a neurotropic, mosquito-borne flavivirus that can cause lethal meningoencephalitis. Type I interferon (IFN) plays a critical role in controlling WNV replication, spread, and tropism. In this study, we begin to examine the effector mechanisms by which type I IFN inhibits WNV infection. Mice lacking both the interferon-induced, double-stranded-RNA-activated protein kinase (PKR) and the endoribonuclease of the 2',5'-oligoadenylate synthetase-RNase L system (PKR(-/-) x RL(-/-)) were highly susceptible to subcutaneous WNV infection, with a 90% mortality rate compared to the 30% mortality rate observed in congenic wild-type mice. PKR(-/-) x RL(-/-) mice had increased viral loads in their draining lymph nodes, sera, and spleens, which led to early viral entry into the central nervous system (CNS) and higher viral burden in neuronal tissues. Although mice lacking RNase L showed a higher CNS viral burden and an increased mortality, they were less susceptible than the PKR(-/-) x RL(-/-) mice; thus, we also infer an antiviral role for PKR in the control of WNV infection. Notably, a deficiency in both PKR and

RNase L resulted in a decreased ability of type I IFN to inhibit WNV in primary macrophages and cortical neurons. In contrast, the peripheral neurons of the superior cervical ganglia of PKR(-/-) x RL(-/-) mice showed no deficiency in the IFN-mediated inhibition of WNV. Our data suggest that PKR and RNase L contribute to IFN-mediated protection in a cell-restricted manner and control WNV infection in peripheral tissues and some neuronal subtypes.
Descriptors: endoribonucleases, meningoencephalitis, neurons, virus replication genetics, West Nile fever, West Nile virus metabolism, cerebellar cortex, endoribonucleases deficiency, type II interferon metabolism, macrophages, meningoencephalitis, knockout mice, organ specificity, superior cervical ganglion.

Sanchez, M.D., T.C. Pierson, D. McAllister, S.L. Hanna, B.A. Puffer, L.E. Valentine, M.M. Murtadha, J.A. Hoxie, and R.W. Doms (2005). **Characterization of neutralizing antibodies to West Nile virus.** *Virology* 336(1): 70-82. ISSN: 0042-6822.
Abstract: We produced nine monoclonal antibodies (MAbs) directed against the West Nile virus E glycoprotein using three different immunization strategies: inactivated virus, naked DNA, and recombinant protein. Most of the MAbs bound to conformation dependent epitopes in domain III of the E protein. Four of the MAbs neutralized WNV infection and bound to the same region of domain III with high affinity. The neutralizing MAbs were obtained from mice immunized with inactivated virus alone or in combination with a DNA plasmid. In contrast, MAbs obtained by immunization with a soluble version of the E glycoprotein did not exhibit neutralizing activity. These non-neutralizing antibodies were cross-reactive with several other flaviviruses, including Saint Louis encephalitis, Japanese encephalitis, Yellow Fever and Powassan viruses. Interestingly, some non-neutralizing MAbs bound with high affinity to domains I or III, indicating that both affinity and the precise epitope recognized by an antibody are important determinants of WNV neutralization.
Descriptors: monoclonal antibodies, viral immunology, West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus, tick borne diseases , enzyme linked immunosorbent assay, epitope mapping, glycoproteins, mice, tertiary protein structure, yellow fever virus.

Secko, D. (2005). **Immunotherapy for West Nile virus infection.** *Canadian Medical Association Journal* 173(6): 591.
Descriptors: monoclonal antibodies, immunotherapy trends, West Nile fever, West Nile virus, mice, survival analysis.

Seligman, S.J. (2006). **Single nucleotide polymorphisms in human genes and increased susceptibility to West Nile Virus disease.** *Journal of Infectious Diseases* 193(8): 1187-1188; Author Reply 1188.
Descriptors: single nucleotide polymorphism genetics, West Nile fever, genetic pre-

disposition to disease, natural immunity genetics, mice, West Nile virus (WNV).
Notes: Comment On: J Infect Dis. 2005 Nov 15;192(10):1741-8.

Seregin, A., R. Nistler, V. Borisevich, G. Yamshchikov, E. Chaporgina, C.W. Kwok, and V. Yamshchikov (2006). **Immunogenicity of West Nile virus infectious DNA and its noninfectious derivatives.** *Virology* 356(1-2): 115-125. ISSN: 0042-6822.
Abstract: The exceptionally high virulence of the West Nile NY99 strain makes its suitability in the development of a live WN vaccine uncertain. The aim of this study is to investigate the immunogenicity of noninfectious virus derivatives carrying pseudolethal mutations, which preclude virion formation without affecting preceding steps of the viral infectious cycle. When administered using DNA immunization, such constructs initiate an infectious cycle but cannot lead to a viremia. While the magnitude of the immune response to a noninfectious replication-competent construct was lower than that of virus or infectious DNA, its overall quality and the protective effect were similar. In contrast, a nonreplicating construct of similar length induced only a marginally detectable immune response in the dose range used. Thus, replication-competent noninfectious constructs derived from infectious DNA may offer an advantageous combination of the safety of noninfectious formulations with the quality of the immune response characteristic of infectious vaccines.
Descriptors: West Nile virus, immunogenicity, DNA, infectious, non infectious, virulence, immune response, NY99 strain.

Sheets, R.L., J. Stein, T.S. Manetz, C. Andrews, R. Bailer, J. Rathmann, and P.L. Gomez (2006). **Toxicological safety evaluation of DNA plasmid vaccines against HIV-1, Ebola, Severe Acute Respiratory Syndrome, or West Nile virus is similar despite differing plasmid backbones or gene-inserts.** *Toxicological Sciences* 91(2): 620-630.
Abstract: The Vaccine Research Center has developed a number of vaccine candidates for different diseases/infectious agents (HIV-1, Severe Acute Respiratory Syndrome virus, West Nile virus, and Ebola virus, plus a plasmid cytokine adjuvant-IL-2/Ig) based on a DNA plasmid vaccine platform. To support the clinical development of each of these vaccine candidates, preclinical studies were performed to screen for potential toxicities (intrinsic and immunotoxicities). All treatment-related toxicities identified in these repeated-dose toxicology studies have been confined primarily to the sites of injection and seem to be the result of both the delivery method (as they are seen in both control and treated animals) and the intended immune response to the vaccine (as they occur with greater frequency and severity in treated animals). Reactogenicity at the site of injection is generally seen to be reversible as the frequency and severity diminished between doses and between the immediate and recovery termination time points. This observation also correlated with the biodistribution data reported in the companion article (Sheets et al., 2006), in which DNA plasmid vaccine was shown to remain at the site of injection, rather

than biodistributing widely, and to clear over time. The results of these safety studies have been submitted to the Food and Drug Administration to support the safety of initiating clinical studies with these and related DNA plasmid vaccines. Thus far, standard repeated-dose toxicology studies have not identified any target organs for toxicity (other than the injection site) for our DNA plasmid vaccines at doses up to 8 mg per immunization, regardless of disease indication (i.e., expressed gene-insert) and despite differences (strengths) in the promoters used to drive this expression. As clinical data accumulate with these products, it will be possible to retrospectively compare the safety profiles of the products in the clinic to the results of the repeated-dose toxicology studies, in order to determine the utility of such toxicology studies for signaling potential immunotoxicities or intrinsic toxicities from DNA vaccines. These data build on the biodistribution studies performed (see companion article, Sheets et al., 2006) to demonstrate the safety and suitability for investigational human use of DNA plasmid vaccine candidates for a variety of infectious disease prevention indications.

Descriptors: DNA vaccines, viral vaccines, acquired immunodeficiency syndrome, ebola like viruses, HIV-1 genetics, hemorrhagic fever, rabbits, severe acute respiratory syndrome, tissue distribution, West Nile fever genetics, West Nile virus.

Sheets, R.L., J. Stein, T.S. Manetz, C. Duffy, M. Nason, C. Andrews, W.P. Kong, G.J. Nabel, and P.L. Gomez (2006). **Biodistribution of DNA plasmid vaccines against HIV-1, Ebola, Severe Acute Respiratory Syndrome, or West Nile virus is similar, without integration, despite differing plasmid backbones or gene inserts.** *Toxicological Sciences* 91(2): 610-619.

Abstract: The Vaccine Research Center has developed a number of vaccine candidates for different diseases/infectious agents (HIV-1, Severe Acute Respiratory Syndrome virus, West Nile virus, and Ebola virus, plus a plasmid cytokine adjuvant-IL-2/Ig) based on a DNA plasmid vaccine platform. To support the clinical development of each of these vaccine candidates, preclinical studies have been performed in mice or rabbits to determine where in the body these plasmid vaccines would biodistribute and how rapidly they would clear. In the course of these studies, it has been observed that regardless of the gene insert (expressing the vaccine immunogen or cytokine adjuvant) and regardless of the promoter used to drive expression of the gene insert in the plasmid backbone, the plasmid vaccines do not biodistribute widely and remain essentially in the site of injection, in the muscle and overlying subcutis. Even though approximately 10(14) molecules are inoculated in the studies in rabbits, by day 8 or 9 (approximately 1 week postinoculation), already all but on the order of 10(4)-10(6) molecules per microgram of DNA extracted from tissue have been cleared at the injection site. Over the course of 2 months, the plasmid clears from the site of injection with only a small percentage of animals (generally 10-20%)

retaining a small number of copies (generally around 100 copies) in the muscle at the injection site. This pattern of biodistribution (confined to the injection site) and clearance (within 2 months) is consistent regardless of differences in the promoter in the plasmid backbone or differences in the gene insert being expressed by the plasmid vaccine. In addition, integration has not been observed with plasmid vaccine candidates inoculated i.m. by Biojector 2000 or by needle and syringe. These data build on the repeated-dose toxicology studies performed (see companion article, Sheets et al., 2006) to demonstrate the safety and suitability for investigational human use of DNA plasmid vaccine candidates for a variety of infectious disease prevention indications.

Descriptors: vaccines, viral vaccines, acquired immunodeficiency syndrome, ebola-like viruses, HIV-1, hemorrhagic fever, inbred strains of mice, plasmids, promoter regions, severe acute respiratory syndrome, DNA administration, West Nile virus.

Shirato, K., H. Miyoshi, H. Kariwa, and I. Takashima (2006). **The kinetics of proinflammatory cytokines in murine peritoneal macrophages infected with envelope protein-glycosylated or non-glycosylated West Nile virus.** *Virus Research* 121(1): 11-16. ISSN: 0168-1702.

Abstract: The envelope (E) protein glycosylation status of the New York strain of West Nile (WN) virus is an important determinant of virus neuroinvasiveness. To elucidate the determinant of the difference between E protein-glycosylated and non-glycosylated WN virus infections, the cytokine expression of murine peritoneal macrophages infected with each virus was examined. Tumor necrosis factor (TNF) alpha and interleukin (IL)-1beta were up-regulated with replication of the E protein-glycosylated virus. Interferon (IFN) beta and IL-6 were up-regulated with the clearance of both viruses. These results suggest that TNFalpha and IL-1beta expression are related to the virulence of E protein-glycosylated WN virus.

Descriptors: interleukin 1 metabolism, peritoneal macrophages, tumor necrosis factor, alpha metabolism, viral envelope proteins, West Nile fever, cultured cells, glycosylation , inbred Balb C mice, up-regulation.

Shrestha, B., T. Wang, M.A. Samuel, K. Whitby, J. Craft, E. Fikrig, and M.S. Diamond (2006). **Gamma interferon plays a crucial early antiviral role in protection against West Nile virus infection.** *Journal of Virology* 80(11): 5338-5348. ISSN: 0022-538X.

Abstract: West Nile virus (WNV) causes a severe central nervous system (CNS) infection in humans, primarily in the elderly and immunocompromised. Prior studies have established an essential protective role of several innate immune response elements, including alpha/beta interferon (IFN-alpha/beta), immunoglobulin M, gammadelta T cells, and complement against WNV infection. In this study, we demonstrate that a lack of IFN-gamma production or signaling results in increased

vulnerability to lethal WNV infection by a subcutaneous route in mice, with a rise in mortality from 30% (wild-type mice) to 90% (IFN-gamma(-/-) or IFN-gammaR(-/-) mice) and a decrease in the average survival time. This survival pattern in IFN-gamma(-/-) and IFN-gammaR(-/-) mice correlated with higher viremia and greater viral replication in lymphoid tissues. The increase in peripheral infection led to early CNS seeding since infectious WNV was detected several days earlier in the brains and spinal cords of IFN-gamma(-/-) or IFN-gammaR(-/-) mice. Bone marrow reconstitution experiments showed that gammadelta T cells require IFN-gamma to limit dissemination by WNV. Moreover, treatment of primary dendritic cells with IFN-gamma reduced WNV production by 130-fold. Collectively, our experiments suggest that the dominant protective role of IFN-gamma against WNV is antiviral in nature, occurs in peripheral lymphoid tissues, and prevents viral dissemination to the CNS.
Descriptors: West Nile virus, gamma interferon, infection, antiviral, CNS, mice, alpha-beta interferon, virus replication, dendritic cells.

Siger, L., R. Bowen, K. Karaca, M. Murray, S. Jagannatha, B. Echols, R. Nordgren, and J.M. Minke (2006). **Evaluation of the efficacy provided by a Recombinant Canarypox-Vectored Equine West Nile Virus vaccine against an experimental West Nile Virus intrathecal challenge in horses.** *Veterinary Therapeutics Research in Applied Veterinary Medicine* 7(3): 249-256.
Abstract: Efficacy of the Recombitek Equine West Nile Virus (WNV) vaccine was evaluated against a WNV intrathecal challenge model that results in WNV-induced clinical disease. Ten vaccinated (twice at days 0 and 35) and 10 control horses were challenged 2 weeks after administration of the second vaccine with a virulent WNV by intrathecal administration. After the challenge, eight of 10 controls developed clinical signs of encephalomyelitis whereas one vaccinate exhibited muscle fasciculation only once. Nine controls and one vaccinate developed a fever. Histopathology revealed mild to moderate nonsuppurative encephalitis in eight controls and one vaccinate. None of the vaccinates and all of the controls developed WNV viremia after challenge. All vaccinated horses developed antibodies to WNV after vaccination. These and results of previous studies demonstrate efficacy of the Recombitek WNV vaccine against WNV-induced clinical disease and natural challenge with WNV-infected mosquitoes.
Descriptors: Recombitek Equine West Nile Virus vaccine, WNV intrathecal challenge model, clinical studies, horses, development of encephalomyelitis, histopathology, antibodies to WNV, mosquito vectors.

Sitati, E.M. and M.S. Diamond (2006). **CD4+ T-cell responses are required for clearance of West Nile virus from the central nervous system.** *Journal of Virology* 80(24): 12060-12069. ISSN: 0022-538X.
Abstract: Although studies have established that innate and adaptive immune

responses are important in controlling West Nile virus (WNV) infection, the function of CD4(+) T lymphocytes in modulating viral pathogenesis is less well characterized. Using a mouse model, we examined the role of CD4(+) T cells in coordinating protection against WNV infection. A genetic or acquired deficiency of CD4(+) T cells resulted in a protracted WNV infection in the central nervous system (CNS) that culminated in uniform lethality by 50 days after infection. Mice surviving past day 10 had high-level persistent WNV infection in the CNS compared to wild-type mice, even 45 days following infection. The absence of CD4(+) T-cell help did not affect the kinetics of WNV infection in the spleen and serum, suggesting a role for CD4-independent clearance mechanisms in peripheral tissues. WNV-specific immunoglobulin M (IgM) levels were similar to those of wild-type mice in CD4-deficient mice early during infection but dropped approximately 20-fold at day 15 postinfection, whereas IgG levels in CD4-deficient mice were approximately 100- to 1,000-fold lower than in wild-type mice throughout the course of infection. WNV-specific CD8(+) T-cell activation and trafficking to the CNS were unaffected by the absence of CD4(+) T cells at day 9 postinfection but were markedly compromised at day 15. Our experiments suggest that the dominant protective role of CD4(+) T cells during primary WNV infection is to provide help for antibody responses and sustain WNV-specific CD8(+) T-cell responses in the CNS that enable viral clearance.
Descriptors: West Nile virus, CNS, clearance, CD4+T cell responses, immune responses, spleen, serum, viral clearance.

Throsby, M., C. Geuijen, J. Goudsmit, A.Q. Bakker, J. Korimbocus, R.A. Kramer, M. Clijsters van der Horst, M. de Jong, M. Jongeneelen, S. Thijsse, R. Smit, T.J. Visser, N. Bijl, W.E. Marissen, M. Loeb, D.J. Kelvin, W. Preiser, J. ter Meulen, and J. de Kruif (2006). **Isolation and characterization of human monoclonal antibodies from individuals infected with West Nile Virus.** *Journal of Virology* 80(14): 6982-6992. ISSN: 0022-538X.
Abstract: Monoclonal antibodies (MAbs) neutralizing West Nile Virus (WNV) have been shown to protect against infection in animal models and have been identified as a correlate of protection in WNV vaccine studies. In the present study, antibody repertoires from three convalescent WNV-infected patients were cloned into an scFv phage library, and 138 human MAbs binding to WNV were identified. One hundred twenty-one MAbs specifically bound to the viral envelope (E) protein and four MAbs to the premembrane (prM) protein. Enzyme-linked immunosorbent assay-based competitive-binding assays with representative E protein-specific MAbs demonstrated that 24/51 (47%) bound to domain II while only 4/51 (8%) targeted domain III. In vitro neutralizing activity was demonstrated for 12 MAbs, and two of these, CR4374 and CR4353, protected mice from lethal WNV challenge at 50% protective doses of 12.9 and 357 mug/kg of body weight, respectively. Our data analyzing three infected

individuals suggest that the human anti-WNV repertoire after natural infection is dominated by nonneutralizing or weakly neutralizing MAbs binding to domain II of the E protein, while domain III-binding MAbs able to potently neutralize WNV in vitro and in vivo are rare.
Descriptors: monoclonal antibodies immunology, viral immunology, viral envelope proteins, West Nile fever, West Nile virus, monoclonal genetics, viral genetics, antibody specificity, molecular cloning, mice, protein structure.

Wang, T., Y. Gao, E. Scully, C.T. Davis, J.F. Anderson, T. Welte, M. Ledizet, R. Koski, J.A. Madri, A. Barrett, Z. Yin, J. Craft, and E. Fikrig (2006). **Gamma delta T cells facilitate adaptive immunity against West Nile virus infection in mice.** *Journal of Immunology* 177(3): 1825-1832.
Abstract: West Nile (WN) virus causes fatal meningoencephalitis in laboratory mice, and gammadelta T cells are involved in the protective immune response against viral challenge. We have now examined whether gammadelta T cells contribute to the development of adaptive immune responses that help control WN virus infection. Approximately 15% of TCRdelta(-/-) mice survived primary infection with WN virus compared with 80-85% of the wild-type mice. These mice were more susceptible to secondary challenge with WN virus than the wild-type mice that survived primary challenge with the virus. Depletion of gammadelta T cells in wild-type mice that survived the primary infection, however, does not affect host susceptibility during secondary challenge with WN virus. Furthermore, gammadelta T cells do not influence the development of Ab responses during primary and at the early stages of secondary infection with WN virus. Adoptive transfer of CD8(+) T cells from wild-type mice that survived primary infection with WN virus to naive mice afforded partial protection from lethal infection. In contrast, transfer of CD8(+) T cells from TCRdelta(-/-) mice that survived primary challenge with WN virus failed to alter infection in naive mice. This difference in survival correlated with the numeric and functional reduction of CD8 memory T cells in these mice. These data demonstrate that gammadelta T cells directly link innate and adaptive immunity during WN virus infection.
Descriptors: gamma delta T cells, T lymphocyte subsets, West Nile fever, West Nile virus, adoptive transfer, CD8 positive T lymphocytes, genetic predisposition to disease genetics, cellular genetics, immunoglobulin G biosynthesis, immunoglobulin M biosynthesis, immunologic memory genetics, lymphocyte depletion, knockout inbred c57bl, mice, receptors.

Wilkins, P.A., A.L. Glaser, and S.M. McDonnell (2006). **Passive transfer of naturally acquired specific immunity against West Nile Virus to foals in a semi-feral pony herd.** *Journal of Veterinary Internal Medicine* 20(4): 1045-1047.
Abstract: Horses naturally exposed to West Nile Virus (WNV) or vaccinated against

WNV develop humoral immunity thought to be protective against development of clinical disease in exposed or infected animals. No reports evaluate the efficacy of passive transfer of naturally acquired specific WNV humoral immunity from dam to foal. The purpose of this study was to investigate passive transfer of naturally acquired immunity to WNV to foals born in a herd of semi-feral ponies, not vaccinated against WNV, in an endemic area, with many dams having seroconverted because of natural exposure. Microwell serum neutralization titers against WNV were determined in all mares and foals. Serum IgG concentration was determined in foals by serial radial immunodiffusion. Differences in IgG concentration between seropositive and seronegative foals were examined by means of the Mann-Whitney U-test. Linear regression was used to evaluate the association between mare and foal titers. Seventeen mare-foal pairs were studied; 1 foal had inadequate IgG concentration. IgG concentration was not different between seronegative and seropositive foals (P = .24). Mare and foal titers were significantly correlated in foals with adequate passive transfer of immunity (Spearman's rho = .84; P < .001); >90% of the foal's titer was explained by the mare's titer (R2 = 0.91; P < .001). Passive transfer of specific immunity to WNV is present in pony foals with adequate passive transfer of immunity born to seroconverted mares.

Descriptors: horse diseases, maternally acquired immunology, West Nile fever, West Nile virus, horses.

Wolf, R.F., J.F. Papin, R. Hines Boykin, M. Chavez Suarez, G.L. White, M. Sakalian, and D.P. Dittmer (2006). **Baboon model for West Nile virus infection and vaccine evaluation.** *Virology* 355(1): 44-51. ISSN: 0042-6822.

Abstract: Animal models that closely mimic the human condition are of paramount significance to study pathogenic mechanisms, vaccine and therapy scenarios. This is particularly true for investigations that involve emerging infectious diseases. Non-human primate species represent an alternative to the more intensively investigated rodent animal models and in a number of instances have been shown to represent a more reliable predictor of the human response to infection. West Nile virus (WNV) has emerged as a new pathogen in the Americas. It has a 5% fatality rate, predominantly in the elderly and immune compromised. Typically, infections are cleared by neutralizing antibodies, which suggests that a vaccine would be efficacious. Previously, only macaques had been evaluated as a primate model for WNV vaccine design. The macaques did not develop WNV disease nor express the full complement of IgG subclasses that is found in humans. We therefore explored baboons, which exhibit the similar four IgG subclasses observed in humans as a new model for WNV infection and vaccine evaluation. In this present report, we describe the experimental infection of baboons with WNV and test the efficacy of an inactivated WNV vaccination strategy. All experimentally infected animals developed transient viremia and

subsequent neutralizing antibodies. Anti-WNV IgM antibodies peaked at 20 days post-infection. Anti-WNV IgG antibodies appeared later and persisted past 60 days. Prior vaccination with chemically inactivated virus induced neutralizing titers and a fast, high titer IgG recall response, which resulted in lower viremia upon challenge. This report is the first to describe the development of the baboon model for WNV experimental infection and the utility of this model to characterize the immunologic response against WNV and a candidate WNV vaccine.
Descriptors: animal disease models, Papio, West Nile fever, West Nile virus vaccines, immunoglobulin G, immunoglobulin M, neutralization tests, viremia.

Yamshchikov, G., V. Borisevich, C.W. Kwok, R. Nistler, J. Kohlmeier, A. Seregin, E. Chaporgina, S. Benedict, and V. Yamshchikov (2005). **The suitability of yellow fever and Japanese encephalitis vaccines for immunization against West Nile virus**. *Vaccine* 23(39): 4785-4792. ISSN: 0264-410X.
Descriptors: West Nile virus, yellow fever, Japanese encephalitis, vaccine suitability, drug therapy.

Zohrabian, A., E.B. Hayes, and L.R. Petersen (2006). **Cost-effectiveness of West Nile virus vaccination.** *Emerging Infectious Diseases* 12(3): 375-380. ISSN: 1080-6040 .
Abstract: West Nile virus (WNV) was first detected in the Western Hemisphere in 1999 in New York City. From 1999 through 2004, >16,600 cases of WNV-related illnesses were reported in the United States, of which >7,000 were neuroinvasive disease and >600 were fatal. Several approaches are under way to develop a human vaccine. Through simulations and sensitivity analysis that incorporated uncertainties regarding future transmission patterns of WNV and costs of health outcomes, we estimated that the range of values for the cost per case of WNV illness prevented by vaccination was US 20,000 dollars-59,000 dollars(mean 36,000 dollars). Cost-effectiveness was most sensitive to changes in the risk for infection, probability of symptomatic illness, and vaccination cost. Analysis indicated that universal vaccination against WNV disease would be unlikely to result in societal monetary savings unless disease incidence increases substantially over what has been seen in the past 6 years.
Descriptors: vaccination economics, viral vaccines, West Nile fever prevention and control, cost benefit analysis, decision trees, health care costs, mortality.

Vector Control Programs

Bowman, C., A.B. Gumel, P. van den Driessche, J. Wu, and H. Zhu (2005). **A mathematical model for assessing control strategies against West Nile virus.** *Bulletin of Mathematical Biology* 67(5): 1107-33.

Abstract: Since its incursion into North America in 1999, West Nile virus (WNV) has spread rapidly across the continent resulting in numerous human infections and deaths. Owing to the absence of an effective diagnostic test and therapeutic treatment against WNV, public health officials have focussed on the use of preventive measures in an attempt to halt the spread of WNV in humans. The aim of this paper is to use mathematical modelling and analysis to assess two main anti-WNV preventive strategies, namely: mosquito reduction strategies and personal protection. We propose a single-season ordinary differential equation model for the transmission dynamics of WNV in a mosquito-bird-human community, with birds as reservoir hosts and culicine mosquitoes as vectors. The model exhibits two equilibria; namely the disease-free equilibrium and a unique endemic equilibrium. Stability analysis of the model shows that the disease-free equilibrium is globally asymptotically stable if a certain threshold quantity (R0), which depends solely on parameters associated with the mosquito-bird cycle, is less than unity. The public health implication of this is that WNV can be eradicated from the mosquito-bird cycle (and, consequently, from the human population) if the adopted mosquito reduction strategy (or strategies) can make R0<1. On the other hand, it is shown, using a novel and robust technique that is based on the theory of monotone dynamical systems coupled with a regular perturbation argument and a Liapunov function, that if R0>1, then the unique endemic equilibrium is globally stable for small WNV-induced avian mortality. Thus, in this case, WNV persists in the mosquito-bird population.

Descriptors: theoretical models, West Nile fever, birds, communicable disease control methods, culicidae, insect control methods, insect repellents, protective clothing, West Nile virus.

Han, X.N., C.Y. Li, D. Feng, L.Q. Fang, and W.C. Cao (2006). **Modeling the dynamics of West Nile virus and evaluation of control measure.** *Jishengchong Yu Yixue Kunchong Xuebao* 13(2): 89-94. ISSN: 1005-0507.

Abstract: After the surprising detection of West Nile virus (WNV) in New York City in 1999 , the virus has spread dramatically in the Western Hemisphere, resulting in the largest epidemics since reported. In a total, just one year of 2005, 2799 human cases were reported in U. S till Dec. 20 . The epidemic through all the world warn us the introduction of WNV and increase the information about WNV transmission.

In this work we formulate and analyze a mathematics model for the transmission of WNV infect between vector (mosquito) and host (avian population and human population) , according to the ecology of WNV. The model concludes three populations (mosquito, avian and human) and provides the relationship between the populations. We build the formula of the Basic Reproductive Number (R0) in terms of WN epidemiology. R0 is the threshold condition that determines the dynamics of WNV infection. We can take the relevant control measure according to the changed parameters and provide a new platform for further research in preventing and controlling WNV disease.

Descriptors: aves, mathematical techniques, viral disease transmission model, dipteran vector, dipteran parasites, culicidae, West Nile virus vector, transmission model and control evaluation, viral diseases, West Nile virus.

Language of Text: Chinese; Summary in Chinese, English.

Kronenwetter Koepel, T.A., J.K. Meece, C.A. Miller, and K.D. Reed (2005). **Surveillance of above- and below-ground mosquito breeding habitats in a rural midwestern community: baseline data for larvicidal control measures against West Nile Virus vectors.** *Clinical Medicine and Research* 3(1): 3-12.

Abstract: BACKGROUND: Mosquitoes in the genus Culex are thought to play a major role as vectors in the transmission cycle of West Nile virus (WNV) and other arboviruses in the United States. Effective control of mosquitoes through larviciding and adulticiding is expensive for communities and should be guided by reliable surveillance data on the distribution of mosquito breeding habitats. However, few small to medium sized cities in rural areas of the midwestern United States have this type of baseline information available. OBJECTIVE: During the summer of 2004, we investigated the characteristics of Culex and other mosquito-breeding habitats in a rural central Wisconsin community with a population of approximately 19,000. Such baseline information will aid in the development of rational strategies to control mosquito populations and prevent human exposure to WNV and other mosquito-transmitted viruses. METHODS: Mosquito larvae were collected and identified weekly from 14 below-ground storm water catch basins and 10 above-ground standing water sites distributed throughout the community. Collection began June 4, 2004 and continued through September 24, 2004. For each collection site the primary and adjacent land use patterns were determined. RESULTS: Over the study period, 1,244 larvae were collected from catch basins; 94% were Culex species. Breeding activity was first detected in early July. Peak breeding was observed during a period of several weeks when average daily temperatures were at the maximum observed and rainfall had declined. Organically enriched catch basins in low intensity urban sites adjacent to forests and wetlands were found to be more productive breeding habitats compared to catch basins having little organic debris located in isolated high

intensity urban sites. Above-ground standing water sites produced 1,504 larvae; 66% of which were Culex species. Flood control ditches and permanent wetlands with stagnant water were most productive, while ditches with moving water were least productive habitats. Larvae were produced earlier in the season by above-ground sites than were produced by catch basins. However, larvae production was more variable in above-ground sites since half the sites became dry at some point during the study period. CONCLUSION: The observed differences in Culex larvae production based on the variables of habitat-type, temperature, and precipitation support the need for ongoing surveillance in communities to guide public health officials in planning for and prioritizing mosquito control efforts.
Descriptors: Culex, insect vectors, mosquito control, West Nile Virus, breeding environment, insect control methods, rain, rural population, seasons, temperature, time factors, water, weather, West Nile fever prevention and control, Wisconsin.

Macedo, P.A., R.K. Peterson, and R. Davis (2006). **Human-health risk assessment for West Nile virus and insecticides used in mosquito management**. *Abstracts of Papers American Chemical Society* 231: 8-AGRO. ISSN: 0065-7727.
Descriptors: West Nile virus, health risk assessment, insecticides, health risk, mosquito management, vector control, pesticides, nervous system disease, diagnostic techniques, public concern, meeting abstract.
Notes: Meeting Information: 231st National Meeting of the American-Chemical-Society, Atlanta, Georgia, USA; March 26 -30, 2006.

Palmisano, C., V. Taylor, K. Caillouet, B. Byrd, and D. Wesson (2005). **Impact of West Nile virus outbreak upon St. Tammany Parish mosquito abatement district.** *Journal of the American Mosquito Control Association.* 21(1): 33-38. ISSN: 8756-971X.
Descriptors: *Culex pipiens*, insect vectors, West Nile virus, disease outbreaks, disease surveillance, sentinel animals, dead animals, humans, disease incidence, vector control, mosquito control, adulticides, larvicides, Louisiana, *Culex-pipiens-quinquefasciatus*, arbovirus-surveillance, sentinel-chickens, dead-birds.

Park, H.W., D.K. Bideshi, M.C. Wirth, J.J. Johnson, W.E. Walton, and B.A. Federici (2005). **Recombinant larvicidal bacteria with markedly improved efficacy against culex vectors of West Nile virus.** *American Journal of Tropical Medicine and Hygiene* 72(6): 732-738. ISSN: antibodies.
Abstract: An urgent need exists for new agents to control mosquito vectors of disease. Mosquito larvicides based on the bacteria *Bacillus thuringiensis* subsp. *israelensis* (Bti) or *B. sphaericus* (Bs) are effective in many habitats, but use is limited by their high cost. Moreover, mosquito resistance evolves rapidly to Bs where it is used intensively. The efficacy of these bacteria is due to a binary protein (BsB) in Bs and four proteins (Cry4A, Cry4B, Cry11A, and Cyt1A) in Bti. Here we report the use

of cyt1A promoters and a 5' mRNA stabilizing sequence to synthesize high levels of Bs2362 binary toxin in Bti strains. The recombinant BtiIPS-82/BsB showed high potency against fourth instars of *Culex quinquefasciatus*, a vector of West Nile virus, being 21-fold as potent as BtiIPS-82, and 32-fold as potent as Bs2362. Similar improved efficacy was obtained against larvae of Cx. tarsalis. Moreover, BtiIPS-82/BsB suppressed resistance to Bs2362 in *Cx. quinquefasciatus*.
Descriptors: bacillus pathogenicity, culex mosquitoes virology, insect vectors, biological pest control, West Nile fever, West Nile virus isolation and purification, polyacrylamide gel electrophoresis, genetic recombination genetic, species specificity.

Peterson, R.K., P.A. Macedo, and R.S. Davis (2006). **A human-health risk assessment for West Nile virus and insecticides used in mosquito management.** *Environmental Health Perspectives* 114(3): 366-372.
Abstract: West Nile virus (WNV) has been a major public health concern in North America since 1999, when the first outbreak in the Western Hemisphere occurred in New York City. As a result of this ongoing disease outbreak, management of mosquitoes that vector WNV throughout the United States and Canada has necessitated using insecticides in areas where they traditionally have not been used or have been used less frequently. This has resulted in concerns by the public about the risks from insecticide use. The objective of this study was to use reasonable worst-case risk assessment methodologies to evaluate human-health risks for WNV and the insecticides most commonly used to control adult mosquitoes. We evaluated documented health effects from WNV infection and determined potential population risks based on reported frequencies. We determined potential acute (1-day) and subchronic (90-day) multiroute residential exposures from each insecticide for several human subgroups during a WNV disease outbreak scenario. We then compared potential insecticide exposures to toxicologic and regulatory effect levels. Risk quotients (RQs, the ratio of exposure to toxicologic effect) were < 1.0 for all subgroups. Acute RQs ranged from 0.0004 to 0.4726, and subchronic RQs ranged from 0.00014 to 0.2074. Results from our risk assessment and the current weight of scientific evidence indicate that human-health risks from residential exposure to mosquito insecticides are low and are not likely to exceed levels of concern. Further, our results indicate that, based on human-health criteria, the risks from WNV exceed the risks from exposure to mosquito insecticides.
Descriptors: environmental exposure to West Nile virus (WNV), environmental pollutants, insecticides, piperonyl butoxide, theoretical modelling, mosquito control.

Reddy, M.R., A. Spielman, T.J. Lepore, D. Henley, A.E. Kiszewski, and P. Reiter (2006). **Efficacy of resmethrin aerosols applied from the road for suppressing Culex vectors of West Nile virus.** *Vector Borne and Zoonotic Diseases* 6(2): 117-127. ISSN: 1530-3667.

Abstract: We determined whether aerosol applications of resmethrin, delivered from the road, suppress the reproductive activity of *Culex pipiens pipiens* and *Cx. restuan*s mosquitoes in suburban sites located near Boston. Oviposition implies a prior blood-feeding event and hence a potential West Nile virus (WNV) transmission-related event. Droplet size, rate of delivery and meteorological conditions were monitored. The target populations proved to be fully susceptible to the insecticide that was used. The roads in the test sites generally gave adequate opportunity for insecticidal coverage. We found that the aerosol plume may have failed to contact the target mosquitoes and conclude that such insecticidal aerosols, delivered from the road, may not effectively reduce the force of transmission of WNV in our test sites.
Descriptors: culex mosquitos, insect vectors, insecticides, mosquito control methods, pyrethrins, dosage, Boston, Massachusetts, oviposition, weather, West Nile fever.

Zou, L., S.N. Miller, and E.T. Schmidtmann (2006). **Mosquito larval habitat mapping using remote sensing and GIS: implications of coalbed methane development and West Nile virus.** *Journal of Medical Entomology* 43(5): 1034-1041. ISSN: 0022-2585.

Abstract: Potential larval habitats of the mosquito *Culex tarsalis* (Coquillett), implicated as a primary vector of West Nile virus in Wyoming, were identified using integrated remote sensing and geographic information system (GIS) analyses. The study area is in the Powder River Basin of north central Wyoming, an area that has been undergoing a significant increase in coalbed methane gas extractions since the late 1990s. Large volumes of water are discharged, impounded, and released during the extraction of methane gas, creating aquatic habitats that have the potential to support immature mosquito development. Landsat TM and ETM+ data were initially classified into spectrally distinct water and vegetation classes, which were in turn used to identify suitable larval habitat sites. This initial habitat classification was refined using knowledge-based GIS techniques requiring spatial data layers for topography, streams, and soils to reduce the potential for overestimation of habitat. Accuracy assessment was carried out using field data and high-resolution aerial photography commensurate with one of the Landsat images. The classifier can identify likely habitat for ponds larger than 0.8 ha (2 acres) with generally satisfactory results (72.1%) with a lower detection limit of approximately 0.4 ha (1 acre). Results show a 75% increase in potential larval habitats from 1999 to 2004 in the study area, primarily because of the large increase in small coalbed methane water discharge ponds. These results may facilitate mosquito abatement programs in the Powder River Basin with the potential for application throughout the state and region.
Descriptors: potential larval habitats, coalbed methane gas, aquatic habitats, immature mosquitoe development, water discharge ponds.

Vectors and Transmission Factors

Anderson, J.F., T.G. Andreadis, A.J. Main, F.J. Ferrandino, and C.R. Vossbrinck (2006). **West Nile virus from female and male mosquitoes (Diptera: Culicidae) in subterranean, ground, and canopy habitats in Connecticut.** *Journal of Medical Entomology* 43(5): 1010-1019.

Abstract: In total, 93,532 female mosquitoes (Diptera: Culicidae) were captured in traps placed in subterranean (catch basin), ground (approximately 1.5 m above ground), and canopy (approximately 7.0 m above ground) habitats in Stamford and Stratford, CT, during 2003-2005. *Culex pipiens L*. was the most abundant (64.8%) of the 31 species identified. Significantly greater numbers of *Cx. pipiens* were captured in canopy-placed mosquito magnet experimental traps, and significantly greater numbers were collected in catch basin-placed (Centers for Disease Control) CDC traps than in CDC traps placed elsewhere. *Culex restuans Theobald* was captured in significantly greater numbers in traps placed in catch basins. *Aedes vexans* (Meigen), *Aedes cinereus* Meigen, and *Aedes cantator* (Coquillett) were significantly more abundant in ground traps. In total, 429 isolations of West Nile virus (WNV) were made from seven species of mosquitoes from late June through the end of October during 2003 through 2005. Three hundred ninety-eight (92.8%) isolates were from *Cx. pipiens*. Others were from *Cx. restuans* (n = 16), *Culex salinarius* Coquillett (n = 5), *Ae. vexans* (n = 4), *Ae. cantator* (n = 3), *Aedes triseriatus* (Say) (n = 2), and *Ae. cinereus* (n = 1). Multiple isolates from *Cx. pipiens* were made each week, primarily during the later part of July through the end of September. Weekly minimum infection rates (MIRs) were lower in 2004 (highest weekly MIR = 7.1) when no human cases were reported in Connecticut in comparison with 2003 and 2005 (highest weekly MIR = 83.9) when human cases were documented. Frequencies of infected pools were significantly higher in *Cx. pipiens* captured in traps in the canopy and significantly higher in catch basin placed traps than in traps at ground level. The physiological age structure of *Cx. pipiens* captured in the canopy was significantly different from that of *Cx. pipiens* collected in catch basins. Invariably, *Cx. pipiens* captured in the canopy were nulliparous or parous with ovaries in Christophers' stage 2, whereas 58.7% of the females captured in catch basins possessed ovaries filled with mature oocytes in Christophers' stage 5. Our results suggest that females in the canopy are seeking hosts, and after digestion of the bloodmeal and development of mature oocytes, they descend to catch basins for shelter and deposition of eggs. WNV was isolated from three, one, and two pools of male *Cx. pipiens* captured in catch basin-, ground-, and canopy-placed traps, respectively, and from six nulliparous *Cx. pipiens* females collected in the canopy. Weekly MIR ranged from 1.2 to 31.1 per 1,000 male specimens. These

data show that mosquitoes become infected by means other than by blood feeding, possibly by transovarial transmission. The placement of traps in tree canopies and in catch basins can be used to augment current practices of placement of traps near the ground for surveillance of mosquitoes infected with WNV and for studies of the ecology of WNV.
Descriptors: West Nile virus, mosquitoes, culex, aedes, traps, subterranean, above ground, CDC, male, female, surveillance.

Anderson, J.F. and A.J. Main (2006). **Importance of vertical and horizontal transmission of West Nile virus by *Culex pipiens* in the Northeastern United States.** *Journal of Infectious Diseases* 194(11): 1577-1579.
Abstract: West Nile virus (WNV) has become established in the northeastern United States, where mosquitoes are inactive during winter. There have been no documented studies to explain how this virus survives winter and reinitiates infection in spring. We report that WNV was vertically transmitted to 2 F(1) female *Culex pipiens* from a naturally infected female collected in Stratford, Connecticut. One vertically infected F(1) female, which was 168 days old, fed on a hamster that died 8 days later of West Nile disease. This suggests that WNV survives winter in unfed, vertically infected *C. pipiens* with amplification initiated in spring by horizontal transmission.
Descriptors: West Nile virus, *Culex pipens*, winter, infection, vertrical transmission, horozontil transmission, mosquitoes, spring.

Balenghien, T., F. Fouque, P. Sabatier, and D.J. Bicout (2006). **Horse-, bird-, and human-seeking behavior and seasonal abundance of mosquitoes in a West Nile virus focus of southern France.** *Journal of Medical Entomology* 43(5): 936-946.
Abstract: After 35 yr of disease absence, West Nile virus (family Flaviviridae, genus Flavivirus, WNV) circulation has been regularly detected in the Camargue region (southern France) since 2000. WNV was isolated from *Culex modestus Ficalbi*, which was considered the main vector in southern France after horse outbreaks in the 1960s. Recent WNV transmissions outside of the *Cx. modestus* distribution suggested the existence of other vectors. To study potential WNV vectors, horse- and bird-baited traps and human landing collections of mosquitoes were carried out weekly from May to October 2004 at two Camargue sites: one site in a wet area and the other site in a dry area, both chosen for their past history of WNV transmission. At the wet site, the most abundant species in bird-baited traps were *Culex pipiens L.* and *Cx. modestus*; both species also were found in lower proportions on horses and humans. The most abundant species in horse-baited traps and human landing collections were *Aedes caspius* (Pallas), *Aedes vexans* (Meigen), and *Anopheles hyrcanus* (Pallas) sensu lato; some of these species were occasionally collected with avian blood at the end of the summer. *Anopheles maculipennis* Meigen sensu lato was an abundant horse feeder, but it was rarely collected landing on human bait and never contained

avian blood. At the dry site, *Cx. pipiens* was the most abundant species in bird- and horse-baited traps. The seasonal and circadian dynamics of these species are analyzed, and their potential in WNV transmission in Camargue discussed.
Descriptors: West Nile virus, bird, human, horse, mosquitoes, seeking behavior, seasonal abundance, southern France, transmission.

Bell, J.A., N.J. Mickelson, and J.A. Vaughan (2005). **West Nile virus in host-seeking mosquitoes within a residential neighborhood in Grand Forks, North Dakota.** *Vector Borne and Zoonotic Diseases* 5(4): 373-382.
Abstract: West Nile virus (WNV) was first recovered in North Dakota near the city of Grand Forks in June 2002. During 2002, 2003, and 2004, we collected mosquitoes from Grand Forks using Mosquito Magnet traps and tested them for WNV. The seasonal abundance, species composition, and reproductive status of female mosquitoes were correlated with local environmental temperature and state surveillance data on WNV to determine the factors affecting local transmission of WNV. Over 90% of the mosquitoes collected were *Aedes vexans, Ochlerotatus dorsalis*, and *Culex tarsalis*, but WNV was detected only in *Cx. tarsalis*. Average summertime temperatures and relative abundance of mosquitoes were highest in 2002 but no WNV-positive mosquitoes were detected until the following summer. In 2003, nulliparous *Cx. tarsalis* appeared in mid-June (first summer brood), and parous *Cx. tarsalis* appeared in mid-July. The first WNV-positive pool occurred 21 July, and minimum daily infections rates increased thereafter until 27 August. The minimum infection rate (MIR) for *Cx. tarsalis* during the season was 5.7 infected mosquitoes per 1,000 tested, with the highest infection rates occurring at the end of the season as *Cx. tarsalis* populations started to decline. Mid-to-late August was identified as the period of highest risk for being bitten by a WNV-infected mosquito in Grand Forks during 2003. In 2004, viral activity in Grand Forks was low, due to very cool temperatures throughout the summer. To examine the genetic diversity of the 2003 WNV isolates from Grand Forks, we sequenced a 366-nucleotide region of the capsid and premembrane gene. Thirteen (46%) of the 28 WNV isolates contained at least one nucleotide substitution when compared to the homologous region of the progenitor WN NY-99 strain, and seven of these 13 substitutions coded for amino acid changes. Thus, WNV is established in North Dakota, it appears to be evolving and it is vectored primarily by *Cx. tarsalis*.
Descriptors: West Nile virus, host seeking mosquitoes, seasonal abundance, traps, *Culex tarsalis*, North Dakota, vectors.

Colton, L., B.J. Biggerstaff, A. Johnson, and R.S. Nasci (2005). **Quantification of West Nile virus in vector mosquito saliva.** *Journal of the American Mosquito Control Association* 21(1): 49-53.
Abstract: Saliva was collected from 4 species of mosquitoes intrathoracically inocu-

lated with West Nile virus (WNV). The amount of infectious virus in the saliva was quantified by plaque assay and the number of WNV genomic equivalents (GE) was measured by reverse transcriptase-polymerase chain reaction. *Ochlerotatus triseriatus* had the greatest mean amount of infectious virus per saliva collection, followed by *Aedes albopictus, Culex pipiens*, and *Cx. quinquefasciatus*. The mean GE/saliva collection was also greatest in *Oc. triseriatus*, followed by *Cx. quinquefasciatus, Cx. pipiens*, and *Ae. albopictus*. The variance of log GE/saliva collection for *Ae. albopictus* was significantly lower than the variance for the other 3 species. This study provides a basis for comparing this component of vector competence and for determining the amounts of virus inoculated into vertebrates in experimental host competence studies.

Descriptors: culicidae virology, insect vectors virology, saliva virology, West Nile virus, aedes virology, culex virology, ochlerotatus virology, polymerase chain reaction.

Colton, L. and R.S. Nasci (2006). **Quantification of West Nile virus in the saliva of Culex species collected from the southern United States.** *Journal of the American Mosquito Control Association* 22(1): 57-63.

Abstract: *Culex quinquefasciatus. Cx. restuans, Cx. pipiens complex*, and *Cx. nigripalpus* were collected as larvae or egg rafts from the southern USA. Adult female mosquitoes were intrathoracically inoculated with approximately 1,000 plaque-forming units of West Nile virus (WNV) and saliva was collected from them 5 days later. The amount of infectious WNV in the saliva samples was quantified by plaque assay and WNV RNA was detected by reverse transcriptase polymerase chain reaction (RT-PCR). More than 90% of the mosquitoes had either infectious virus or viral RNA in their saliva. The RT-PCR assay detected a greater percent of samples with WNV RNA than the plaque assay detected infectious virus. Pairwise comparisons revealed 6 significant differences between the 7 groups surveyed. The *Cx. nigripalpus* secreted lower mean amounts of WNV than 3 other groups, and a difference was found between early- and late-season *Cx. quinquefasciatus* collected in Louisiana.

Descriptors: culex virology, West Nile virus, Florida, insect vectors virology, Louisiana, plaque assay, reverse transcriptase polymerase chain reaction, saliva virology, Tennessee, West Nile fever transmission.

Cooke, W.H.3., K. Grala, and R.C. Wallis (2006). **Avian GIS models signal human risk for West Nile virus in Mississippi.** *International Journal of Health Geographics* 5: 36.

Abstract: BACKGROUND: West Nile virus (WNV) poses a significant health risk for residents of Mississippi. Physicians and state health officials are interested in new and efficient methods for monitoring disease spread and predicting future outbreaks. Geographic Information Systems (GIS) models have the potential to support these efforts. Environmental conditions favorable for mosquito habitat were modeled using GIS to derive WNV risk maps for Mississippi. Variables important

to WNV dissemination were selected and classified as static and dynamic. The static variables included road density, stream density, slope, and vegetation. The dynamic variable represented seasonal water budget and was calculated using precipitation and evaporation estimates. Significance tests provided deterministic evidence of variable importance to the models. RESULTS: Several models were developed to estimate WNV risk including a landscape-base model and seasonal climatic sub-models. P-values from t-tests guided variable importance ranking. Variables were ranked and weights assigned as follows: road density (0.4), stream density (0.3), slope (0.2) and vegetation (0.1). This landscape-base model was modified by climatic conditions to assess the importance of climate to WNV risk. Human case data at the zip code level were used to validate modeling results. All models were summarized by zip codes for interpretation and model validation. For all models, estimated risk was higher for zip codes with at least one human case than for zip codes where no human cases were recorded. Overall median measure of risk by zip code indicated that 67% of human cases occurred in the high-risk category. CONCLUSION: Modeling results indicated that dead bird occurrences are correlated with human WNV risk and can facilitate the assessment of environmental variables that contribute to that risk. Each variable's importance in GIS-based risk predictions was assigned deterministically. Our models indicated non-uniform distribution of risk across the state and showed elevated risk in urban and as well as rural areas. Model limitations include resolution of human data, zip code aggregation issues, and quality/availability of vegetation and stream density layers. Our approach verified that WNV risk can be modeled at the state level and can be modified for risk predictions of other vector-borne diseases in varied ecological regions.

Descriptors: disease outbreaks, environment, geographic information systems, models, theoretical, West Nile fever epidemiology, West Nile virus isolation and purification, birds, mississippi epidemiology, risk factors, West Nile virus.

Davis, A., M. Bunning, P. Gordy, N. Panella, B. Blitvich, and R. Bowen (2005). **Experimental and natural infection of North American bats with West Nile virus.** *American Journal of Tropical Medicine and Hygiene* 73(2): 467-469. ISSN: 0002-9637.

Abstract: Big brown (*Eptesicus fuscus*) and Mexican free-tailed (*Tadarida brasiliensis*) bats were inoculated with the New York 99 strain of West Nile virus to assess their potential to serve as amplifying hosts and determine the clinical effect of infection. Groups of three or four bats were bled at daily intervals between 1 and 6 days after inoculation to determine the pattern of viremia. Beginning 2 days after inoculation, virus was isolated each day from one or more *E. fuscus* bats, in titers ranging from 10 to 180 plaque-forming units per milliliter of serum. Virus was not isolated from any of the sera collected from *T brasiliensis bats*. None of the bats from either species showed clinical signs associated with exposure to virus. Sera from an additional 149

bats collected in Louisiana in 2002 during an epizootic of West Nile fever were tested for antibodies to virus, and two were found to be positive. These data suggest that bats from these two widely distributed species are unlikely to serve as amplifying hosts for West Nile virus.

Descriptors: *Eptesicus fuscus*, *Tadarida brasiliensis*, transmission of viruses, West Nile virus, experimental and natural infection, USA.

Diuk Wasser, M.A., H.E. Brown, T.G. Andreadis, and D. Fish (2006). **Modeling the spatial distribution of mosquito vectors for west Nile virus in Connecticut, USA.** *Vector Borne and Zoonotic Diseases* 6(3): 283-295. ISSN: 1530-3667.

Abstract: The risk of transmission of West Nile virus (WNV) to humans is associated with the density of infected vector mosquitoes in a given area. Current technology for estimating vector distribution and abundance is primarily based on Centers for Disease Control and Prevention (CDC) light trap collections, which provide only point data. In order to estimate mosquito abundance in areas not sampled by traps, we developed logistic regression models for five mosquito species implicated as the most likely vectors of WNV in Connecticut. Using data from 32 traps in Fairfield County from 2001 to 2003, the models were developed to predict high and low abundance for every 30 X 30 m pixel in the County. They were then tested with an independent dataset from 16 traps in adjacent New Haven County. Environmental predictors of abundance were extracted from remotely sensed data. The best predictive models included non-forested areas for *Culex pipiens*, surface water and distance to estuaries for *Cx. salinarius*, surface water and grasslands/agriculture for *Aedes vexans* and seasonal difference in the normalized difference vegetation index and distance to palustrine habitats for *Culiseta melanura*. No significant predictors were found for *Cx. restuans*. The sensitivity of the models ranged from 75% to 87.5% and the specificity from 75% to 93.8%. In New Haven County, the models correctly classified 81.3% of the traps for *Cx. pipiens*, 75.0% for *Cx. salinarius*, 62.5% for *Ae. vexans*, and 75.0% for *Cs. melanura*. Continuous surface maps of habitat suitability were generated for each species for both counties, which could contribute to future surveillance and intervention activities.

Descriptors: culicidae, ecological techniques, transmission of viruses, flavivirus, modelling vector spatial distribution and abundance, population density, Connecticut.

Ebel, G., I. Rochlin, J. Longacker, and L. Kramer (2005). ***Culex restuans* (Diptera: Culicidae) relative abundance and vector competence for West Nile virus.** *Journal of Medical Entomology.* 42(5): 838-843. ISSN: 0022-2585.

Abstract: The abundance and vector competence of *Culex restuans* Theobald and *Culex pipiens* L. were compared to determine the relative importance of these species as West Nile virus (WNV) vectors in the northeastern United States. Abundance

was estimated from egg raft collections at 12 sites in Albany, Suffolk, and Richmond counties, New York, during July, August, and September 2002 and 2003. *Cx. restuans* was more abundant than *Cx. pipiens* in both urban and rural areas, comprising 86% of 1,623 egg rafts collected. Vector competence for WNV was estimated after feeding on an artificial bloodmeal and in vitro transmission assays. The vector competence of the two species for WNV was similar, but the dynamics of infection seems to be mosquito species dependent. These findings suggest an important role for *Cx. restuans* in WNV transmission cycles in New York.
Descriptors: *Culex restuans*, *Culex pipiens*, population density, insect vectors, West Nile virus, vector competence, hematophagy, infection, virus transmission, virus replication, vector potential, New York.

Erickson, S.M., K.B. Platt, B.J. Tucker, R. Evans, S. Tiawsirisup, and W.A. Rowley (2006). **The potential of *Aedes triseriatus* (Diptera: Culicidae) as an enzootic vector of West Nile virus.** *Journal of Medical Entomology* 43(5): 966-970. ISSN: 0022-2585.
Abstract: The susceptibility of *Aedes triseriatus* (Say) (Diptera: Culicidae) to low levels of West Nile virus (family Flaviviridae, genus Flavivirus, WNV) was determined and compared with that of *Culex pipiens L.* to assess the likelihood of its participation in an enzootic cycle involving mammals. *Ae. triseriatus* and *Cx. pipiens* were exposed to WNV by feeding on baby chickens with WNV serum titers ranging from 10(4.1 +/- 0.1) to 10(8.6 +/- 0.1) plaque-forming units (PFU)/ml and from 10(4.1 +/- 0.1) to 10(7.0) PFU/ml, respectively. Infection rates and 95% confidence intervals (CIs) of 8% (4, 14) and 25% (15, 38) occurred in *Ae. triseriatus* and *Cx. pipiens* after feeding on chickens with WNV titers of 10(4.1 +/- 0.1) PFU/ml and increased to 65% (49, 79) and 100% (72, 100) in *Ae. triseriatus* and *Cx. pipiens* after feeding on chickens with titers of 10(7.1 +/- 0.1) PFU/ml. The mean infection rate of *Ae. triseriatus* ranged from 97% (84, 100) to 100% (79, 100) after feeding on chickens with WNV titers of > or = 10(8.2) PFU/ml. The infectious dose (ID)50 values for *Ae. triseriatus* and *Cx. pipiens* were 10(6.5) (6.4, 6.7) and 10(4.9) (4.6, 5.1) PFU/ml, respectively. The combined estimated transmission rate of *Ae. triseriatus* at 14 and 18 d after feeding on chickens with a mean WNV titer of 10(8.6 +/- 0.1) PFU/ml was 55%. Although *Ae. triseriatus* is significantly less susceptible to WNV than *Cx. pipiens*, the susceptibility of *Ae. triseriatus* to WNV titers < 10(5.0) PFU/ml and its ability to transmit WNV suggest that *Ae. triseriatus* has the potential to be an enzootic vector among mammalian populations.
Descriptors: West Nile virus, *Aedes triseriatus*, *Culex pipiens*, susceptibility, feeding, baby chickens, infection rate, enzootic vector.

Formosinho, P. and M.M. Santos Silva (2006). **Experimental infection of *Hyalomma marginatum* ticks with West Nile virus.** *Acta Virologica* 50(3): 175-180.
Abstract: To define the possible role of *Hyalomma marginatum* ticks in the trans-

mission of West Nile virus (WNV) in Portugal an experimental infection was established. Ticks were fed on viremic rabbits previously infected with WNV. In different developmental stage of *H. marginatum* virus isolation and detection of viral antigen and viral RNA were attempted. The oral infection rates were 3%, 33% and 75% for engorged larvae, nymphs and females after oviposition, respectively. Transstadial transmission rates for nymphs exposed to virus as larvae, for adults exposed as larvae, and for adults exposed as nymphs were 33%, 11% and 46%, respectively. No evidence of transovarial transmission was obtained. Ticks in the stages of nymphs and adults were able to transmit the infection to uninfected hosts. This study demonstrated that *H. marginatum* could be involved in the natural circulation of WNV in Portugal.

Descriptors: West Nile virus, ticks, experimental infection, vector, *Hyalomma marginatum*, viremic rabbits, infection rates, transviral transmission.

Furumizo, R., W. Warashina, and H. Savage (2005). **First collection of *Anopheles punctipennis* (Say) on Oahu, Hawaii: Implications for the potential introduction of West Nile virus.** *Journal of the American Mosquito Control Association.* 21(2): 225-226. ISSN: 8756-971X.

Descriptors: *Anopheles punctipennis*, mosquitoes, geographical distribution, new geographic records, introduced species, insect vectors, West Nile virus, pest monitoring, disease surveillance, quarantine, Hawaii, pest-introduction, vector-surveillance.

Fyodorova, M.V., H.M. Savage, J.V. Lopatina, T.A. Bulgakova, A.V. Ivanitsky, O.V. Platonova, and A.E. Platonov (2006). **Evaluation of potential West Nile virus vectors in Volgograd region, Russia, 2003 (Diptera: Culicidae): Species composition, bloodmeal host utilization, and virus infection rates of mosquitoes.** *Journal of Medical Entomology* 43(3): 552-563. ISSN: 0022-2585.

Abstract: Potential West Nile virus (family Flaviviridae, genus Flavivirus, WNV) vectors were assessed during 2003 at indoor and outdoor collection sites in urban Volgograd, Russia, and in three nearby towns and surrounding rural areas. In total, 9,182 female mosquitoes comprising 13 species in six genera were collected. Relative abundance and bloodmeal host utilization differed temporarily and spatially. During June and July in Volgograd, *Aedes vexans* (Meigen) (85.4%) and *Culex p. pipiens* L. (7.6%) were the two most abundant species collected indoors, whereas during August, *Cx. p. pipiens* was the dominant species, accounting for 87.9% of specimens collected. Two WNV-positive mosquito pools were detected in August: one pool was composed of *Cx. p. pipiens* and the other pool of *Culex modestus* Ficalbi. *Anopheles messeae* Falleroni, *Aedes caspius* (Pallas), *Ae. vexans*, *Cx. modestus*, and *Cx. p. pipiens* used both humans and birds as bloodmeal sources. In urban areas, 20.4% of the *Cx. p. pipiens* fed on humans, 58.1% fed on chickens, and six specimens were positive for both chicken and human blood. *Culex p. pipiens* collected from flooded basements

were predominantly autogenous (91.7%), whereas adult females resting in buildings with dry basements were composed of 67.5% anautogenous and 32.5% autogenous specimens. Our data suggest that the primary WNV vectors in the Volgograd region were *Cx. p. pipiens* and *Cx. modestus* and that intense transmission of WNV to humans in urban areas during the epidemic of 1999 may have been facilitated by the abundance and concentration of anautogenous *Cx. p. pipiens* in multistory buildings. The role of autogenous *Cx. p. pipiens* in urban transmission remains unresolved.
Descriptors: insect vectors, West Nile virus, climate, environment, feeding behavior, host parasite relations, Russia, urban population, West Nile fever epidemiology.

Gibbs, S.E., M.C. Wimberly, M. Madden, J. Masour, M.J. Yabsley, and D.E. Stallknecht (2006). **Factors affecting the geographic distribution of West Nile virus in Georgia, USA: 2002-2004.** *Vector Borne and Zoonotic Diseases* 6(1): 73-82. ISSN: 1530-3667.

Abstract: The distribution of West Nile virus (WNV) is dependent on the occurrence of both susceptible avian reservoir hosts and competent mosquito vectors. Both factors can be influenced by geographic variables such as land use/landcover, elevation, human population density, physiographic region, and temperature. The current study uses geographic information systems (GIS) and logistic regression analyses to model the distribution of WNV in the state of Georgia based on a wild bird indicator system, and to identify human and environmental predictor variables that are important in the determination of WNV distribution. A database for Georgia was constructed that included (1) location points of all the avian samples tested for WNV, (2) local land use classifications, including temperature, physiographic divisions, land use/landcover, and elevation, (3) human demographic data from the U.S. Census, and (4) statistics summarizing land cover, elevation, and climate within a 1-km-radius landscape around each sample point. Logistic regression analysis was carried out using the serostatus of avian collection sites as the dependent variable. Temperature, housing density, urban/suburban land use, and mountain physiographic region were important variables in predicting the distribution of WNV in the state of Georgia. While weak, the positive correlation between WNV-antibody positive sites and the urban/suburban environment was consistent throughout the study period. The risks associated with WNV endemicity appear to be increased in urban/suburban areas and decreased in the mountainous region of the state. This information may be used in addressing regional public health needs and mosquito control programs.
Descriptors: West Nile virus distribution, avian reservoir hosts, vectors, geographic variables, control programs.

Gingrich, J.B., R.D. Anderson, G.M. Williams, L. O'Connor, and K. Harkins (2006). **Stormwater ponds, constructed wetlands, and other best management practices as potential breeding sites for West Nile virus vectors in Delaware during 2004.** *Journal of the American Mosquito Control Association* 22(2): 282-291.

Abstract: We performed longitudinal surveys of mosquito larval abundance (mean mosquito larvae per dip) in 87 stormwater ponds and constructed wetland in Delaware from June to September 2004. We analyzed selected water quality factors, water depth, types of vegetation, degree of shade, and level of insect predation in relation to mosquito abundance. The 2004 season was atypical, with most ponds remaining wet for the entire summer. In terms of West Nile virus (WNV) vectors, wetlands predominantly produce *Aedes vexans, Culex pipiens pipiens*, and *Culex restuans*. Retention ponds generally produced the same species as wetlands, except that *Cx. p. pipiens* was more abundant than *Cx. restuans* in retention ponds. *Aedes vexans* and *Culex salinarius* were the most abundant species to Conservation Restoration Enhancement Program ponds. Sand filters uniquely produced high numbers of *Cx. restuans, Cx. p. pipiens*, and *Aedes japonicus japonicus*, a newly invasive vector species. Site that alternately dried and flooded, mostly detention ponds, forebays of retention ponds, and some wetlands often produced Ae. vexans, an occasional WNV bridge vector species. Overall, seasonal distribution of vectors was bimodal, with peaks occurring during early and late summer. Ponds with shallow sides and heavy shade generally produced an abundance of mosquitoes, unless insect predators were abundant. Bright, sunny ponds with steep sides and little vegetation generally produced the fewest mosquitoes. The associations among mosquito species and selected vegetation types are discussed.

Descriptors: Culicidae virology, insect vectors, West Nile virus, Aedes, Anopheles, Culex virology, Delaware, fresh water ecology, population density, seasons , waste disposal.

Gingrich, J.B. and G.M. Williams (2005). **Host feeding patterns of suspected West Nile virus mosquito vectors in Delaware, 2001-2002.** *Journal of the American Mosquito Control Association* 21(2): 194-200. ISSN: 8756-971X.

Abstract: Paucity of data on host-feeding patterns and behavior of 43 mosquito species that are reported as suspected West Nile virus (WN) vectors has limited full evaluation of their vectorial capacity. Recent studies addressing this issue need additional confirmation and should also be expanded to include collections of species or subpopulations attracted to humans. We used 4 types of collection methods to collect mosquitoes, including omnidirectional Fay-Prince traps, Centers for Disease Control-type light traps, gravid traps, and human-landing collections. Mosquitoes were collected during 2 full WN transmission seasons in 2001 and 2002, at 9 different sites across Delaware. We collected from various habitats, including salt marshes, brackish water areas, woodlands, a tire dump, a racetrack, and a mushroom farm.

Blood-meal analyses were performed on parous mosquitoes by using a sandwich-type enzyme-linked immunosorbent assay. We tested primarily for 5 common host species, including rabbits, dogs, deer, horses, and chickens. We obtained substantial host-feeding data from 8 mosquito species. The most mammalophilic species were *Anopheles quadrimaculatus*, *Coquillettidia perturbans*, and *Aedes albopictus*, whereas the most ornithophilic species was *Culex pipiens*. *Aedes albopictus* was the most anthropophilic species, whereas *Ae. vexans* and *Cq. perturbans* exhibited relatively low attraction to humans. *Culex salinarius* was the species with the most diverse host-feeding activity. Based on feeding behavior, *Cx. pipiens* and *Cx. salinarius* appear to be the most likely bridge vectors. Other species may have opportunities to be bridge vectors under special circumstances, as discussed in the paper.
Descriptors: culicidae, feeding behaviour, avian hosts, human hosts, mammalian hosts, transmission of viruses, West Nile virus, avian, mammalian and human host preferences and feeding behaviour, suspected vectors, Delaware.

Girard, Y., V. Popov, J. Wen, V. Han, and S. Higgs (2005). **Ultrastructural study of West Nile virus pathogenesis in *Culex pipiens quinquefasciatus* (Diptera: Culicidae).** *Journal of Medical Entomology.* 42(3): 429-444. ISSN: 0022-2585.
Abstract: The ultrastructural features of West Nile virus (WNV) replication and dissemination in orally infected *Culex pipiens quinquefasciatus* Say were analyzed over a 25-d infection period. To investigate the effects of virus replication on membrane induction, cellular organization, and cell viability in midgut and salivary gland tissues, midguts were dissected on days 3, 7, 14, and 21, and salivary glands were collected on days 7, 14, 21, and 25 postinfection (d.p.i.) for examination by transmission electron microscopy (TEM). Whole mosquito heads were embedded for TEM analysis 14 d.p.i. to localize WNV particles and to investigate the effects of replication on nervous tissues of the brain. Membrane proliferation was induced by WNV in the midgut epithelium, midgut muscles, and salivary glands, although extensive endoplasmic reticulum swelling was a unique feature of salivary gland infection. TEM revealed WNV-induced pathology in salivary glands at 14, 21, and 25 d.p.i., and we hypothesize that long-term virus infection of this tissue results in severe cellular degeneration and apoptotic-like cell death. This finding indicates that the efficiency of WNV transmission may decrease with mosquito age postinfection.
Descriptors: *Culex pipiens*, insect vectors, West Nile virus, infection, pathogenesis, tissue distribution, histopathology, midgut, salivary glands, brain, ultrastructure, apoptosis, virus replication, virus-dissemination.

Godsey, M.S.J., R. Nasci, H.M. Savage, S. Aspen, R. King, A.M. Powers, K. Burkhalter, L. Colton, D. Charnetzky, S. Lasater, V. Taylor, and C.T. Palmisano (2005). **West Nile virus-infected mosquitoes, Louisiana, 2002.** *Emerging Infectious Diseases* 11(9): 1399-1404. ISSN: 1080-6040.

Abstract: Human cases of West Nile virus (WNV) disease appeared in St. Tammany and Tangipahoa Parishes in southeastern Louisiana in June 2002. Cases peaked during July, then rapidly declined. We conducted mosquito collections from August 3 to August 15 at residences of patients with confirmed and suspected WNV disease to estimate species composition, relative abundance, and WNV infection rates. A total of 31,215 mosquitoes repre senting 25 species were collected by using primarily gravid traps and CO2-baited light traps. Mosquitoes containing WNV RNA were obtained from 5 of 11 confirmed case sites and from 1 of 3 sites with non-WNV disease. WNV RNA was detected in 9 mosquito pools, including 7 *Culex quinquefasciatus*, 1 *Cx salinarius*, and 1 *Coquillettidia perturbans* . Mosquito infection rates among sites ranged from 0.8/1,000 to 10.9/1,000. Results suggest that *Cx. quinquefasciatus* was the primary epizootic/epidemic vector, with other species possibly playing a secondary role.
Descriptors: culicidae, human hosts, transmission of viruses, West Nile virus, vector community structure and human disease prevalence relationships, community structure, population density, Louisiana, St Tammany and Tangipahoa Parishes.

Godsey, M.J., M. Blackmore, N. Panella, K. Burkhalter, K. Gottfried, L. Halsey, R. Rutledge, S. Langevin, R. Gates, and K. Lamonte (2005). **West Nile virus epizootiology in the southeastern United States, 2001.** *Vector Borne and Zoonotic Diseases.* 5(1): 82-89. ISSN: 1530-3667.
Descriptors: West Nile virus, virus transmission, insect vectors, Culex, *Culiseta melanura*, mosquitoes, disease reservoirs, birds, disease detection, antibody detection, seroprevalence, chickens, sentinel animals, Southeastern United States, Florida , Georgia.

Gu, W., R. Lampman, N. Krasavin, R. Berry, and R. Novak (2006). **Spatio-temporal analyses of West Nile virus transmission in culex mosquitoes in northern Illinois, USA, 2004.** *Vector Borne and Zoonotic Diseases* 6(1): 91-98 . ISSN: 1530-3667.
Abstract: After a severe outbreak of West Nile virus (WNV) in Cook County, Illinois, in 2002, detections of WNV in mosquitoes were frequent across the state in the following years despite small numbers of human cases. We conducted a spatio-temporal analysis of Culex (subgenus Culex) mosquitoes collected in 2004 in three mosquito abatement districts (MAD) in Cook County by calculating monthly estimates of mosquito density, prevalence of infected mosquitoes, and exposure intensity, which in turn is a product of mosquito density and infection rates. Mosquito infections were detected early at three sites in late May and were widely detected throughout the three MADs in the summer with infection rates as high as 13 per 1000 in August. Exposure intensities were higher at sites adjacent to the Des Plaines River, especially in August and September. The aggregated pattern of WNV transmission along the river might be related to the existence of substantial forest preserves

and wetlands that might produce ecological conditions favorable for mosquito proliferation and interactions between mosquitoes and birds.
Descriptors: West Nile virus, culex mosquitoes, infection rate, wetlands, birds, prevalence, ecological conditions.

Hahn, D.C., N.M. Nemeth, E. Edwards, P.R. Bright, and N. Komar (2006). **Passive West Nile virus antibody transfer from maternal Eastern screech-owls (*Megascops asio*) to progeny.** *Avian Diseases* 50(3): 454-455. ISSN: 0005-2086.
Abstract: Transovarial antibody transfer in owls has not been demonstrated for West Nile virus (WNV). We sampled chicks from captive adult WNV-antibody-positive Eastern Screech-Owls (*Megascops asio*) to evaluate the prevalence of transovarial maternal antibody transfer, as well as titers and duration of maternal antibodies. Twenty-four owlets aged 1 to 27 days old circulated detectable antibodies with neutralizing antibody titers ranging from 20 to 1600 (median 1:40). Demonstrating that WNV antibodies are passively transferred transovarially is important for accurate interpretation of serologic data from young birds.
Descriptors: viral blood antibodies, bird diseases, maternally acquired immunity, West Nile virus, screech owls.

Hayes, E.B., N. Komar, R.S. Nasci, S.P. Montgomery, D.R. O'Leary, and G.L. Campbell (2005). **Epidemiology and transmission dynamics of West Nile virus disease.** *Emerging Infectious Diseases* 11(8): 1167-1173. ISSN: 1080-6040.
Abstract: From 1937 until 1999, West Nile virus (WNV) garnered scant medical attention as the cause of febrile illness and sporadic encephalitis in parts of Africa, Asia, and Europe. After the surprising detection of WNV in New York City in 1999, the virus has spread dramatically westward across the United States, southward into Central America and the Caribbean, and northward into Canada, resulting in the largest epidemics of neuroinvasive WNV disease ever reported. From 1999 to 2004, >7,000 neuroinvasive WNV disease cases were reported in the United States. In 2002, WNV transmission through blood transfusion and organ transplantation was described for the first time, intrauterine transmission was first documented, and possible transmission through breastfeeding was reported. This review highlights new information regarding the epidemiology and dynamics of WNV transmission, providing a new platform for further research into preventing and controlling WNV disease.
Descriptors: disease outbreaks, insect vectors, West Nile fever epidemiology, West Nile virus growth and development, humans, United States.

Higgs, S., B. Schneider, D. Vanlandingham, K. Klingler, and E. Gould (2005). **Nonviremic transmission of West Nile virus.** *Proceedings of the National Academy of Sciences of the United States of America.* 102(25): 8871-8874. ISSN: 0027-8424.

Abstract: West Nile virus (WNV) is now the predominant circulating arthropod-borne virus in the United States with >15,000 human cases and >600 fatalities since 1999. Conventionally, mosquitoes become infected when feeding on viremic birds and subsequently transmit the virus to susceptible hosts. Here, we demonstrate non-viremic transmission of WNV between cofeeding mosquitoes. Donor, *Culex pipiens quinquefasciatus* mosquitoes infected with WNV were fed simultaneously with uninfected "recipient" mosquitoes on naive mice. At all times, donor and recipient mosquitoes were housed in separate sealed containers, precluding the possibility of mixing. Recipients became infected in all five trials, with infection rates as high as 5.8% and no detectable viremia in the hosts. Remarkably, a 2.3% infection rate was observed when 87 uninfected mosquitoes fed adjacent to a single infected mosquito. This phenomenon could potentially enhance virus survival, transmission, and dispersion and obviate the requirement for viremia. All vertebrates, including immune and insusceptible animals, might therefore facilitate mosquito infection. Our findings question the status of dead-end hosts in the WNV transmission cycle and may partly explain the success with which WNV established and rapidly dispersed throughout North America.
Descriptors: West Nile virus, disease transmission, *Culex quinquefasciatus*, *Culex pipiens*, mosquitoes , insect vectors, hematophagy, mice, *Culex-pipiens-quinquefasciatus.*

Hutcheson, H.J., C.H. Gorham, C. Machain Williams, M.A. Lorono Pino, A.M. James, N.L. Marlenee, B. Winn, B.J. Beaty, and C.D. Blair (2005). **Experimental transmission of West Nile virus (Flaviviridae: Flavivirus) by *Carios capensis* ticks from North America.** *Vector Borne and Zoonotic Diseases* 5(3): 293-295. ISSN: 1530-3667.
Abstract: Seabird soft ticks, *Carios capensis* (Ixodida: Argasidae), originally collected from coastal Georgia, USA, were allowed to ingest a blood meal from pekin ducklings (*Anas domesticus*) infected with WNV. After 35 days of extrinsic incubation, the ticks transmitted virus to naive ducklings. WNV was detected via plaque assay and RTPCR in ticks and in tissues and serum of ducklings 7 days post infestation.
Descriptors: arachnid vectors, bird diseases, ducks, ticks, West Nile fever, viral RNA analysis, reverse transcriptase polymerase chain reaction, viremia.

Kilpatrick, A.M., P. Daszak, M.J. Jones, P.P. Marra, and L.D. Kramer (2006). **Host heterogeneity dominates West Nile virus transmission.** *Proceedings. Biological Sciences The Royal Society* 273(1599): 2327-2333.
Abstract: Heterogeneity in host populations and communities can have large effects on the transmission and control of a pathogen. In extreme cases, a few individuals give rise to the majority of secondary infections, which have been termed super spreading events. Here, we show that transmission of West Nile virus (WNV) is dominated by extreme heterogeneity in the host community, resulting in highly

inflated reproductive ratios. A single relatively uncommon avian species, American robin (*Turdus migratorius*), appeared to be responsible for the majority of WNV-infectious mosquitoes and acted as the species equivalent of a super spreader for this multi-host pathogen. Crows were also highly preferred by mosquitoes at some sites, while house sparrows were significantly avoided. Nonetheless, due to their relative rarity, corvids (crows and jays) were relatively unimportant in WNV amplification. These results challenge current beliefs about the role of certain avian species in WNV amplification and demonstrate the importance of determining contact rates between vectors and host species to understand pathogen transmission dynamics.
Descriptors: birds, Culicidae physiology, feeding behavior, West Nile fever transmission, West Nile virus isolation and purification.

Kilpatrick, A.M., L.D. Kramer, S.R. Campbell, E.O. Alleyne, A.P. Dobson, and P. Daszak (2005). **West Nile virus risk assessment and the bridge vector paradigm.** *Emerging Infectious Diseases* 11(3): 425-429. ISSN: 1080-6040.
Abstract: In the northeast United States, control of West Nile virus (WNV) vectors has been unfocused because of a lack of accurate knowledge about the roles different mosquitoes play in WNV transmission. We analyzed the risk posed by 10 species of mosquitoes for transmitting WNV to humans by using a novel risk-assessment measure that combines information on the abundance, infection prevalence, vector competence, and biting behavior of vectors. This analysis suggests that 2 species (*Culex pipiens* L. and *Cx. restuans* Theobald [Diptera: Cilicidae]) not previously considered important in transmitting WNV to humans may be responsible for up to 80% of human WNV infections in this region. This finding suggests that control efforts should be focused on these species which may reduce effects on nontarget wetland organisms. Our risk measure has broad applicability to other regions and diseases and can be adapted for use as a predictive tool of future human WNV infections.
Descriptors: culex mosquitoes physiology, insect vectors, West Nile fever transmission, New England, risk, West Nile virus.

Kilpatrick, A.M., L.D. Kramer, M.J. Jones, P.P. Marra, and P. Daszak (2006). **West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior.** *PLoS Biology* 4(4): E82.
Online: http://biology.plosjournals.org/perlserv/?request=get-document&doi=10.1371/journal.pbio.0040082
Abstract: West Nile virus (WNV) has caused repeated large-scale human epidemics in North America since it was first detected in 1999 and is now the dominant vector-borne disease in this continent. Understanding the factors that determine the intensity of the spillover of this zoonotic pathogen from birds to humans (via mosquitoes) is a prerequisite for predicting and preventing human epidemics. We

integrated mosquito feeding behavior with data on the population dynamics and WNV epidemiology of mosquitoes, birds, and humans. We show that *Culex pipiens*, the dominant enzootic (bird-to-bird) and bridge (bird-to-human) vector of WNV in urbanized areas in the northeast and north-central United States, shifted its feeding preferences from birds to humans by 7-fold during late summer and early fall, coinciding with the dispersal of its preferred host (American robins, *Turdus migratorius*) and the rise in human WNV infections. We also show that feeding shifts in *Cx. tarsalis* amplify human WNV epidemics in Colorado and California and occur during periods of robin dispersal and migration. Our results provide a direct explanation for the timing and intensity of human WNV epidemics. Shifts in feeding from competent avian hosts early in an epidemic to incompetent humans after mosquito infection prevalences are high result in synergistic effects that greatly amplify the number of human infections of this and other pathogens. Our results underscore the dramatic effects of vector behavior in driving the transmission of zoonotic pathogens to humans.

Descriptors: West Nile virus, vector-borne diseases, mosquito hosts, *Culex pipens*, feeding preferences of mosquitos, birds to human shift in preference, seasonal shift, WNV epidemics.

Kipp, A.M., J.A. Lehman, R.A. Bowen, P.E. Fox, M.R. Stephens, K. Klenk, N. Komar, and M.L. Bunning (2006). **West Nile virus quantification in feces of experimentally infected American and fish crows.** *American Journal of Tropical Medicine and Hygiene* 75(4): 688-690. ISSN: 0002-9637.

Abstract: To better understand the potential environmental health risk presented by West Nile virus (WNV)-contaminated feces, we quantified the amount of WNV present in the feces of experimentally infected American crows (*Corvus brachyrhynchos*) and fish crows (*Corvus ossifragus*). Peak fecal titers ranged from 10(3.5) to 10(8.8) plaque-forming units (PFU)/g for 10 American crows and from 10(2.3) to 10(6.4) PFU/g for 10 fish crows. The presence of infectious WNV in bird feces indicates a potential for direct transmission of WNV. Thus, handlers of sick or dead birds should take appropriate precautions to avoid exposure to fecal material.

Descriptors: bird diseases, crows, feces, virus shedding, West Nile fever, West Nile virus isolation and purification.

Komar, N., N.A. Panella, S.A. Langevin, A.C. Brault, M. Amador, E. Edwards, and J.C. Owen (2005). **Avian hosts for West Nile Virus in St. Tammany Parish, Louisiana, 2002.** *American Journal of Tropical Medicine and Hygiene* 73(6): 1031-1037. ISSN: 0002-9637.

Abstract: West Nile virus (WNV) infections in free-ranging birds were studied in Slidell, St. Tammany Parish, Louisiana, after a human encephalitis outbreak peaked there in July 2002. Seroprevalence in resident, free-ranging wild birds in one sub-

urban site was 25% and 24% in August and October, respectively, indicating that most transmission had ceased by early August. Mortality rates, seroprevalence rates, host competence, and crude population estimates were used in mathematical models to predict actual infection rates, population impacts, and importance as amplifying hosts for several common passerine birds. Northern cardinal (*Cardinalis cardinalis*) and house sparrow (*Passer domesticus*) were the principal amplifying hosts, but blue jay (*Cyanocitta cristata*) and northern mockingbird (*Mimus polyglottos*) also contributed. The blue jay population was reduced by an estimated 47%. A variety of passerine bird species combined to play an important role as amplifying hosts in the WNV transmission cycle.

Descriptors: aves, viral diseases, West Nile virus, prevalence survey and role in transmission cycle, transmission of viruses, hosts role in transmission cycle, Luisiana, St Tammany parish, viral disease prevalence survey and role in transmission cycle.

Kunkel, K.E., R.J. Novak, R.L. Lampman, and W. Gu (2006). **Modeling the impact of variable climatic factors on the crossover of *Culex restauns* [restuans] and *Culex pipiens* (Diptera: Culicidae), vectors of West Nile virus in Illinois.** *American Journal of Tropical Medicine and Hygiene* 74(1): 168-173. ISSN: 0002-9637.

Abstract: The aim of this study was to model the impact of temperature on the timing of the seasonal shift in relative proportion of *Culex restuans* Theobald and *Culex pipiens* L. in Illinois. The temporal pattern of West Nile virus (WNV) and St. Louis encephalitis virus transmission in the midwest exhibits a late summer to early fall peak in activity, which parallels the temporal increase in the abundance of *Cx. pipiens*. The daily number of egg rafts oviposited by each species has been monitored at multiple surveillance sites in Urbana-Champaign in central Illinois for more than 13 years. The time when the two Culex species are in equal abundance (crossover) varies considerably from year to year. Our investigation of several thermal measures indicated that this variation was related in large part to climatic conditions with warmer (cooler) temperatures correlated to earlier (later) crossover dates. Models based on degree days and the number of days in which the daily maximum temperature exceeded an upper temperature threshold explained more than 60% of the variance in crossover dates. In contrast, models based on the number of days in which the daily minimum temperature exceeded a lower temperature threshold explained no more than 52% of the variance. An evaluation of these models demonstrated that they provide relatively simple and accurate estimates of crossover date from daily temperature data, a necessary component for developing an overall climatic index for the risk of WNV transmission in Illinois.

Descriptors: *Culex pipiens*, *Culex restuans*, viral diseases, West Nile virus, transmission of viruses, temperature effect on seasonal change in relative abundance relations,

relative abundance, climate and weather, temperature, Illinois, Urbana Champaign region.

Lampman, R., M. Slamecka, N. Krasavin, K. Kunkel, and R. Novak (2006). **Culex population dynamics and West Nile virus transmission in east-central Illinois.** *Journal of the American Mosquito Control Association* 22(3): 390-400.
Abstract: Temporal changes in the abundance *Culex restuans* and *Culex pipiens* were monitored in east-central Illinois for over a decade using infusion-baited oviposition traps. The 2 species typically exhibited a seasonal shift in relative abundance with a mean crossover date (when the proportion of egg rafts from both species is equal) of August 10 or 11, depending on leap year, with a 95% confidence interval of +/- 10.7 days. The date of crossover was linearly related to the date of last spring frost and occurred on average about 123 days after the last spring frost. Despite the predictability of crossover, the weekly pattern in the proportion of *Cx. pipiens* before and after crossover varied considerably, even between years with similar crossover dates. After West Nile virus became established in our area, we found that transmission based on Culex from gravid traps did not increase until *Cx. pipiens* abundance increased in oviposition traps. Infection rates peaked within the half-month period after crossover. The peak in *Cx. pipiens* abundance in oviposition traps during this 3-year period was between the 2nd half of August and the end of September. A higher magnitude of transmission in 2002 coincided with warmer temperatures during July and August and an extended period in which the 2 Culex species were in relatively equal abundance.
Descriptors: *Culex sp*., West Nile fever transmission, West Nile virus, Illinois, epidemiology, insect vectors virology, oviposition, population dynamics, seasons, time factors.

Medlock, J., K. Snow, and S. Leach (2005). **Potential transmission of West Nile virus in the British Isles: an ecological review of candidate mosquito bridge vectors.** *Medical and Veterinary Entomology.* 19(1): 2-21. ISSN: 0269-283X.
Descriptors: West Nile virus, arboviruses, insect vectors, Culicidae, mosquitoes, virus transmission, vector potential, insect ecology, zoonoses, literature reviews, British Isles, Ireland, United Kingdom, vector-ecology.

Miramontes, R.J., W.E. Lafferty, B.K. Lind, and M.W. Oberle (2006). **Is agricultural activity linked to the incidence of human West Nile virus?** *American Journal of Preventive Medicine* 30(2): 160-163.
Abstract: BACKGROUND: West Nile virus (WNV) has spread throughout the contiguous United States. During the 2002-2003 period, there were 14,023 laboratory-confirmed human cases of WNV in 45 states and 541 associated deaths. Factors that affect case distribution are poorly understood. This study assessed the

relationship of environmental factors and agricultural activity with the presence of human WNV cases. METHODS: County-level data were collected that included 2002 and 2003 WNV surveillance data, temperature, dairy revenue, precipitation, total irrigated acres, and total crop revenue. Logistic regression models were used to determine which risk factors were significantly associated with WNV human cases. RESULTS: Significant independent predictors for counties with human WNV cases were population (odds ratio [OR]=1.20, p<0.0001); higher average daily temperature in April through October (OR=1.19 for each additional degree Fahrenheit, p<0.0001); and total crop sales (OR=1.14 (p<0.001). The ORs for these predictors increased in an analysis of counties with ten or more cases. CONCLUSIONS: Higher temperature and farming activity may be strongly associated with the incidence of human WNV infection. Larger studies of more agricultural centers are warranted to determine which environmental factors increase the risk of human infection and how these infections can be prevented.
Descriptors: agriculture, Culex mosquitoes, environmental microbiology, insect vectors, West Nile fever epidemiology, logistic models, risk factors, United States.

Molaei, G. and T.G. Andreadis (2006). **Identification of avian- and mammalian-derived bloodmeals in *Aedes vexans* and *Culiseta melanura* (Diptera: Culicidae) and its implication for West Nile virus transmission in Connecticut, U.S.A.** *Journal of Medical Entomology* 43(5): 1088-1093. ISSN: 0022-2585.

Abstract: To evaluate the host-feeding patterns of *Aedes vexans* (Meigen) and *Culiseta melanura* (Coquillett) as secondary vectors of West Nile virus (family Flaviviridae, genus Flavivirus, WNV) in Northeastern United States, we identified the source of vertebrate bloodmeals by sequencing portions of the cytochrome b gene of mitochondrial DNA. Analysis of polymerase chain reaction products from a total of 119 *Ae. vexans* revealed that 92.4% of individuals acquired blood solely from mammalian and 2.5% from avian hosts. Mixed bloodmeals from both avian and mammalian hosts were detected in 5% of individuals of this species. *Ae. vexans* obtained vertebrate bloodmeals most frequently from white-tailed deer (80%) followed by domestic horse, American robin, eastern cottontail, and domestic cat. In contrast, *Cs. melanura* fed predominantly on avian species (89.6%) but exhibited some inclination for mammalian blood (4.2%). Individual mosquitoes containing mixed bloodmeals were also identified in 6% of *Cs. melanura*. American robin was the most common source of vertebrate blood for *Cs. melanura* (23%), followed by wood thrush and gray catbird. American crow represented only 2% of the bloodmeals identified in *Cs. melanura*, as was similarly found with other recognized Culex vectors of WNV in the northeast. These findings support the view that *Ae. vexans* is likely to be a relatively important "bridge vector" to large mammals, including deer and horse, whereas *Cs. melanura* likely plays a secondary role in enzootic transmission of WNV among free-ranging

birds in more rural environs.
Descriptors: *Aedes vexans*, *Culiseta melanura*, vectors, bloodmeals, mitochondrial DNA, polymerase chain reaction, horse, American robin, Eastern cottontail rabbit.

Molaei, G., T.G. Andreadis, P.M. Armstrong, J.F. Anderson, and C.R. Vossbrinck (2006). **Host feeding patterns of Culex mosquitoes and West Nile virus transmission, northeastern United States.** *Emerging Infectious Diseases* 12(3): 468-474. ISSN: 1080-6040.
Abstract: To evaluate the role of Culex mosquitoes as enzootic and epidemic vectors for WNV, we identified the source of vertebrate blood by polymerase chain reaction amplification and sequencing portions of the cytochrome b gene of mitochondrial DNA. All Cx. restuans and 93% of *Cx. pipiens* acquired blood from avian hosts; *Cx. salinarius* fed frequently on both mammals (53%) and birds (36%). Mixed-blood meals were detected in 11% and 4% of *Cx. salinarius* and *Cx. pipiens*, respectively. American robin was the most common source of vertebrate blood for *Cx. pipiens* (38%) and *Cx. restuans* (37%). American crow represented 1% of the blood meals in *Cx. pipiens*. and none in *Cx. restuans*. Human-derived blood meals were identified from 2 *Cx. salinarius* and 1 *Cx. pipiens*. Results suggest that *Cx. salinarius* is an important bridge vector to humans, while *Cx. pipiens* and *Cx. restuans* are more efficient enzootic vectors in the northeastern United States.
Descriptors: aves, mammalia, dipteran parasites, culex, host feeding patterns and role in viral disease transmission, viral diseases, West Nile virus, dipteran vector role inferred from host feeding patterns.

Mumcuoglu, K.Y., C. Banet Noach, M. Malkinson, U. Shalom, and R. Galun (2005). **Argasid ticks as possible vectors of West Nile virus in Israel.** *Vector Borne and Zoonotic Diseases* 5(1): 65-71. ISSN: 1530-3667.
Abstract: Mites and soft ticks collected directly from wild and domestic birds and their nests were tested for the presence of West Nile virus (WNV). The cattle egret argas, *Argas arboreus*, was collected from the nests of seven cattle egret colonies. Out of 1,000 *A. arboreus* pools examined, 16 were positive for WNV based on RT-PCR technique. The positive pools were from four nesting colonies of birds. Out of 37 cattle egret squabs examined, 37.8% had serum-neutralizing antibodies to WNV. WNV RNA was also detected in one out of 15 pools of *R. turanicus*, in one out of 21 pools of *O. sylviarum*, and in one out of 18 pools of *D. gallinae*, while 63 pools of *A. reflexus*, 11 of *R. sanguineus*, and 30 of Hyalomma spec. were negative. The role of mites and ticks in maintaining the endemic state of WNV in Israel is discussed.
Descriptors: arachnid vectors, argasidae, bird diseases, West Nile fever transmission, West Nile virus isolation and purification, birds as disease vectors, Israel, reverse transcriptase polymerase chain reaction, tick infestations, ticks, West Nile fever, zoonoses.

Ozer, N. (2006). **Bati Nil virusu ve vektorleri. [West Nile virus and its vectors]**. *Mikrobiyoloji Bulteni* 40(1-2): 121-128.

Abstract: There are more than five hundred known arthropod-borne viruses (arboviruses) all around the world and approximately hundred of them may cause disease in humans. During the past 20 years there has been a dramatic resurgence or emergence of epidemic arboviral diseases affecting both humans and domestic animals. Many factors play important roles in the emergence of arboviral diseases like Yellow Fever, Dengue, West Nile encephalitis, and of other diseases such as malaria and leishmaniasis in countries where they have not been previously encountered and in the increase in incidences where they have been under control. Some of these are demographic factors such as global population increase and uncontrolled urbanization; social changes such as modern transportation, human encroachment on natural disease hotspots; changes in agricultural activities such as the use of new irrigation techniques; deforestation; genetic changes in the pathogens; preventive measures and probably global climate changes. Mosquitoes are among the most important vectors carrying viruses belonging to Alphavirus, Flavivirus, Bunyavirus and Phlebovirus genera. All of the above factors have contributed to the increase in mosquito populations and closer contact between humans and mosquito vectors. West Nile virus notable after the epidemic of 1996 in Romania in Europe is one of the latest examples indicating that viruses can jump continents and produce epidemics. In this review article, the distribution of West Nile virus and its principal vectors and also its importance by means of public health, have been discussed.

Descriptors: West Nile virus, arthropod-borne arboviruses, mosquito populations, literature review.

Language of Text: Turkish.

Paz, S. (2006). **The West Nile Virus outbreak in Israel (2000) from a new perspective: The regional impact of climate change.** *International Journal of Environmental Health Research* 16(1): 1-13.

Abstract: The West Nile Virus (WNV) outbreak in Israel in 2000 appeared after medical and climatic warning signs. Re-analysis of the epidemic from a new viewpoint, the regional impact of global warming, especially the worsening in the summers' heat conditions, is presented. The disease appeared averagely at a lag of 3-9 weeks (strongest correlation = lag of 7 weeks). The minimum temperature was found as the most important climatic factor that encourages the disease earlier appearance. Extreme heat is more significant than high air humidity for increasing WNV cases. An early extreme rise in the summer temperature could be a good indicator of increased vector populations. While 93.5% of cases were in the metropolitan areas, the disease was not reported in the sub-arid regions. The outbreak development was comparable to the cases from Romania (1996) and NYC (1999). Each of those epi-

demics appeared after a long heatwave.
Descriptors: disease outbreaks, disease vectors, West Nile fever epidemiology, West Nile virus, climate, humidity, Israel epidemiology, seasons, temperature, time factors, urban population.

Phillips, R.A. and K. Christensen (2006). **Field-caught *Culex erythrothorax* larvae found naturally infected with West Nile virus in Grand County, Utah.** *Journal of the American Mosquito Control Association* 22(3): 561-562.
Abstract: *Culex erythrothorax* larvae were collected from a bulrush-cattail marsh near Moab, Grand County, Utah, October 28, 2004, and found positive for West Nile virus (WNV) RNA by real-time reverse-transcriptase polymerase chain reaction (RT-PCR). This demonstrates WNV vertical transmission in this species in the wild and suggests that vertical transmission in overwintering Cx. erythrothorax larvae may contribute to WNV overwintering.
Descriptors: *Culex sp*., West Nile virus, larva virology, reverse transcriptase polymerase chain reaction, Utah.

Reisen, W.K., C.M. Barker, R. Carney, H.D. Lothrop, S.S. Wheeler, J.L. Wilson, M.B. Madon, R. Takahashi, B. Carroll, S. Garcia, Y. Fang, M. Shafii, N. Kahl, S. Ashtari , V. Kramer, C. Glaser, and C. Jean (2006). **Role of corvids in epidemiology of west Nile virus in southern California.** *Journal of Medical Entomology* 43(2): 356-367. ISSN: 0022-2585.
Abstract: The invasion of different southern California landscapes by West Nile virus (WNV) and its subsequent amplification to epidemic levels during 2004 enabled us to study the impact of differing corvid populations in three biomes: the hot Colorado desert with few corvids (Coachella Valley), the southern San Joaquin Valley (Kern County) with large western scrub-jay but small American crow populations, and the cool maritime coast (Los Angeles) with a large clustered American crow population. Similar surveillance programs in all three areas monitored infection rates in mosquitoes, seroconversion rates in sentinel chickens, seroprevalence in wild birds, numbers of dead birds reported by the public, and the occurrence of human cases. Infection rates in *Culex tarsalis Coquillett* and sentinel chicken seroconversion rates were statistically similar among all three areas, indicating that highly competent mosquito hosts were capable of maintaining enzootic WNV transmission among less competent and widely distributed avian hosts, most likely house sparrows and house finches. In contrast, infection rates in *Culex pipiens quinquefasciatus* Say were statistically higher in Kern and Los Angeles counties with elevated corvid populations than in Coachella Valley with few corvids. Spatial analyses of dead corvids showed significant clusters near known American crow roosts in Los Angeles that were congruent with clusters of human cases. In this area, the incidence of human and *Cx. p. quinquefasciatus* infection was significantly greater within corvid clusters than without, indicating

their importance in virus amplification and as a risk factor for human infection. In contrast the uniform dispersion by territorial western scrub-jays resulted in a high, but evenly distributed, incidence of human disease in Kern County.
Descriptors: crows, culex mosquitoes, insect vectors, West Nile fever epidemiology, West Nile virus, California, chickens, incidence, population density, population surveillance, sentinel surveillance, zoonoses transmission.

Reisen, W.K., S.S. Wheeler, S. Yamamoto, Y. Fang, and S. Garcia (2005). **Nesting Ardeid colonies are not a focus of elevated West Nile virus activity in southern California.** *Vector Borne and Zoonotic Diseases* 5(3): 258-266. ISSN: 1530-3667.
Abstract: A large nesting colony of Ardeid birds at the Finney-Ramer Wildlife Refuge in Imperial County, California, did not appear to be a focus of West Nile virus (WNV) amplification during the summer of 2004. Blood samples taken during June and July from 155 nestlings of four species of Ardeid birds (cattle egrets, black-crowned night herons, great egrets, and snowy egrets) and five nestling double-crested cormorants yielded a single WNV isolation from a 3-week-old cattle egret. Antibody was detected by enzyme immunoassay from 20 nestlings (13%), 14 (70%) of which were confirmed as positive by plaque reduction neutralization test (PRNT). However, titration end points against WNV and St. Louis encephalitis virus (SLEV) were similar precluding viral identification. The grouping of positives within few nests, highest PRNT titers in youngest birds (<1 weeks of age), the decline of titer with nestling age, and the lack of antibody specificity indicated that antibody may have been acquired maternally and did not represent new infections. Infection rates in Culex tarsalis mosquitoes collected near the Ardeid colony at Ramer Lake (3.1 per 1,000) were statistically similar to rates estimated at the nearby Wister Unit wetlands (5.3 per 1,000) that lacked an Ardeid nesting colony. Black-crowned night heron nestlings experimentally infected with the NY99 strain of WNV produced viremias >5 log10 plaque forming units (PFU)/mL and were considered moderately competent hosts, whereas cattle egret nestlings had viremias that remained <5 log10 PFU/mL and were incompetent hosts.
Descriptors: viral blood antibodies, bird diseases, culex mosquitoes, insect vectors, West Nile fever, West Nile virus, wild animals, birds, California, disease reservoirs, St. Louis encephalitis virus, neutralization tests, species specificity, zoonoses.

Reisen, W.K., Y. Fang, H.D. Lothrop, V.M. Martinez, J. Wilson, P. O'Connor, R. Carney, B. Cahoon Young, M. Shafii, and A.C. Brault (2006). **Overwintering of West Nile virus in southern California.** *Journal of Medical Entomology* 43(2): 344-355. ISSN: 0022-2585.
Abstract: West Nile virus (family Flaviviridae, genus Flavivirus, WNV) invaded southern California dining 2003, successfully overwintered, amplified to epidemic levels, and then dispersed to every county in the state. Although surveillance pro-

grams successfully tracked and measured these events, mechanisms that allowed the efficient overwintering and subsequent amplification of WNV have not been elucidated. Our current research provided evidence for three mechanisms whereby WNV may have persisted in southern California during the winters of 2003-2004 and 2004-2005: 1) continued enzootic transmission, 2) vertical transmission by Culex mosquitoes, and 3) chronic infection in birds. WNV was detected in 140 dead birds comprising 32 species, including 60 dead American crows, thereby verifying transmission during the November-March winter period. Dead American crows provide evidence of recent transmission because this species always succumbs rapidly after infection. However, WNV RNA was not detected concurrently in 43,043 reproductively active female mosquitoes comprising 11 species and tested in 1,258 pools or antibody in sera from 190 sentinel chickens maintained in 19 flocks. Although efficient vertical transmission by WNV was demonstrated experimentally for Culex tarsalis Coquillett infected per os, 369 females collected diapausing in Kern County and tested in 32 pools were negative for WNV. Vertical transmission was detected in Culex pipiens quinquefinciatus Say adults reared from field-collected immatures collected from Kern County and Los Angeles during the summer transmission period. Chronic infection was detected by finding WNV RNA in 34 of 82 birds that were inoculated with WNV experimentally, held for >6 wk after infection, and then necropsied. Frequent detection of WNV RNA in kidney tissue in experimentally infected birds >6 wk postinfection may explain, in part, the repeated detection of WNV RNA in dead birds recovered during winter, especially in species such as mourning doves that typically do not die after experimental infection. In summary, our study provides limited evidence to support multiple modes of WNV persistence in southern California. Continued transmission and vertical transmission by *Culex p. quinquefasciatus* Say seem likely candidates for further study.
Descriptors: aves, viral diseases, West Nile virus, overwintering, transmission of viruses, California, south, viral disease overwintering.

Reisen, W.K., Y. Fang, and V.M. Martinez (2006). **Effects of temperature on the transmission of West Nile virus by *Culex tarsalis* (Diptera: Culicidae).** *Journal of Medical Entomology* 43(2): 309-317. ISSN: 0022-2585.
Abstract: *Culex tarsalis Coquillett* females were infected with the NY99 strain of West Nile virus (family Flaviviridae, genus Flavivirus, WNV) and then incubated under constant temperatures of 10-30[degree]C. At selected time intervals, transmission was attempted using an in vitro capillary tube assay. The median time from imbibing an infectious bloodmeal until infected females transmitted WNV (median extrinsic incubation period, EIP50) was estimated by probit analysis. By regressing the EIP rate (inverse of EIP50) as a function of temperature from 14 to 30[degree]C, the EIP was estimated to require 109 degree-days (DD) and the point of zero virus

development (x-intercept) was estimated to be 14.3[degree]C. The resulting degree-day model showed that the NY99 WNV strain responded to temperature differently than a lineage II strain of WNV from South Africa and approximated our previous estimates for St. Louis encephalitis virus (family Flaviviridae, genus Flavivirus, SLEV). The invading NY99 WNV strain therefore required warm temperatures for efficient transmission. The time for completion of the EIP was estimated monthly from temperatures recorded at Coachella Valley, Los Angeles, and Kern County, California, during the 2004 epidemic year and related to the duration of the Cx. tarsalis gonotrophic cycle and measures of WNV activity. Enzootic WNV activity commenced after temperatures increased, the duration of the EIP decreased, and virus potentially was transmitted in two or less gonotrophic cycles. Temperatures in the United States during the epidemic summers of 2002-2004 indicated that WNV dispersal and resulting epicenters were linked closely to above-average summer temperatures.

Descriptors: *Culex tarsalis*, viral diseases, West Nile virus, temperature effect on transmission, transmission of viruses, temperature.

Root, J.J., P.T. Oesterle, N.M. Nemeth, K. Klenk, D.H. Gould, R.G. McLean, L. Clark, and J.S. Hall (2006). **Experimental infection of fox squirrels (*Sciurus niger*) with West Nile virus.** *American Journal of Tropical Medicine and Hygiene* 75(4): 697-701. ISSN: 0002-9637.

Abstract: Tree squirrels (Sciurus spp.) have exhibited high seroprevalence rates, suggesting that they are commonly exposed to West Nile virus (WNV). Many characteristics of WNV infections in tree squirrels, such as the durations and levels of viremia, remain unknown. To better understand WNV infections in fox squirrels (*S. niger*), we subcutaneously inoculated fourteen fox squirrels with WNV. Peak viremias ranged from 10(4.00) plaque-forming units (PFU)/mL of serum on day 2 post-infection (DPI) to 10(4.98) PFU/mL on 3 DPI, although viremias varied between individuals. Oral secretions of some fox squirrels were positive for WNV viral RNA, occasionally to moderate levels (10(3.2) PFU equivalent/swab). WNV PFU equivalents in organs were low or undetectable on 12 DPI; gross and histologic lesions were rare. The viremia profiles of fox squirrels indicate that they could serve as amplifying hosts in nature. In addition, viral RNA in the oral cavity and feces indicate that this species could contribute to alternative WNV transmission in suburban communities.

Descriptors: rodent diseases, squirrels, West Nile fever, West Nile virus, viral biosynthesis, brain pathology, kidney pathology, liver pathology, myocardium pathology, viral RNA analysis, random allocation, reverse transcriptase polymerase chain reaction, virus shedding, mortality, pathology.

Savage, H.M., M. Anderson, E. Gordon, L. McMillen, L. Colton, D. Charnetzky, M. Delorey, S. Aspen, K. Burkhalter, B.J. Biggerstaff, and M. Godsey (2006). **Oviposition activity patterns and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (Diptera: Culicidae).** *Journal of Medical Entomology* 43(6): 1227-1238. ISSN: 0022-2585.

Abstract: Oviposition activity and West Nile virus (family Flaviviridae, genus Flavivirus, WNV) infection rates were assessed for members of the *Culex pipiens* complex from July through December 2002 by using gravid traps placed at four ecologically different sites in the southern portion of the hybrid zone in Shelby County, TN. Molecular assays identified three members of the *Cx. pipiens* complex: *Cx. pipiens pipiens* L., *Cx. p. quinquefasciatus* Say, and *Cx. p. pipiens-Cx. p. quinquefasciatus* hybrids (hybrids). The *Cx. pipiens* complex accounted for 90% of mosquitoes collected in gravid traps. All 285 WNV-positive mosquitoes were Culex mosquitoes, and 277 (97%) were *Cx. pipiens* complex mosquitoes. Infection rates among members of the *Cx. pipiens* complex were not significantly different. Infection rates were significantly higher at two urban sites than at a rural site, and WNV was not detected at a forested site. At urban sites, abundances of members of the *Cx. pipiens* complex corresponded to a simple latitude model of the hybrid zone. *Cx. p. quinquefasciatus* was most abundant (46.4%), followed by hybrids (34.1%) and *Cx. p. pipiens* (19.5%). The relative abundances at a rural site were reversed with *Cx. p. pipiens* (48.4%) being most abundant. This demonstrates that spatial habitat variation may profoundly influence the distribution of members of the *Cx. pipiens* complex within the hybrid zone. Members of the *Cx. pipiens* complex did not display different oviposition patterns. However, oviposition patterns assessed hourly at urban and rural sites were significantly different. At urban sites, oviposition activity of *Cx. pipiens* complex mosquitoes was bimodal with an evening peak associated with sunset and a morning peak associated with sunrise. At the rural site, the evening peak was pronounced and the morning peak weak and similar to nighttime activity.

Descriptors: West Nile virus, Culex mosquitoes, traps, habitat variation, infection rates, oviposition activity, evening peak, morning peak.

Shaman, J., J. Day, and M. Stieglitz (2005). **Drought-induced amplification and epidemic transmission of West Nile virus in southern Florida.** *Journal of Medical Entomology.* 42(2): 134-141. ISSN: 0022-2585.

Abstract: We show that the spatial-temporal variability of human West Nile (WN) cases and the transmission of West Nile virus (WNV) to sentinel chickens are associated with the spatial-temporal variability of drought and wetting in southern Florida. Land surface wetness conditions at 52 sites in 31 counties in southern Florida for 2001-2003 were simulated and compared with the occurrence of human WN cases

and the transmission of WNV to sentinel chickens within these counties. Both WNV transmission to sentinel chickens and the occurrence of human WN cases were associated with drought 2-6 mo prior and land surface wetting 0.5-1.5 mo prior. These dynamics are similar to the amplification and transmission patterns found in southern Florida for the closely related St. Louis encephalitis virus. Drought brings avian hosts and vector mosquitoes into close contact and facilitates the epizootic cycling and amplification of the arboviruses within these populations. Southern Florida has not recorded a severe, widespread drought since the introduction of WNV into the state in 2001. Our results indicate that widespread drought in the spring followed by wetting during summer greatly increase the probability of a WNV epidemic in southern Florida.

Descriptors: West Nile virus, virus transmission, drought, disease outbreaks, sentinel animals, chickens, humans, spatial variation, temporal variation, insect vectors, *Culex nigripalpus*, Culicidae, simulation models, Florida, virus-amplification, land-surface-wetting, hydrology-models.

Steinman, A., C. Banet Noach, L. Simanov, N. Grinfeld, Z. Aizenberg, O. Levi, D. Lahav, M. Malkinson, S. Perk, and N.Y. Shpigel (2006). **Experimental Infection of Common Garter Snakes (*Thamnophis sirtalis*) with West Nile Virus.** *Vector Borne and Zoonotic Diseases* 6(4): 361-368. ISSN: 1530-3667.

Abstract: The role of various reptilian species in the infectious cycle of several arboviruses is documented, but their role in that of West Nile virus (WNV) is uncertain. Common garter snakes (*Thamnophis sirtalis*) were infected subcutaneously with 10(5) plaque forming units (PFU) WNV-Isr 98, five of nine snakes became viremic, and five exhibited persistent low levels of neutralizing antibodies. Four of the parentally infected snakes died and high titers of virus were found in multiple organ samples. In contrast, orally infected garter snakes did not become viremic, but viral RNA was detected in cloacal swabs. Since oral infection of predator birds by WNV is known, their ingestion of infected snakes may also result in their becoming infected.

Descriptors: reptilian species, West Nile virus, garter snakes, subcutaneous infection, oral infection, viremic, RNA, predator birds.

Stephen, C., N. Plamondon, and P. Belton (2006). **Notes on the distribution of mosquito species that could potentially transmit West Nile virus on Vancouver Island, British Columbia.** *Journal of the American Mosquito Control Association* 22(3): 553-556.

Abstract: We record the spatial and temporal distribution of 8 potential vectors of West Nile virus (WNV) on Vancouver Island in 2003 and 2004. Five species were widely distributed, but the other 3 were restricted to specific larval habitats. Adults were trapped from early April to September. The findings indicate a potential for WNV transmission if the virus arrives on the island. The results extend the published

range of 5 mosquito species.
Descriptors: Culicidae virology, West Nile virus, Aedes, Anopheles, British Columbia, insect vectors, larva, population surveillance, West Nile fever transmission.

Sullivan, H., G. Linz, L. Clark, and M. Salman (2006). **West Nile virus antibody prevalence in red-winged blackbirds (*Agelaius phoeniceus*) from North Dakota, USA (2003-2004).** *Vector Borne and Zoonotic Diseases* 6(3): 305-309. ISSN: 1530-3667.
Abstract: This study was designed to explore the role that red-winged blackbirds (*Agelaius phoeniceus*) may have played in disseminating West Nile virus (WNV) across the United States. Using enzyme-linked immunosorbent assays designed to detect WNV antibodies in avian species we were able to determine the WNV antibody prevalence in a cohort of red-winged blackbirds in central North Dakota in 2003 and 2004. The peak WNV antibody prevalence was 22.0% in August of 2003 and 18.3% in July of 2004. The results of this study suggest that red-winged blackbird migratory populations may be an important viral dispersal mechanism with the ability to spread arboviruses such as WNV across the United States.
Descriptors: West Nile virus, red winged black birds, antibody prevalence, migratory populations, disseminating, disperal, spread.

Tachiiri, K., B. Klinkenberg, S. Mak, and J. Kazmi (2006). **Predicting outbreaks: A spatial risk assessment of West Nile virus in British Columbia.** *International Journal of Health Geographics* 5: 21.
Abstract: BACKGROUND: West Nile virus (WNv) has recently emerged as a health threat to the North American population. After the initial disease outbreak in New York City in 1999, WNv has spread widely and quickly across North America to every contiguous American state and Canadian province, with the exceptions of British Columbia (BC), Prince Edward Island and Newfoundland. In this study we develop models of mosquito population dynamics for *Culex tarsalis* and *C. pipiens*, and create a spatial risk assessment of WNv prior to its arrival in BC by creating a raster-based mosquito abundance model using basic geographic and temperature data. Among the parameters included in the model are spatial factors determined from the locations of BC Centre for Disease Control mosquito traps (e.g., distance of the trap from the closest wetland or lake), while other parameters were obtained from the literature. Factors not considered in the current assessment but which could influence the results are also discussed. RESULTS: Since the model performs much better for *C. tarsalis* than for *C. pipiens*, the risk assessment is carried out using the output of *C. tarsalis* model. The result of the spatially-explicit mosquito abundance model indicates that the Okanagan Valley, the Thompson Region, Greater Vancouver, the Fraser Valley and southeastern Vancouver Island have the highest potential abundance of the mosquitoes. After including human population data, Greater Vancouver, due to its high population density, increases in significance relative to the other areas.

CONCLUSION: Creating a raster-based mosquito abundance map enabled us to quantitatively evaluate WNv risk throughout BC and to identify the areas of greatest potential risk, prior to WNv introduction. In producing the map important gaps in our knowledge related to mosquito ecology in BC were identified, as well, it became evident that increased efforts in bird and mosquito surveillance are required if more accurate models and maps are to be produced. Access to real time climatic data is the key for developing a real time early warning system for forecasting vector borne disease outbreaks, while including social factors is important when producing a detailed assessment in urban areas.
Descriptors: *Culex sp*., forecasting methods, West Nile virus, British Columbia, geographic information systems (GIS), theoretical models, population density, population dynamics, risk, temperature.

Ternovoi, V.A., E.V. Protopopova, S.G. Surmach, M.V. Gazetdinov, S.I. Zolotykh, A.M. Shestopalov, E.V. Pavlenko, G.N. Leonova, and V.B. Loktev (2006). **[The genotyping of the West Nile virus in birds in the far eastern region of Russia in 2002-2004]**. *Molekuliarnaia Genetika, Mikrobiologiia i Virusologiia*(4): 30-35.
Abstract: Samples from 20 species of trapped and dead birds were collected in the Far Eastern Region in 2002-2004 and were analyzed by the anti-WNV MAb-modified immunoenzyme assay for antigen detection and RT-PCR for viral RNA detection. Five positive samples from cinereous vultures (*Aegypius monachus*) and two positive samples from cattle egret (*Bubulcus ibis*) were found in both tests. The sequencing of the 322 bp fragments of protein E gene showed 99-99.67% homology with the strain WNV/LEIV-VlgOO-27924 of the WNV isolated in Volgograd, Russia, 2000. Additionally, five positive samples from birds (*Pica pica, Corvus macrorhynchos, Larus crossirostris, Parus minor, Emberiza spodocephala*) collected in autumn 2004 were found during screening with anti-WNV MAb-modified ELISA. These results confirm that the WNV is circulating in the Far Eastern Region of Russia and outbreaks of WN fever in humans may be possible. This demonstrates that the genotype 1a of the West Nile virus could spread in the southern regions of the Far East by migrating birds and introduction of the WNV into other southern regions of the Asian part of Russia are probably.
Descriptors: birds, West Nile fever, viral genotype, immunoenzyme techniques, reverse transcriptase polymerase chain reaction, Russia, West Nile virus classification.
Language of Text: Russian.

Tiawsirisup, S., K.B. Platt, R.B. Evans, and W.A. Rowley (2005). **A comparision of West Nile Virus transmission by *Ochlerotatus trivittatus* (COQ.), *Culex pipiens* (L.), and *Aedes albopictus* (Skuse).** *Vector Borne and Zoonotic Diseases* 5(1): 40-47. ISSN: 1530-3667.
Abstract: Transmission of West Nile virus (WNV) by *Ochlerotatus trivittatus, Culex*

pipiens, and *Aedes albopictus* were compared 14 days after taking blood meals from viremic chickens with titers ranging from 10(2.5) to 10(9.5) cell infective dose (50)s (CID50s)/mL serum. Transmission occurred in one of four (25%) *Oc. trivittatus* and one of 25 (4%) *Cx. pipiens* that fed on chickens with titers of 10(5.5) CID50s/mL. No transmission occurred among two of 16 (13%) *Oc. trivittatus* or one of 25 (4%) *Cx. pipiens* that became infected after blood meals with titers of 10(5.0) and 10(4.5) CID50s/mL, the next lowest blood meal titers evaluated. Seventeen of 28 (61%) *Ae. albopictus* transmitted WNV after blood meals with titers of 10(7.0) CID50s/mL, but no infection or transmission was observed among 21 *Ae. albopictus* that fed on chickens with titers of 10(5.0) CID50s/mL, the next lowest titer evaluated. Transmission by all three species increased dramatically after blood meals with WNV titers of > or = 10(5.5) CID50s/mL. No significant differences occurred in dissemination and transmission rates of the three species after taking blood meals with titers of > 10(7.0) CID50s/mL. The cumulative mean +/- SE transmission rates of *Oc. trivittatus, Cx. pipiens* , and *Ae. albopictus* after blood meals with titers of > or = 10(7.0) CID50s/mL were 45.5 +/- 4.1%, 46.8 +/- 4.5%, and 72.4 +/- 5.5%. The cumulative mean dissemination rates of the three species were 78.3 +/- 6.7%, 74.8 +/- 2.6%, and 88.6 +/- 2.1%. The rates of transmission by the three species that developed disseminated infections after blood meals with titers of > or = 10(7.0) CID50s/mL were 58.8 +/- 4.4%, 62.6 +/- 5.8%, and 81.6 +/- 5.4%, respectively. In a previous study, we found that susceptibility of the three species to WNV was essentially the same when fed on chickens with WNV titers of > 10(7.0) CID50s/mL, but *Oc. trivittatus* and *Cx. pipiens* were more susceptible than *Ae. albopictus* to WNV at lower virus titers. The current study strongly suggests that *Ae. albopictus* is a more efficient vector than *Oc. trivittatus* and *Cx. pipiens* when fed blood meals with titers of > 10(7.0) CID50s/mL. However, *Oc. trivittatus* and *Cx. pipiens* might be more efficient as vectors when infected by blood meals with titers of < 10(7.0) CID50s/mL.

Descriptors: insect vectors, West Nile fever transmission, West Nile virus isolation and purification, chickens, culex mosquitoes, disease susceptibility, disease vectors, ochlerotatus, species specificity, viral load.

Tiawsirisup, S., K.B. Platt, B.J. Tucker, and W.A. Rowley (2005). **Eastern cottontail rabbits (*Sylvilagus floridanus*) develop West Nile virus viremias sufficient for infecting select mosquito species.** *Vector Borne and Zoonotic Diseases* 5(4): 342-350. ISSN: 1530-3667.

Abstract: The potential of the eastern cottontail rabbit (CTR; *Sylvilagus floridanus*) to contribute to an enzootic West Nile virus (WNV) cycle was demonstrated by characterizing the WNV viremia profile of 15 CTRs and demonstrating that mosquitoes could become infected by feeding on these CTRs. Eight CTRs were infected with a titer of 10(5.0) cell-infectious dose 50% endpoints (CID50s) of WNV (NY99-Crow)

by needle and seven CTRs by bite of one or more WNV-infected mosquitoes. There were no marked differences between the WNV viremia profiles of CTRs infected by either method. West Nile virus was detected in serums of all CTRs by 24 h p.i. The daily mean titers of all 15 CTRs on days 1-4 p.i. were 10(4.1+/-0.4), 10(4.7+/-0.3), 10(4.1+/-0.6), and 10(3.7+/-0.6) respectively, declining to 10(1.2+/-0.1) CID50s/ml of serum by day 6 p.i. No virus was detected in the blood of any CTR on day 7 p.i. The average duration of WNV titers of >or=10(4.3) and <10(5.0) CID50s/mL for all CTRs was 2.2 +/- 0.6 and 1.0 +/- 0.1 days, respectively. The minimum estimated infection rates (MEIRs) of *Culex pipiens* (L.) and *Culex salinarius* (Coq.) that fed on CTRs with titers of >or=10(4.3) and >10(5.0) were 11.5 +/- 5.5 and 21 +/- 6.0%, respectively. These rates increased to 20.5 +/- 6.4% and 25.0 +/- 3.0% when CTR serum titers were >10(5.0) CID50s/mL. Neither *Aedes aegypti* (L.) nor *Aedes albopictus* (Skuse) were infected by feeding on CTRs with titers of <10(5.0) CID50s/mL. The MEIRs of these two species were 11.5 +/- 3.5% and 1.5 +/- 0.5% after feeding on CTRs with titers of >10(5.0) CID50s/ml. None of the CTRs infected by mosquito bite or by needle showed any symptoms of WNV disease.
Descriptors: cottontail rabbit, West Nile virus, viremia, Culex mosquitos, infection rates.

Turell, M., D. Dohm, M. Sardelis, M. O' Guinn, T. Andreadis, and J. Blow (2005). **An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus.** *Journal of Medical Entomology.* 42(1): 57-62. ISSN: 0022-2585.
Abstract: Since first discovered in the New York City area in 1999, West Nile virus (WNV) has become established over much of the continental United States and has been responsible for >10,000 cases of severe disease and 400 human fatalities, as well as thousands of fatal infections in horses. To develop appropriate surveillance and control strategies, the identification of which mosquito species are competent vectors and how various factors influence their ability to transmit this virus must be determined. Therefore, we evaluated numerous mosquito species for their ability to transmit WNV under laboratory conditions. This report contains data for several mosquito species not reported previously, as well as a summary of transmission data compiled from previously reported studies. Mosquitoes were allowed to feed on chickens infected with WNV isolated from a crow that died during the 1999 outbreak in New York City. These mosquitoes were tested approximately equal to 2 wk later to determine infection, dissemination, and transmission rates. All Culex species tested were competent vectors in the laboratory and varied from highly efficient vectors (e.g., *Culex tarsalis Coquillett*) to moderately efficient ones (e.g., *Culex nigripalpus Theobald*). Nearly all of the Culex species tested could serve as efficient enzootic or amplifying vectors for WNV. Several container-breeding Aedes and

Ochlerotatus species were highly efficient vectors under laboratory conditions, but because of their feeding preferences, would probably not be involved in the maintenance of WNV in nature. However, they would be potential bridge vectors between the avian-Culex cycle and mammalian hosts. In contrast, most of the surface pool-breeding Aedes and Ochlerotatus species tested were relatively inefficient vectors under laboratory conditions and would probably not play a significant role in transmitting WNV in nature. In determining the potential for a mosquito species to become involved in transmitting WNV, it is necessary to consider not only its laboratory vector competence but also its abundance, host-feeding preference, involvement with other viruses with similar transmission cycles, and whether WNV has been isolated from this species under natural conditions.

Descriptors: Culicidae, Culex, mosquitoes, insect vectors, West Nile virus, vector potential, vector competence, disease transmission, infection, vectorial capacity, infection-rate, dissemination-rate, transmission-rate.

Van Dyken, M., B. Bolling, C. Moore, C. Blair, B. Beaty, W.I. Black, and B. Foy (2006). **Molecular evidence for trypanosomatids in Culex mosquitoes collected during a West Nile virus survey.** *International Journal for Parasitology.* 36(9): 1015-1023. ISSN: 0020-7519.

Abstract: Adult mosquitoes were previously collected and tested for West Nile virus during an intense WNV outbreak in 2003-2004 along the Cache la Poudre River in Colorado, USA. A subset of these mosquitoes was also tested for infection with trypanosomatids using nested PCR to amplify 18S rRNA. Of the 69 pools of *Culex pipiens* that were screened for both pathogens, 4.3% were positive for WNV and 11.6% tested positive for trypanosomes; no pools were found to be co-infected with both pathogens. One hundred and forty-three pools of *Culex tarsalis*, considered to be the principal WNV vector in this area, were tested in the same manner. 7.7% were positive for WNV and 20.3% of these pools tested positive for trypanosomes. Five pools of *C. tarsalis* were found to be co-infected with both pathogens, which was approximately 2.2 times more frequent than would be expected if these pathogens are independent of each other. Sequencing and maximum parsimony analysis of 18S rRNA revealed that four of the isolates arise in or near clades of described avian trypanosomes, likely indicating that these are vectored pathogens between birds and mosquitoes. Unexpectedly, the majority (24/28, 86%) of our positive samples form their own separate clade within the order Trypanosomatida with 100% bootstrap support. We have identified a potential new clade of trypanosomatids that exist within important mosquito vectors and discuss the potential ecological connections between these trypanosomes, arboviruses and mosquitoes.

Descriptors: *Culex pipiens*, *Culex tarsalis*, Trypanosoma, disease detection, pathogen identification, ribosomal DNA, nucleotide sequences, phylogeny, insect vectors,

mixed infection, trypanosomiasis, West Nile virus , disease surveillance, Colorado, Internet-resource.

Ward, M.P., A. Raim, S. Yaremych Hamer, R. Lampman, and R.J. Novak (2006). **Does the roosting behavior of birds affect transmission dynamics of West Nile virus?** *American Journal of Tropical Medicine and Hygiene* 75(2): 350-355. ISSN: subcutaneous.
Abstract: The potential role of many urban passerine birds in the transmission of West Nile virus (WNV) is well-documented by studies on host competency, seroprevalence in wild birds, and identification of vector blood meal source. In contrast, the impact of bird behavior on transmission dynamics is largely unexplored. Bird roosting (perching) behavior may be a critical component regulating WNV transmission because of the crepuscular/nocturnal feeding behavior of Culex mosquitoes, the primary vectors of WNV. We used radio telemetry to determine the roosting behavior of American crows (*Corvus brachyrhynchos*) and northern cardinals (*Cardinalus cardinalus*). On average, healthy crows moved slightly shorter distances between roosts than viremic crows, 1,038.3 meters versus 1,255.5 meters, while cardinals only moved 54.7 meters. Given the average movements of crows and cardinals between roosts, crows, which are viremic for five days, could spread the virus throughout a mean +/- SE area of 20.84 +/- 0.79 km(2), while viremic cardinals would, on average, only spread the virus over a mean +/- SE area of 0.03 +/- 0.01 km(2). Because the crow population in Illinois is decreasing at a rate of 11.5% per year and up to 35.6% per year in certain locations, crows are becoming scarce in some areas, thus reducing their role as wild bird sentinels. We suggest that if crows are important in dispersing WNV, large decreases in their abundance will shift transmission cycles to a more focal nature because of the differences in roosting behavior of crows compared with other urban birds, such as cardinals.
Descriptors: transmission of bird diseases, passeriformes physiology, West Nile fever, animal behavior and physiology, crows, Illinois, population dynamics, radio waves, time factors, West Nile virus.

Ward, M.R., D.E. Stallknecht, J. Willis, M.J. Conroy, and W.R. Davidson (2006). **Wild bird mortality and West Nile virus surveillance: biases associated with detection, reporting, and carcass persistence.** *Journal of Wildlife Diseases* 42(1): 92-106. ISSN: 0090-3558.
Abstract: Surveillance targeting dead wild birds, in particular American crows (*Corvus brachyrhynchos*), plays a critical role in West Nile virus (WNV) surveillance in the United States. Using crow decoy surrogates, detection and reporting of crow carcasses within urban and rural environments of DeKalb County, Georgia were assessed for potential biases that might occur in the county's WNV surveillance program.
In each of two replicated trials, during July and September 2003, 400 decoys were labeled with reporting instructions and distributed along randomly chosen routes

throughout designated urban and rural areas within DeKalb County. Information-theoretic methods were used to compare alternative models incorporating the effects of area and trial on probabilities of detection and reporting. The model with the best empirical support included the effects of area on both detection and reporting of decoys. The proportion of decoys detected in the urban area (0.605, SE = 0.024) was approximately twice that of the rural area (0.293, SE = 0.023), and the proportion of decoys reported in the urban area (0.273, SE = 0.023) was approximately three times that of the rural area (0.103, SE = 0.028). These results suggest that human density and associated factors can substantially influence dead crow detection and reporting and, thus, the perceived distribution of WNV. In a second and separate study, the persistence and fate of American crow and house sparrow (*Passer domesticus*) carcasses were assessed in urban and rural environments in Athens-Clarke, Madison, and Oconee counties, Georgia. Two replicated trials using 96 carcasses of each species were conducted during July and September 2004. For a portion of the carcasses, motion sensitive cameras were used to monitor scavenging species visits. Most carcasses (82%) disappeared or were decayed by the end of the 6-day study. Carcass persistence averaged 1.6 days in rural areas and 2.1 days in urban areas. We analyzed carcass persistence rates using a known-fate model framework in program MARK. Model selection based on Akaike's Information Criteria (AIC) indicated that the best model explaining carcass persistence rates included species and number of days of exposure; however, the model including area and number of days of exposure received approximately equal support. Model-averaged carcass persistence rates were higher for urban areas and for crow carcasses. Six mammalian and one avian species were documented scavenging upon carcasses. Dead wild birds could represent potential sources of oral WNV exposure to these scavenging species. Species composition of the scavenger assemblage was similar in urban and rural areas but "scavenging pressure" was greater in rural areas.

Descriptors: aves, ecological techniques, wild bird mortality monitoring and application in West Nile virus surveillance, biases, pathological techniques, viral diseases, West Nile virus, wild bird mortality monitoring application in disease surveillance, mortality, Georgia USA.

Ward, M. (2005). **Epidemic West Nile virus encephalomyelitis: A temperature-dependent, spatial model of disease dynamics.** *Preventive Veterinary Medicine.* 71(3-4): 253-264. ISSN: 0167-5877.

Descriptors: West Nile virus, viral encephalitis, disease outbreaks, ambient temperature, epidemiological studies, epidemiology, spatial distribution, horses, horse diseases, heat sums, risk factors, geographic information systems, biogeography, disease transmission, models, Indiana.

Notes: In the Special Issue: Proceedings of GISVET'04/edited by W. Martin and P. Durr. Proceedings of a conference held June 23-25, 2004, Ontario, Canada.

Ward, M., B. Ramsay, and K. Gallo (2005). **Rural cases of equine West Nile virus encephalomyelitis and the normalized difference vegetation index.** *Vector Borne and Zoonotic Diseases.* 5(2): 181-188. ISSN: 1530-3667.
Descriptors: horses, West Nile virus, viral encephalitis, disease outbreaks, vegetation cover, spatial distribution, spatial variation, temporal variation, remote sensing, satellites, image analysis, rural areas, Indiana, disease-foci.

White, B.J., D.R. Andrew, N.Z. Mans, O.A. Ohajuruka, and M.C. Garvin (2006). **West Nile virus in mosquitoes of northern Ohio, 2003.** *American Journal of Tropical Medicine and Hygiene* 75(2): 346-349. ISSN: subcutaneous.
Abstract: From June 19, 2003 to August 18, 2003, we surveyed the mosquitoes of Oberlin, OH, for West Nile Virus (WNV) infection using reverse transcriptase-polymerase chain reaction. A total of 12,055 mosquitoes, representing 17 species or species groups and 4 genera, were collected in gravid traps at seven sites throughout the city, with *Culex pipiens/restuans* being the most abundant and showing the highest minimum infection rate (MIR) of 0.78. This represents a decrease in WNV enzootic activity from the previous year. Both *Cx. pipiens/restuans* abundance and MIR increased significantly with date. However, we found no correlation between *Cx. pipiens/restuans* abundance and MIR.
Descriptors: culex mosquitos, insect vectors, West Nile virus isolation and purification, culicidae classification, Ohio, population density, population surveillance methods, reverse transcriptase polymerase chain reaction methods, time factors, West Nile fever transmission.

Wonham, M.J., M.A. Lewis, J. Renc+or awowicz, and P.v.d. Driessche (2006). **Transmission assumptions generate conflicting predictions in host-vector disease models: a case study in West Nile virus.** *Ecology Letters.* 9(6): 706-725. ISSN: 1461-023X.
Abstract: This review synthesizes the conflicting outbreak predictions generated by different biological assumptions in host-vector disease models. It is motivated by the North American outbreak of West Nile virus, an emerging infectious disease that has prompted at least five dynamical modelling studies. Mathematical models have long proven successful in investigating the dynamics and control of infectious disease systems. The underlying assumptions in these epidemiological models determine their mathematical structure, and therefore influence their predictions. A crucial assumption is the host-vector interaction encapsulated in the disease-transmission term, and a key prediction is the basic reproduction number, R[subscript 0]. We connect these two model elements by demonstrating how the choice of transmission term qualitatively and quantitatively alters R[subscript 0] and therefore alters

predicted disease dynamics and control implications. Whereas some transmission terms predict that reducing the host population will reduce disease outbreaks, others predict that this will exacerbate infection risk. These conflicting predictions are reconciled by understanding that different transmission terms apply biologically only at certain population densities, outside which they can generate erroneous predictions. For West Nile virus, R[subscript 0] estimates for six common North American bird species indicate that all would be effective outbreak hosts.
Descriptors: disease control, West Nile virus, Arboviral-encephalitis, basic-reproduction-number, emerging-infectious-disease, R[subscript-0], transmission-dynamics, Internet-resource.

Xia, Y. and L. Zwiebel (2006). **Identification and characterization of an odorant receptor from the West Nile Virus mosquito, Culex quinquefasciatus.** *Insect Biochemistry and Molecular Biology.* 36(3): 169-176. ISSN: 0965-1748.
Abstract: Members of the *Culex pipens* mosquito group including *C. quinquefasciatus* are responsible for the transmission of *Bancroftian filarisis* as well as West Nile Virus (WNV) in the United States. As is the case for other mosquitoes, the host preference of this disease vector relies on olfaction and accordingly mediated via G-protein coupled signal transduction pathways. Here, we identify and characterize CqOR7, the first candidate member of the odorant receptor gene family from *C. quinquefasciatus*. CqOR7 displays extremely high primary amino acid conservation with other apparent orthologs including AaOR7, from the Dengue virus vector mosquito *Aedes aegypti*, AgOR7 from the malaria vector *Anopheles gambiae* and DOr83b from the fruit fly *Drosophila melanogaster* that form an essential non-conventional odorant receptor sub-family. CqOR7 transcripts can be detected in adult chemosensory tissues and during several pre-adult stages of *C. quinquefasciatus*, and the CqOR7 protein is localized to characteristic olfactory tissues such as the antennae and maxillary palps as well as the proboscis, a typically gustatory appendage. These results suggest that CqOR7 and its orthologs are likely to play a role in the chemosensory processes of Culicine and other mosquitoes that underlie their vectorial capacity.
Descriptors: *Culex quinquefasciatus*, odor compounds, olfactory receptors, multigene family, nucleotide sequences, amino acid sequences, sequence homology, gene expression, tissue distribution, smell, insect vectors, molecular-sequence-data, Internet-resource.

Yang, W.f., M.h. Zhou, J. Sun, and et al (2005). **Investigation of potential mosquito-vector of West Nile virus disease in Jiangsu Province.** *Zhongguo Meijieshengwuxue Ji Kongzhi Zazhi* 16(4): 267-269. ISSN: 1003-8280.
Descriptors: culicidae, transmission of viruses, West Nile virus, potential vectors community structure and density variation, community structure, population

density, Jiangsu, Lianyungang, Suzhou and Zhenjiang cities.
Language of Text: Chinese; Summary in Chinese, English.

Yiannakoulias, N.W., D.P. Schopflocher, and L.W. Svenson (2006). **Modelling geographic variations in West Nile virus.** *Canadian Journal of Public Health* 97(5): 374-378.
Abstract: BACKGROUND: This paper applies a method for modelling the spatial variation of West Nile virus (WNv) in humans using bird, environmental and human testing data. METHODS: We used data collected from 503 Alberta municipalities. In order to manage the effects of residual spatial autocorrelation, we used generalized linear mixed models (GLMM) to model the incidence of infection. RESULTS: There were 275 confirmed cases of WNv in the 2003 calendar year in Alberta. Our spatial model indicates that living in the grasslands natural region and levels of human testing are significant positive predictors of WNv; living in an urban area is a significant negative predictor. CONCLUSION: Infected bird data contribute little to our model. The variability of West Nile virus incidence in Alberta may be partly confounded by the variations in the rate of testing in different parts of the province. However, variation in infection is also associated with known environmental risk factors. Our findings are consistent with existing knowledge of WNv in North America.
Descriptors: ecology, linear models, rural health, urban health, West Nile fever, Alberta, birds, seroepidemiologic studies.

Zou, L., S.N. Miller, and E.T. Schmidtmann (2006). **Mosquito larval habitat mapping using remote sensing and GIS: implications of coalbed methane development and West Nile virus.** *Journal of Medical Entomology* 43(5): 1034-1041. ISSN: 0022-2585.
Abstract: Potential larval habitats of the mosquito *Culex tarsalis* (Coquillett), implicated as a primary vector of West Nile virus in Wyoming, were identified using integrated remote sensing and geographic information system (GIS) analyses. The study area is in the Powder River Basin of north central Wyoming, an area that has been undergoing a significant increase in coalbed methane gas extractions since the late 1990s. Large volumes of water are discharged, impounded, and released during the extraction of methane gas, creating aquatic habitats that have the potential to support immature mosquito development. Landsat TM and ETM+ data were initially classified into spectrally distinct water and vegetation classes, which were in turn used to identify suitable larval habitat sites. This initial habitat classification was refined using knowledge-based GIS techniques requiring spatial data layers for topography, streams, and soils to reduce the potential for overestimation of habitat. Accuracy assessment was carried out using field data and high-resolution aerial photography commensurate with one of the Landsat images. The classifier can identify likely habitat for ponds larger than 0.8 ha (2 acres) with generally satisfactory results

(72.1%) with a lower detection limit of approximately 0.4 ha (1 acre). Results show a 75% increase in potential larval habitats from 1999 to 2004 in the study area, primarily because of the large increase in small coalbed methane water discharge ponds. These results may facilitate mosquito abatement programs in the Powder River Basin with the potential for application throughout the state and region.

Descriptors: potential larval habitats, coalbed methane gas, aquatic habitats, immature mosquitoe development, water discharge ponds.

Viral Genetics and Strain Differentiation

Bakonyi, T., E. Ivanics, K. Erdely, K. Ursu, E. Ferenczi, H. Weissenbock, and N. Nowotny (2006). **Lineage 1 and 2 strains of encephalitic West Nile virus, central Europe.** *Emerging Infectious Diseases* 12(4): 618-623. ISSN: 1080-6040.

Abstract: Two different West Nile virus (WNV) strains caused lethal encephalitis in a flock of geese and a goshawk in southeastern Hungary in 2003 and 2004, respectively. During the outbreak in geese, 14 confirmed human cases of WNV encephalitis and meningitis were reported in the same area. Sequencing of complete genomes of both WNV strains and phylogenetic analyses showed that the goose-derived strain exhibits closest genetic relationship to strains isolated in 1998 in Israel and to the strain that emerged in 1999 in the United States. WNV derived from the goshawk showed the highest identity to WNV strains of lineage 2 isolated in central Africa. The same strain reemerged in 2005 in the same location, which suggests that the virus may have overwintered in Europe. The emergence of an exotic WNV strain in Hungary emphasizes the role of migrating birds in introducing new viruses to Europe.

Descriptors: *Accipiter gentilis*, *Anser anser domesticus*, viral diseases, West Nile virus, records and epidemiological implications, Hungary.

Beasley, D.W., M.C. Whiteman, and S. Zhang (2005). **Envelope Protein Glycosylation Status Influences Mouse Neuroinvasion Phenotype of Genetic Lineage 1 West Nile Virus Strains**. *Journal of Virology*: 8339-8347.

Descriptors: West Nile virus, strains, genetic linkeage, protein glycosylation, mouse neuroinvasion phenotype.

Benzaghou, I., I. Bougie, F. Picard Jean, and M. Bisaillon (2006). **Energetics of RNA binding by the West Nile virus RNA triphosphatase.** *FEBS Letters* 580(3): 867-877.

Abstract: The West Nile virus (WNV) RNA genome harbors the characteristic methylated cap structure present at the 5' end of eukaryotic mRNAs. In the present study, we report a detailed study of the binding energetics and thermodynamic parameters involved in the interaction between RNA and the WNV RNA triphosphatase, an enzyme involved in the synthesis of the RNA cap structure. Fluorescence spectroscopy assays revealed that the initial interaction between RNA and the enzyme is characterized by a high enthalpy of association and that the minimal RNA binding site of NS3 is 13 nucleotides. In order to provide insight into the relationship between the enzyme structure and RNA binding, we also correlated the effect

of RNA binding on protein structure using both circular dichroism and denaturation studies as structural indicators. Our data indicate that the protein undergoes structural modifications upon RNA binding, although the interaction does not significantly modify the stability of the protein.
Descriptors: acid anhydride hydrolases, viral RNA, West Nile virus genetics.

Davis, C.T., G.D. Ebel, R.S. Lanciotti, A.C. Brault, H. Guzman, M. Siirin, A. Lambert, R.E. Parsons, D.W. Beasley, R.J. Novak, D. Elizondo Quiroga, E.N. Green, D.S. Young, L.M. Stark, M.A. Drebot, H. Artsob, R.B. Tesh, L.D. Kramer, and A.D. Barrett (2005). **Phylogenetic analysis of North American West Nile virus isolates, 2001-2004: Evidence for the emergence of a dominant genotype.** *Virology* 342(2): 252-265.
Abstract: The distribution of West Nile virus has expanded in the past 6 years to include the 48 contiguous United States and seven Canadian provinces, as well as Mexico, the Caribbean islands, and Colombia. The suggestion of the emergence of a dominant genetic variant has led to an intensive analysis of isolates made across North America. We have sequenced the pre-membrane and envelope genes of 74 isolates and the complete genomes of 25 isolates in order to determine if a dominant genotype has arisen and to better understand how the virus has evolved as its distribution has expanded. Phylogenetic analyses revealed the continued presence of genetic variants that group in a temporally and geographically dependent manner and provide evidence that a dominant variant has emerged across much of North America. The implications of these findings are discussed as they relate to transmission and spread of the virus in the Western Hemisphere.
Descriptors: viral genome, West Nile virus, molecular sequence data, North America, species specificity, variation genetics, viral envelope proteins genetics.

Fayzulin, R., F. Scholle, O. Petrakova, I. Frolov, and P.W. Mason (2006). **Evaluation of replicative capacity and genetic stability of West Nile virus replicons using highly efficient packaging cell lines.** *Virology* 351(1): 196-209. ISSN: 0042-6822.
Abstract: A stable cell system for high-efficiency packaging of West Nile virus (WNV) subgenomic replicons into virus-like particles (VLPs) was developed. VLPs could be propagated on these packaging cells and produced infectious foci similar to foci produced by WNV. Focus size correlated with the replicative capacity of WNV replicons, indicating that genome copy number, rather than amount of trans-complementing structural proteins, was rate-limiting in packaging of VLPs. Comparison of VLP production from replicon genomes encoding partial or complete C genes indicated that portions of C downstream of the cyclization sequence could improve genome replication or that cis expression of C could enhance packaging. Interestingly, a rapid loss of replicon-encoded reporter gene activity was detected within two serial passages of reporter gene-containing VLPs. The loss of reporter activity cor-

related with gene deletion and better VLP growth, indicating a powerful selection pressure for WNV genomes lacking reporter genes.
Descriptors: variation genetics, virus assembly, virus replication physiology, West Nile virus, *Cercopithecus aethiops*, cricetinae, vero cells.

Jerzak, G., K.A. Bernard, L.D. Kramer, and G.D. Ebel (2005). **Genetic variation in West Nile virus from naturally infected mosquitoes and birds suggests quasispecies structure and strong purifying selection.** *Journal of General Virology* 86(8): 2175-2183. ISSN: 0022-1317.
Abstract: Intrahost genetic diversity was analysed in naturally infected mosquitoes and birds to determine whether West Nile virus (WNV) exists in nature as a quasispecies and to quantify selective pressures within and between hosts. WNV was sampled from ten infected birds and ten infected mosquito pools collected on Long Island, NY, USA, during the peak of the 2003 WNV transmission season. A 1938 nt fragment comprising the 3' 1159 nt of the WNV envelope (E) coding region and the 5' 779 nt of the non-structural protein 1 (NS1) coding region was amplified and cloned and 20 clones per specimen were sequenced. Results from this analysis demonstrate that WNV infections are derived from a genetically diverse population of genomes in nature. The mean nucleotide diversity was 0.016 % within individual specimens and the mean percentage of clones that differed from the consensus sequence was 19.5 %. WNV sequences in mosquitoes were significantly more genetically diverse than WNV in birds. No host-dependent bias for particular types of mutations was observed and estimates of genetic diversity did not differ significantly between E and NS1 coding sequences. Non-consensus clones obtained from two avian specimens had highly similar genetic signatures, providing preliminary evidence that WNV genetic diversity may be maintained throughout the enzootic transmission cycle, rather than arising independently during each infection. Evidence of purifying selection was obtained from both intra- and interhost WNV populations. Combined, these data support the observation that WNV populations may be structured as a quasispecies and document strong purifying natural selection in WNV populations.
Descriptors: *Corvus brachyrhynchos*, *Corvus ossifragus*, *Culex pipiens*, *Culex restuans*, *Culex salinarius*, *Cyanocitta cristata*, viral diseases, West Nile virus, virus genetic diversity, New York, Long Island.

Kinney, R.M., C.Y. Huang, M.C. Whiteman, R.A. Bowen, S.A. Langevin, B.R. Miller, and A.C. Brault (2006). **Avian virulence and thermostable replication of the North American strain of West Nile virus.** *Journal of General Virology* 87(Pt 12): 3611-3622.
Abstract: The NY99 genotype of West Nile virus (WNV) introduced into North America has demonstrated high virulence for American crows (AMCRs), whilst a closely related WNV strain (KEN-3829) from Kenya exhibits substantially reduced

virulence in AMCRs [Brault, A. C., Langevin, S. A., Bowen, R. A., Panella, N. A., Biggerstaff, B. J., Miller, B. R. & Nicholas, K. (2004). Emerg Infect Dis 10, 2161-2168]. Viruses rescued from infectious cDNA clones of both the NY99 and KEN-3829 strains demonstrated virulence comparable to that of their parental strains in AMCRs. To begin to define parameters that might explain the different virulence phenotypes between these two viruses, temperature-sensitivity assays were performed for both viruses at the high temperatures experienced in viraemic AMCRs. Growth curves of the two WNV strains were performed in African green monkey kidney (Vero; 37-42 degrees C) and duck embryonic fibroblast (DEF; 37-45 degrees C) cells cultured at temperatures that were tolerated by the cell line. Unlike the NY99 virus, marked decreases in KEN-3829 viral titres were detected between 36 and 120 h post-infection (p.i.) at temperatures above 43 degrees C. Replication of KEN-3829 viral RNA was reduced 6500-fold at 72 h p.i. in DEF cells incubated at 44 degrees C relative to levels of intracellular virus-specific RNA measured at 37 degrees C. In contrast, replication of virus derived from the NY99 infectious cDNA at 44 degrees C demonstrated only a 17-fold reduction in RNA level. These results indicated that the ability of WNV NY99 to replicate at the high temperatures measured in infected AMCRs could be an important factor leading to the increased avian virulence and emergence of this strain of WNV.

Descriptors: bird diseases, crows, virus replication, West Nile fever, West Nile virus, body temperature, cell line, *Cercopithecus aethiops*, ducks, plaque assay, viral RNA biosynthesis, survival analysis, temperature, vero cells.

Kononova, I., V.A. Ternovoi, M. Shchelkanov, E.V. Protopopova, S.I. Zolotykh, A.K. Iurlov, A.V. Druziaka, A.A. Slavskii, A.M. Shestopalov, D.K. L'vov, and V.B. Loktev (2006). **[West Nile virus genotyping among wild birds belonging to ground and tree-brush bird populations on the territories of the Baraba forest-steppe and Kulunda steppe (2003-2004)]**. *Voprosy Virusologii* 51(4): 19-23.

Abstract: The paper gives the results of the 2003-2004 examinations of 104 wild birds belonging to land tree-brush complexes from the Baraba forest-steppe and Kulunda steppe for the detection and genotyping West Nile virus (WNV). ELISA and RT-PCR were used to show that in the forest-steppe and steppe zones of the south of Western Siberia, WNV circulates among both migrating and settled birds. An analysis of the nucleotide sequence of a protein E gene fragment showed the circulation of WNV genotype Ia in the study birds. A number of revealed amino acid substitutions in surface glycoprotein E are unique for the 2003-2004 Western-Siberian WNV variants and absent in the 2002 Western-Siberian variants, which suggests that there are regional features of the evolution of WNV genotype Ia.

Descriptors: wild animals, birds, West Nile virus, amino acid sequence, animals, antigens, ecosystem, evolution, molecular sequence data, phylogeny, sequence align-

ment, Siberia, species specificity, trees, viral envelope proteins genetics.
Language of Text: Russian.

Langevin, S.A., A.C. Brault, N.A. Panella, R.A. Bowen, and N. Komar (2005). **Variation in virulence of West Nile virus strains for house sparrows (*Passer domesticus*).** *American Journal of Tropical Medicine and Hygiene* 72(1): 99-102. ISSN: 0002-9637.
Abstract: The observation of avian mortality associated with West Nile virus (WNV) infection has become a hallmark epiderniologic feature in the recent emergence of this pathogen in Israel and North America. To determine if phenotypic differences exist among different WNV isolates, we exposed house sparrows (*Passer doniesticits*) to low passage, lineage 1 WNV strains from North America (NY99), Kenya (KEN), and Australia (KUN; also known as Kunjin virus). House sparrows inoculated with the NY99 and KEN strains experienced similar mortality rates and viremia profiles. The KUN strain elicited significantly lower-titered viremia when compared with the other strains and induced no mortality. This study suggests that natural mortality in house sparrows due to Old World strains of WNV may be occurring where the KEN strain occurs.
Descriptors: *Passer domesticus*, viral diseases, West Nile virus, virulence, variation between strains, mortality.

Li, J., R. Bhuvanakantham, J. Howe, and M.L. Ng (2006). **The glycosylation site in the envelope protein of West Nile virus (Sarafend) plays an important role in replication and maturation processes.** *Journal of General Virology* 87(Pt 3): 613-622.
Abstract: The complete genome of West Nile (Sarafend) virus [WN(S)V] was sequenced. Phylogenetic trees utilizing the complete genomic sequence, capsid gene, envelope gene and NS5 gene/3' untranslated region of WN(S)V classified WN(S)V as a lineage II virus. A full-length infectious clone of WN(S)V with a point mutation in the glycosylation site of the envelope protein (pWNS-S154A) was constructed. Both growth kinetics and the mode of maturation were affected by this mutation. The titre of the pWNS-S154A virus was lower than the wild-type virus. This defect was corrected by the expression of wild-type envelope protein in trans. The pWNS-S154A virus matured intracellularly instead of at the plasma membrane as shown for the parental WN(S)V.
Descriptors: viral genome, viral envelope proteins, West Nile virus, amino acid sequence, cell line, *Cercopithecus aethiops*, glycosylation, molecular sequence data, sequence alignment, species specificity, vero cells, viral envelope proteins.

Pierson, T., M. Diamond, A. Ahmed, L. Valentine, C. Davis, M. Samuel, S. Hanna, B. Puffer , and R. Doms (2005). **An infectious West Nile Virus that expresses a GFP reporter gene.** *Virology* 334(1): 28-40. ISSN: 0042-6822.
Descriptors: West Nile virus, genetic vectors, green fluorescent protein, reporter

genes, gene expression regulation, neutralization, virus replication, humans, cultured cells, nucleotide sequences, viral-antibodies, molecular-sequence-data, Internet-resource.

Ramanathan, M.P., J.A. Chambers, J. Taylor, B.T. Korber, M.D. Lee, A. Nalca, K. Dang, P. Pankhong, W. Attatippaholkun, and D.B. Weiner (2005). **Expression and evolutionary analysis of West Nile virus (Merion strain).** *Journal of Neurovirology* 11(6): 544-556.

Abstract: The authors report a new strain of West Nile virus (WNV) with the expression analysis of its individual open reading frames. Since its sudden appearance in the summer of 1999 in New York City, the virus has spread rapidly across the continental United States into Canada and Mexico. Besides, its rapid transmission by various vectors, the spread of this virus through organ transplantation, blood transfusion, and mother-child transmission through breast milk is of concern. In order to understand molecular variations of WNV in North America and to generate new tools for understanding WNV biology, a complete clone of WNV has been constructed. Investigations so far have focused only on half of its genes products and a detailed molecular and cell biological aspects on all of WNV gene have yet to be clearly established. The open reading frames of WNV were recovered through an reverse transcriptase-polymerase chain reaction (RT-PCR)-PCR using brain tissue from a dead crow collected in Merion, PA, and cloned into a mammalian expression vector. The deduced amino acid sequences of individual open reading frames were analyzed to determine various structural motifs and functional domains. Expression analysis shows that in neuronal cells, C, NS1, and NS5 proteins are nuclear localized whereas the rest of the antigens are confined to the cytoplasm when they are expressed in the absence of other viral antigens. This is the first report that provides an expression analysis as well as intracellular distribution pattern for all of WNV gene products, cloned from an infected bird. Evolutionary analysis of Merion strain sequences indicates that this strain is distinct phylogenetically from the previously reported WNV strains.

Descriptors: viral gene expression regulation, West Nile fever, West Nile virus, amino acid sequence, bird diseases, birds, molecular sequence data, phylogeny, reverse transcriptase polymerase chain reaction.

Rasgon, J.L., M. Venkatesan, C.J. Westbrook, and M.C. Hauer (2006). **Polymorphic microsatellite loci from the West Nile virus vector *Culex tarsalis*.** *Molecular Ecology Notes.* 6(3): 680-682. ISSN: 1471-8278.

Abstract: Since its introduction in 1999, West Nile virus (WNV) has spread across North America. *Culex tarsalis* is a highly efficient WNV vector species. Many traits such as virus susceptibility, autogeny and host preference vary geographically and temporally in *C. tarsalis*. *Culex tarsalis* genomic libraries were developed and were

highly enriched for microsatellite inserts (42-96%). We identified 12 loci that were polymorphic in wild *C. tarsalis* populations. These microsatellites are the first DNA-based genetic markers developed for *C. tarsalis* and will be useful for investigating population structure and constructing genetic maps in this mosquito.
Descriptors: Internet-resource, West Nile virus, vectors, *C. tarsalis*, genomic libraries, loci, microsatellites, genetic markers.

Scholle, F. and P.W. Mason (2005). **West Nile virus replication interferes with both poly(I:C)-induced interferon gene transcription and response to interferon treatment.** *Virology* 342(1): 77-87. ISSN: 0042-6822.
Abstract: West Nile virus (WNV), the leading cause of viral encephalitis in the United States, is an arthropod-transmitted member of the family Flaviviridae. We have explored the interaction of this positive-strand RNA virus with signaling pathways involved in induction of the host's innate immune response. Phosphorylation of STAT-1 in response to interferon (IFN) treatment and the ability of IFN to establish an antiviral state were reduced in WNV replicon-bearing cell lines. Similarly, the activation of IRF3 and stimulation of IFN-beta transcription in response to the double-stranded RNA (dsRNA) mimetic poly(I:C) were inhibited in replicon-bearing and WNV-infected HeLa cells. In contrast, WNV replicons did not affect IRF3 activation by Sendai virus infection, suggesting that not all IRF3 activating pathways are inhibited by WNV. Taken together, these findings demonstrate that WNV replication in cultured cells interferes with both the response to IFN and synthesis of IFN-beta in response to dsRNA.
Descriptors: interferon beta genetics, West Nile virus, cell nucleus active transport, dimerization, hela cells, natural immunity, interferon regulatory factor 3, viral genetics, signal transduction, transcription, genetic drug effects, virus replication.

Seligman, S.J. (2006). **Single nucleotide polymorphisms in human genes and increased susceptibility to West Nile Virus disease.** *Journal of Infectious Diseases* 193(8): 1187-1188; Author Reply 1188.
Descriptors: single nucleotide polymorphism genetics, West Nile fever, genetic predisposition to disease, natural immunity genetics, mice, West Nile virus (WNV).
Notes: Comment On: J Infect Dis. 2005 Nov 15;192(10):1741-8.

Sheets, R.L., J. Stein, T.S. Manetz, C. Andrews, R. Bailer, J. Rathmann, and P.L. Gomez (2006). **Toxicological safety evaluation of DNA plasmid vaccines against HIV-1, Ebola, Severe Acute Respiratory Syndrome, or West Nile virus is similar despite differing plasmid backbones or gene-inserts.** *Toxicological Sciences* 91(2): 620-630.
Abstract: The Vaccine Research Center has developed a number of vaccine candidates for different diseases/infectious agents (HIV-1, Severe Acute Respiratory Syndrome virus, West Nile virus, and Ebola virus, plus a plasmid cytokine adju-

vant-IL-2/Ig) based on a DNA plasmid vaccine platform. To support the clinical development of each of these vaccine candidates, preclinical studies were performed to screen for potential toxicities (intrinsic and immunotoxicities). All treatment-related toxicities identified in these repeated-dose toxicology studies have been confined primarily to the sites of injection and seem to be the result of both the delivery method (as they are seen in both control and treated animals) and the intended immune response to the vaccine (as they occur with greater frequency and severity in treated animals). Reactogenicity at the site of injection is generally seen to be reversible as the frequency and severity diminished between doses and between the immediate and recovery termination time points. This observation also correlated with the biodistribution data reported in the companion article (Sheets et al., 2006), in which DNA plasmid vaccine was shown to remain at the site of injection, rather than biodistributing widely, and to clear over time. The results of these safety studies have been submitted to the Food and Drug Administration to support the safety of initiating clinical studies with these and related DNA plasmid vaccines. Thus far, standard repeated-dose toxicology studies have not identified any target organs for toxicity (other than the injection site) for our DNA plasmid vaccines at doses up to 8 mg per immunization, regardless of disease indication (i.e., expressed gene-insert) and despite differences (strengths) in the promoters used to drive this expression. As clinical data accumulate with these products, it will be possible to retrospectively compare the safety profiles of the products in the clinic to the results of the repeated-dose toxicology studies, in order to determine the utility of such toxicology studies for signaling potential immunotoxicities or intrinsic toxicities from DNA vaccines. These data build on the biodistribution studies performed (see companion article, Sheets et al., 2006) to demonstrate the safety and suitability for investigational human use of DNA plasmid vaccine candidates for a variety of infectious disease prevention indications.

Descriptors: DNA vaccines, viral vaccines, acquired immunodeficiency syndrome, ebola like viruses, HIV-1 genetics, hemorrhagic fever, rabbits, severe acute respiratory syndrome, tissue distribution, West Nile fever genetics, West Nile virus.

Shirato, K., H. Miyoshi, H. Kariwa, and I. Takashima (2006). **The kinetics of proinflammatory cytokines in murine peritoneal macrophages infected with envelope protein-glycosylated or non-glycosylated West Nile virus.** *Virus Research* 121(1): 11-16. ISSN: 0168-1702.

Abstract: The envelope (E) protein glycosylation status of the New York strain of West Nile (WN) virus is an important determinant of virus neuroinvasiveness. To elucidate the determinant of the difference between E protein-glycosylated and non-glycosylated WN virus infections, the cytokine expression of murine peritoneal macrophages infected with each virus was examined. Tumor necrosis factor

(TNF) alpha and interleukin (IL)-1beta were up-regulated with replication of the E protein-glycosylated virus. Interferon (IFN) beta and IL-6 were up-regulated with the clearance of both viruses. These results suggest that TNFalpha and IL-1beta expression are related to the virulence of E protein-glycosylated WN virus.
Descriptors: interleukin 1 metabolism, peritoneal macrophages, tumor necrosis factor, alpha metabolism, viral envelope proteins, West Nile fever, cultured cells, glycosylation , inbred Balb C mice, up-regulation.

Sitati, E.M. and M.S. Diamond (2006). **CD4+ T-cell responses are required for clearance of West Nile virus from the central nervous system.** *Journal of Virology* 80(24): 12060-12069. ISSN: 0022-538X.
Abstract: Although studies have established that innate and adaptive immune responses are important in controlling West Nile virus (WNV) infection, the function of CD4(+) T lymphocytes in modulating viral pathogenesis is less well characterized. Using a mouse model, we examined the role of CD4(+) T cells in coordinating protection against WNV infection. A genetic or acquired deficiency of CD4(+) T cells resulted in a protracted WNV infection in the central nervous system (CNS) that culminated in uniform lethality by 50 days after infection. Mice surviving past day 10 had high-level persistent WNV infection in the CNS compared to wild-type mice, even 45 days following infection. The absence of CD4(+) T-cell help did not affect the kinetics of WNV infection in the spleen and serum, suggesting a role for CD4-independent clearance mechanisms in peripheral tissues. WNV-specific immunoglobulin M (IgM) levels were similar to those of wild-type mice in CD4-deficient mice early during infection but dropped approximately 20-fold at day 15 postinfection, whereas IgG levels in CD4-deficient mice were approximately 100- to 1,000-fold lower than in wild-type mice throughout the course of infection. WNV-specific CD8(+) T-cell activation and trafficking to the CNS were unaffected by the absence of CD4(+) T cells at day 9 postinfection but were markedly compromised at day 15. Our experiments suggest that the dominant protective role of CD4(+) T cells during primary WNV infection is to provide help for antibody responses and sustain WNV-specific CD8(+) T-cell responses in the CNS that enable viral clearance.
Descriptors: West Nile virus, CNS, clearance, CD4+T cell responses, immune responses, spleen, serum, viral clearance.

Ternovoi, V.A., E.V. Protopopova, S.G. Surmach, M.V. Gazetdinov, S.I. Zolotykh, A.M. Shestopalov, E.V. Pavlenko, G.N. Leonova, and V.B. Loktev (2006). **[The genotyping of the West Nile virus in birds in the far eastern region of Russia in 2002-2004]**. *Molekuliarnaia Genetika, Mikrobiologiia i Virusologiia*(4): 30-35.
Abstract: Samples from 20 species of trapped and dead birds were collected in the Far Eastern Region in 2002-2004 and were analyzed by the anti-WNV MAb-modified immunoenzyme assay for antigen detection and RT-PCR for viral RNA

detection. Five positive samples from cinereous vultures (*Aegypius monachus*) and two positive samples from cattle egret (*Bubulcus ibis*) were found in both tests. The sequencing of the 322 bp fragments of protein E gene showed 99-99.67% homology with the strain WNV/LEIV-VlgOO-27924 of the WNV isolated in Volgograd, Russia, 2000. Additionally, five positive samples from birds (*Pica pica, Corvus macrorhynchos, Larus crossirostris, Parus minor, Emberiza spodocephala*) collected in autumn 2004 were found during screening with anti-WNV MAb-modified ELISA. These results confirm that the WNV is circulating in the Far Eastern Region of Russia and outbreaks of WN fever in humans may be possible. This demonstrates that the genotype 1a of the West Nile virus could spread in the southern regions of the Far East by migrating birds and introduction of the WNV into other southern regions of the Asian part of Russia are probably.
Descriptors: birds, West Nile fever, viral genotype, immunoenzyme techniques, reverse transcriptase polymerase chain reaction, Russia, West Nile virus classification.
Language of Text: Russian.

Venter, M., T.G. Myers, M.A. Wilson, T.J. Kindt, J.T. Paweska, F.J. Burt, P.A. Leman, and R. Swanepoel (2005). **Gene expression in mice infected with West Nile virus strains of different neurovirulence.** *Virology* 342(1): 119-140. ISSN: 0042-6822.
Abstract: West Nile virus causes febrile illness in humans with a proportion of cases progressing to meningoencephalitis, encephalitis, hepatitis, and death. Isolates of the virus fall into two genetic lineages, with differences in neuroinvasiveness for mice occurring between strains within both lineages. We used DNA microarrays to compare gene expression in mice infected peripherally with seven lineage 1 and 2 strains confirmed to be of either high or low neuroinvasiveness in mice and associated with severe or benign infection in humans and birds. The 4 strains with highest neuroinvasiveness induced increased expression of 47 genes in the brain, 111 genes in the liver, and 70 genes in the spleen, relative to the 3 least neuroinvasive strains. Genes involved in interferon signaling pathways, protein degradation, T-cell recruitment, MHC class I and II antigen presentation, and apoptosis were identified that may have both pathogenic and protective effects, but increased expression of certain acute proteins, central nervous system specific proteins and proteins associated with T-cell hepatitis, implicate mechanisms related to exalted virulence.
Descriptors: gene expression, West Nile fever genetics, West Nile virus, brain metabolism, gene expression profiling, liver metabolism, mice, oligonucleotide array sequence analysis, reverse transcriptase polymerase chain reaction, spleen metabolism.

Virus Isolation and Purification

Anon (2006). **West nile virus activity--United States, January 1-November 7, 2006.** *MMWR. Morbidity and Mortality Weekly Report* 55(44): 1204-1205.

Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Standard Time, November 7, 2006. A total of 41 states and the District of Columbia had reported 3,830 cases of human WNV illness to CDC.

Descriptors: West Nile fever, bird diseases, culicidae, horse diseases, rodent diseases, sciuridae, United States, West Nile virus isolation and purification.

Anon (2006). **West Nile virus activity--United States, January 1-October 10, 2006.** *MMWR. Morbidity and Mortality Weekly Report* 55(40): 1097-1098.

Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Daylight Time, October 10, 2006. A total of 41 states and the District of Columbia had reported 3,135 cases of human WNV illness to CDC. A total of 1,717 (55%) cases for which such data were available occurred in males; median age of patients was 50 years (range: 3 months-99 years). Dates of illness onset ranged from January 6 to September 25; a total of 97 cases were fatal.

Descriptors: West Nile fever epidemiology, West Nile virus isolation and purification, bird diseases, culicidae, horse diseases, rodent diseases, sciuridae, United States.

Anon (2006). **West Nile virus activity--United States, January 1-September 12, 2006.** *MMWR. Morbidity and Mortality Weekly Report* 55(36): 996.

Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Daylight Time, September 12, 2006. A total of 36 states and the District of Columbia had reported 1,634 cases of human WNV illness to CDC. A total of 921 (57%) cases for which such data were available occurred in males; median age of patients was 51 years (range: 3 months-95 years). Dates of illness onset ranged from January 6 to September 10; a total of 52 cases were fatal.

Descriptors: West Nile fever, humans, birds, humans, United States, fatality reports.

Anon (2006). **West Nile virus activity--United States, January 1-August 15, 2006.** *Morbidity and Mortality Weekly Report* 55(32): 879-880.

Abstract: This report summarizes West Nile virus (WNV) surveillance data reported to CDC through ArboNET as of 3 a.m. Mountain Daylight Time, August 15, 2006. A total of 26 states had reported 388 cases of human WNV illness to CDC. A total

of 214 (56%) cases for which such data were available occurred in males; median age of patients was 49 years (range: 2-91 years). Dates of illness onset ranged from January 6 to August 10; a total of 13 cases were fatal. A total of 68 presumptive West Nile viremic blood donors (PVDs) have been reported to ArboNET during 2006. Of these, 20 were reported from Nebraska; 18 were reported from Texas; five were reported from California; four were reported from Utah; three each were reported from Oklahoma and South Dakota; two each were reported from Idaho, Iowa, Kentucky, and Mississippi; and one each was reported from Arizona, Colorado, Minnesota, Nevada, North Dakota, Wisconsin, and Wyoming. Of the 68 PVDs, 10 persons (median age: 43 years [range: 18-59 years]) subsequently had West Nile fever.
Descriptors: West Nile fever epidemiology, humans, bird diseases, culicidae, horse diseases, rodent diseases, United StatesWest Nile virus isolation and purification.

Buckley, A., A. Dawson, and E.A. Gould (2006). **Detection of seroconversion to West Nile virus, Usutu virus and Sindbis virus in UK sentinel chickens.** *Virology Journal* 3: 71.
Abstract: We previously reported evidence of West Nile virus (WNV) circulation in UK birds, probably introduced by migratory birds from overseas. We now demonstrate WNV-specific seroconversion in sentinel chickens raised on an English farm. Maternal neutralizing antibodies to WNV in hatchlings declined within three weeks. During the following months, healthy chickens developed WNV neutralizing antibodies that were confirmed by immunoblotting and indirect immunofluorescence tests using WNV antigens. The proportion of seropositive chickens was higher for WNV than for Usutu virus or Sindbis virus. Attempts to isolate infectious virus or to detect viral RNA in the sera, failed.
Descriptors: alphavirus infections, viral blood, chickens, encephalitis, flavivirus, sindbis virus, bird diseases, western blotting, Great Britain, fluorescence microscopy, sentinel surveillance, seroepidemiologic studies , West Nile virus isolation and purification.

D'Arcy, A., M. Chaillet, N. Schiering, F. Villard, S.P. Lim, P. Lefeuvre, and P. Erbel (2006). **Purification and crystallization of dengue and West Nile virus NS2B-NS3 complexes.** *Acta Crystallographica. Section F, Structural Biology and Crystallization Communications* 62(Pt 2): 157-162. ISSN: 1744-3091.
Abstract: Both dengue and West Nile virus infections are an increasing risk to humans, not only in tropical and subtropical areas, but also in North America and parts of Europe. These viral infections are generally transmitted by mosquitoes, but may also be tick-borne. Infection usually results in mild flu-like symptoms, but can also cause encephalitis and fatalities. Approximately 2799 severe West Nile virus cases were reported this year in the United States, resulting in 102 fatalities. With this alarming increase in the number of West Nile virus infections in western coun-

tries and the fact that dengue virus already affects millions of people per year in tropical and subtropical climates, there is a real need for effective medicines. A possible therapeutic target to combat these viruses is the protease, which is essential for virus replication. In order to provide structural information to help to guide a lead identification and optimization program, crystallizations of the NS2B-NS3 protease complexes from both dengue and West Nile viruses have been initiated. Crystals that diffract to high resolution, suitable for three-dimensional structure determinations, have been obtained.

Descriptors: cysteine endopeptidases, dengue virus, serine endopeptidases, viral nonstructural proteins, West Nile virus, crystallography, x ray, multiprotein complexes.

Deardorff, E., J. Estrada Franco, A.C. Brault, R. Navarro Lopez, A. Campomanes Cortes, P. Paz Ramirez, M. Solis Hernandez, W.N. Ramey, C.T. Davis, D.W. Beasley, R.B. Tesh, A.D. Barrett, and S.C. Weaver (2006). **Introductions of West Nile virus strains to Mexico.** *Emerging Infectious Diseases* 12(2): 314-318.

Abstract: Complete genome sequencing of 22 West Nile virus isolates suggested 2 independent introductions into Mexico. A previously identified mouse-attenuated glycosylation variant was introduced into southern Mexico through the southeastern United States, while a common US genotype appears to have been introduced incrementally into northern Mexico through the southwestern United States.

Descriptors: West Nile virus, bird diseases, horse diseases, Mexico, mice, molecular sequence data, United States.

Jiang, S.F., T.Y. Zhao, Y.D. Dong, X.X. Guo, Y.M. Zhang, C.X. Li, X.L. Zhang, and D. Xing (2006). **Isolation and identification of west Nile virus from experimentally infected Culex tritaeniorhynchus and leghorn chicken.** *Acta Entomologica Sinica* 49(4): 588-592. ISSN: 0454-6296.

Abstract: C6/36 culture isolation test, indirect immunofluorescence assay, and reverse transcription PCR and sequencing of the PCR products were carried out to isolate and identify the West Nile virus (WNV) from experimentally infected *Culex tritaeniorhynchus* and Leghorn chicken. The cell pathological effects such as cell fusion and cavitation were observed in. C6/36 inoculated experimentally infected samples. The antigen of WNV was detected by indirect immuno fluorescence assay. Three specific PCR bands with expected size (408 bp, 498 bp, and 559 bp, respectively) were found, and the sequences of these PCR products were concordant with those of WNV Chin-01 strain. Hence, it was confirmed that the WNV from experimentally infected *Cx. tritaeniorhynchu*s and Leghorn chicken was same as WNV Chin-01 strain used in our experiments.

Descriptors: *Culex tritaeniorhynchus*, viral diseases, West Nile virus, isolation and

identification after experimental infection.
Language of Text: Chinese; Summary in Chinese, English.

Kononova, I., V.A. Ternovoi, M. Shchelkanov, E.V. Protopopova, S.I. Zolotykh, A.K. Iurlov, A.V. Druziaka, A.A. Slavskii, A.M. Shestopalov, D.K. L'vov, and V.B. Loktev (2006). **[West Nile virus genotyping among wild birds belonging to ground and tree-brush bird populations on the territories of the Baraba forest-steppe and Kulunda steppe (2003-2004)]**. *Voprosy Virusologii* 51(4): 19-23.
Abstract: The paper gives the results of the 2003-2004 examinations of 104 wild birds belonging to land tree-brush complexes from the Baraba forest-steppe and Kulunda steppe for the detection and genotyping West Nile virus (WNV). ELISA and RT-PCR were used to show that in the forest-steppe and steppe zones of the south of Western Siberia, WNV circulates among both migrating and settled birds. An analysis of the nucleotide sequence of a protein E gene fragment showed the circulation of WNV genotype Ia in the study birds. A number of revealed amino acid substitutions in surface glycoprotein E are unique for the 2003-2004 Western-Siberian WNV variants and absent in the 2002 Western-Siberian variants, which suggests that there are regional features of the evolution of WNV genotype Ia.
Descriptors: wild animals, birds, West Nile virus, amino acid sequence, animals, antigens, ecosystem, evolution, molecular sequence data, phylogeny, sequence alignment, Siberia, species specificity, trees, viral envelope proteins genetics.
Language of Text: Russian.

Zhao, Y.X., J. Ge, Q.L. Song, J. Pace, B. Chen, H. Goldman, J.H. Keffer, and N. Shaikh (2006). **Purification and characterization of human West Nile virus-specific antibodies from patients' plasma using a recombinant WNV envelope protein affinity column**. *Clinical Chemistry* 52(6, Suppl. S): A143-A144. ISSN: 0009-9147.
Descriptors: West Nile virus, virus specific antibodies, plasma PCR, genetic techniques, ELISA, immunologic techniques, gel electrophoresis, surface plasmon resonance, spectrum analysis, envelope protein affinity column.
Notes: Meeting Information: 58th Annual Meeting of the American Association of Clinical Chemistry, Chicago, Illinois, USA; July 23 -27, 2006.

WNV Associated Diseases

Abroug, F., L. Ouanes Besbes, M. Letaief, F. Ben Romdhane, M. Khairallah, H. Triki, and N. Bouzouiaia (2006). **A cluster study of predictors of severe West Nile virus infection.** *Mayo Clinic Proceedings* 81(1): 12-16.

Abstract: OBJECTIVE: To assess the value of multifocal chorioretinitis and of clinical manifestations and biologic parameters in the diagnosis of West Nile virus (WNV) infection. PATIENTS AND METHODS: We conducted a prospective, controlled case series study during an outbreak of WNV infection between August 15 and October 24, 2003, of 64 consecutive patients who presented with clinical manifestations consistent with WNV disease. In each patient, standardized clinical and biologic data were collected. An ophthalmologic examination searching particularly for multifocal chorioretinitis was performed. RESULTS: Of 64 patients who presented primarily with meningitis and/or encephalitis, 36 had IgM antibodies against WNV. The WNV-infected patients tended to be older (median age of 54 years vs 46 years in WNV infection and control groups, respectively) and more frequently had diabetes (30% vs 7% in WNV infection and control groups, respectively; P = .03). Multifocal chorioretinitis was found in 75% of WNV-infected patients but in no patient in the control group (P = .001). Blood glucose and amylase levels were higher in WNV-infected patients, whereas serum sodium levels were lower. The cerebrospinal fluid leukocyte count and protein levels were significantly higher in WNV meningitis or encephalitis. Overall, multifocal chorioretinitis had 100% specificity and 73% sensitivity (88% when only patients with meningitis or encephalitis were analyzed) for the diagnosis of WNV. Multivariate analysis disclosed multifocal chorioretinitis as the only predictor of WNV infection (odds ratio, 62; 95% confidence interval, 6-700; P = .001). CONCLUSION: Multifocal chorioretinitis appears to be a specific marker of WNV infection, particularly in patients who present with meningoencephalitis. An ophthalmologic examination should be part of the routine evaluation of such patients.

Descriptors: chorioretinitis, disease outbreaks, eye infections, West Nile fever, antibodies, viral immunology, enzyme linked immunosorbent assay, prospective studies, severity of illness index, Tunisia, West Nile virus.

Abroug, F., L. Ouanes Besbes, I. Ouanes, N. Nciri, F. Dachraoui, and F. Najjar (2006). **Adrenal insufficiency in severe West Nile Virus infection.** *Intensive Care Medicine* 32(10): 1636-1639.

Abstract: OBJECTIVE: To explore adrenal function in severe West Nile virus (WNV) infection. DESIGN AND SETTING: Prospective interventional cohort

study in a medical ICU of a teaching hospital. PATIENTS: Ten consecutive patients (seven men, mean age 64[Symbol: see text]+/-[Symbol: see text]12[Symbol: see text]years, mean SAPS II 26[Symbol: see text]+/-[Symbol: see text]6) with definite diagnosis of WNV related meningoencephalitis and variable proportion of organ/system failure. All patients had fever (mean body temperature 39[Symbol: see text]+/-[Symbol: see text]1 degrees C) and altered mental status (mean Glasgow Coma Score 11[Symbol: see text]+/-[Symbol: see text]2). Mean SOFA score was 9[Symbol: see text]+/-[Symbol: see text]2; eight patients had systemic inflammatory response syndrome, five septic shock, and six acute respiratory failure (usually from central origin) requiring mechanical ventilation. INTERVENTIONS: A short corticotropin test was performed in each patient to assess the adrenal function. MEASUREMENTS AND RESULTS: Cortisol response was defined as the difference between baseline and corticotropin-stimulated peak. Absolute adrenal insufficiency was defined by a baseline cortisol level below 15[Symbol: see text]mug/dl (415[Symbol: see text]nmol/l). Relative insufficiency was defined by a cortisol response of 9[Symbol: see text]mug/dl (250[Symbol: see text]nmol/l) or less. Relative adrenal insufficiency, defined by a corticotropin response below 9[Symbol: see text]mug/dl, was observed in seven while the remaining three had normal cortisol response; six out of these seven died in the ICU. All patients with normal adrenal function survived. CONCLUSION: Adrenal insufficiency is frequent in severe WNV infection and carries a poor outcome. In the absence of specific effective treatment, our data provide a rational to investigate a supplemental corticosteroid treatment in a controlled trial.
Descriptors: West Nile virus, adrenal function, meningoencephalitis, corticotropin test, adrenal insufficiency, prognosis.

Alexander, J.J., A.S. Lasky, and W.D. Graf (2006). **Stroke associated with central nervous system vasculitis after West Nile virus infection.** *Journal of Child Neurology* 21(7): 623-625.
Abstract: We report the association of West Nile virus infection, isolated vasculitis, and stroke in a 9-year-old girl. West Nile virus is of growing epidemiologic importance and should be considered in the differential diagnosis of stroke etiologies, especially during late summer and in patients with a history of exposure in areas where West Nile virus transmission is present.
Descriptors: cerebrovascular accident, vasculitis, central nervous system, West Nile fever complications, child.

Ali, M., Y. Safriel, J. Sohi, A. Llave, and S. Weathers (2005). **West Nile virus infection: MR imaging findings in the nervous system.** *AJNR. American Journal of Neuroradiology* 26(2): 289-297.
Abstract: BACKGROUND AND PURPOSE: West Nile virus (WNV) infection is an ongoing seasonal epidemic. We correlated the MR imaging findings with the clini-

cal presentations and outcomes of WNV infection. METHODS: We reviewed 14 brain and three spinal MR images: nonenhanced and contrast-enhanced T1-weighted images (T1WIs) and T2-weighted images (T2WIs), nonenhanced fluid-attenuated inversion recovery (FLAIR) images (11 patients) and enhanced FLAIR images (three patients), with diffusion-weighted (DW) images and apparent diffusion coefficient maps. WNV infection was diagnosed by means of enzyme-linked immunosorbent assay with a plaque reduction neutralization test. We also correlated the MR findings with the clinical presentation, course, and outcome to determine their prognostic importance. RESULTS: MR imaging findings included: 1) normal (five patients); 2) DW imaging-only abnormalities in the white matter, corona radiata, and internal capsule (four patients); 3) hyperintensity on FLAIR images and T2WIs in the lobar gray and white matter, cerebellum, basal ganglia, thalamus and internal capsule, pons and midbrain (three patients); 4) meningeal involvement (two patients); and 5) spinal cord, cauda equina, and nerve root involvement (three patients). All patients with finding 1 and all but one with finding 2 recovered completely. Two patients with finding 3 died. Those with finding 4 or 5 had residual neurologic deficits that were severe or moderate to severe, respectively. CONCLUSION: Patients with normal MR images or abnormalities on only DW images had the best prognosis, while those with abnormal signal intensity on T2WI and FLAIR images had the worst outcomes. No definite predilection for any specific area of the brain parenchyma was noted.
Descriptors: central nervous system, viral diseases pathology, magnetic resonance imaging, West Nile fever diagnosis, adolescent, adult, aged, middle aged.

Anon (2006). **West nile virus a looming deadly threat for liver transplantation.** *Transplantation* 82(1 Suppl 2): 150.
Descriptors: West Nile virus, liver transplantation, deadly threat, infection, abstract.

Anon (2005). **West Nile virus infections in organ transplant recipients--New York and Pennsylvania, August-September, 2005.** *Morbidity and Mortality Weekly Report* 54(40): 1021-3.
Abstract: In September 2005, West Nile virus (WNV) infection was confirmed in three of four recipients of organs transplanted from a common donor. Two recipients subsequently had neuroinvasive disease, one recipient had asymptomatic WNV infection, and a fourth recipient apparently was not infected. This report summarizes the ongoing investigation. Clinicians should be aware of the potential for transplant-associated transmission of infectious disease.
Descriptors: organ transplantation, West Nile fever transmission, West Nile virus, New York, Pennsylvania.

Arnold, J.C., G.A. Revivo, M.O. Senac, and J.A. Leake (2005). **West Nile virus encephalitis with thalamic involvement in an immunocompromised child.** *Pediatric Infectious Disease Journal* 24(10): 932-934.

Abstract: West Nile virus has been an increasingly important pathogen in the United States since it was first reported in 1999. Neuroinvasive West Nile virus has been infrequently reported in the pediatric population. We report a case of severe West Nile virus encephalitis with cranial magnetic resonance imaging findings not yet described in children.

Descriptors: immunocompromised host, thalamus, West Nile fever, humans.

Barshes, N.R., E.E. Agee, T. Zgabay, F.C. Brunicardi, J.A. Goss, and M.E. Debakey (2006). **West nile virus encephalopathy following pancreatic islet transplantation.** *American Journal of Transplantation* 6(12): 3037.

Descriptors: West Nile virus, encephalopathy, pancreatic islet transplantation, infection, transplants, surgery.

Batalis, N.I., L. Galup, S.R. Zaki, and J.A. Prahlow (2005). **West Nile virus encephalitis.** *American Journal of Forensic Medicine and Pathology* 26(2): 192-196.

Abstract: West Nile virus (WNV) is a mosquito-borne virus that has caused a large number of deaths in the United States since the first outbreak in New York City in 1998. The outbreak initially was limited to the northeast but has since spread across the entire continental United States. WNV causes a variety of clinical symptoms, but the most severe consequences result from central nervous system infection, resulting in meningitis, encephalitis, or meningoencephalitis. We present a case of a 62-year-old male with metastatic cancer, who died as a result of WNV encephalitis. This is followed by a discussion on the epidemiology of WNV and a detailed summary of the methods and resources available to make a diagnosis of WNV infection postmortem. The material presented in the discussion should provide the forensic pathologist with all the information necessary to make a diagnosis of WNV infection postmortem. If nothing else, the routine collection and storage of serum, cerebrospinal fluid, and tissue for every case can enable the forensic pathologist to make this diagnosis even in cases in which WNV is not suspected until after autopsy.

Descriptors: mosquito vectors, West Nile fever, brain pathology, carcinoma, small cell therapy, chills, confusion, fever, forensic pathology, lung neoplasms, psychomotor agitation.

Batsis, J.A. and M.P. Phy (2005). **West Nile virus meningitis in a chronic immunosuppressed patient with rheumatoid arthritis.** *Clinical Rheumatology* 24(5): 548-550.

Abstract: The clinical presentation of West Nile virus (WNV) can be severe in immunosuppressed patients. A 65-year-old with steroid-dependent rheumatoid arthritis on infliximab and methotrexate presented with meningitis and profound

muscular weakness. Serum WNV IgM and IgG antibody were positive. WNV should be included in the differential diagnosis of neurological symptoms in peak months.
Descriptors: rheumatoid arthritis, immunocompromised host, meningitis, West Nile fever, monoclonal antibodies, antirheumatic agents, immunosuppressive agents, West Nile virus.

Bhangoo, S., R. Chua, C. Hammond, Z. Kimmel, I. Semenov, A. Videnovic, J. Kessler, and M. Borsody (2005). **Focal neurological injury caused by West Nile virus infection may occur independent of patient age and premorbid health.** *Journal of the Neurological Sciences* 234(1-2): 93-98.
Abstract: INTRODUCTION: Limited evidence suggests that focal neurological injury (e.g., acute flaccid paralysis) caused by infection with the West Nile virus (WNV) is more common in older patients. We re-evaluate this association in a series of patients who were infected with the WNV during the 2002 epidemic. METHODS: We performed a retrospective chart review of 34 patients who were hospitalized for treatment of serologically confirmed WNV infection. Measurements included the patient's demographic characteristics, baseline medical diagnoses, the occurrence of symptoms and exam findings, the results of various diagnostic tests, and the patient's clinical outcome. RESULTS: Patients infected with the WNV who developed focal neurological injury were found to be comparable to patients who did not develop focal neurological injury both in terms of patient age and the number of medical conditions the patient had prior to infection. This is in contrast to WNV-infected patients who developed an encephalitis-like clinical course, or who died or were institutionalized after their hospitalization; such patients tended to be older and-in cases with a poor outcome-have more medical conditions prior to WNV infection. CONCLUSIONS: In our patient group, focal neurological injury caused by WNV infection was not related to advanced patient age or to the number of medical conditions the patient had prior to infection. Our findings bring into question commonly held views about the development of focal neurological injury caused by WNV infection, and they raise concerns about the management of future WNV epidemics and the testing and use of potential antiviral treatments against this infection.
Descriptors: health status, nervous system diseases , West Nile fever, cerebrospinal fluid, West Nile virus (WNV).

Bode, A.V., J.J. Sejvar, W.J. Pape, G.L. Campbell, and A.A. Marfin (2006). **West Nile virus disease: A descriptive study of 228 patients hospitalized in a 4-county region of Colorado in 2003.** *Clinical Infectious Diseases an Official Publication of the Infectious Diseases Society of America* 42(9): 1234-1240.
Abstract: BACKGROUND: Risk factors for complications of West Nile virus disease and prognosis in hospitalized patients are incompletely understood.

METHODS: Demographic characteristics and data regarding potential risk factors, hospitalization, and dispositions were abstracted from medical records for residents of 4 Colorado counties who were hospitalized in 2003 with West Nile virus disease. Univariate and multivariate analyses were used to identify factors associated with West Nile encephalitis (WNE), limb weakness, or death by comparing factors among persons with the outcome of interest with factors among those without the outcome of interest. RESULTS: Medical records of 221 patients were reviewed; 103 had West Nile meningitis, 65 had WNE, and 53 had West Nile fever. Respiratory failure, limb weakness, and cardiac arrhythmia occurred in all groups, with significantly more cases of each in the WNE group. Age, alcohol abuse, and diabetes were associated with WNE. Age and WNE were associated with limb weakness. The mortality rate in the WNE group was 18%; age, immunosuppression, requirement of mechanical ventilation, and history of stroke were associated with death. Only 21% of patients with WNE who survived returned to a prehospitalization level of function. The estimated incidence of West Nile fever cases that required hospitalization was 6.0 cases per 100,000 persons; West Nile fever was associated with arrhythmia, limb weakness, and respiratory failure. CONCLUSIONS: Persons with diabetes and a reported history of alcohol abuse and older persons appear to be at increased risk of developing WNE. Patients with WNE who have a history of stroke, who require mechanical ventilation, or who are immunosuppressed appear to be more likely to die. Respiratory failure, limb weakness, and arrhythmia occurred in all 3 categories, but there were significantly more cases of all in the WNE group.

Descriptors: West Nile fever, retrospective studies, risk factors, Colorado, humans.

Notes: Comment In: Clin Infect Dis. 2006 Aug 1;43(3):388-9.

Bragin Sanchez, D. and P.P. Chang (2005). **West Nile virus encephalitis infection in a heart transplant recipient: A case report.** *Journal of Heart and Lung Transplantation the Official Publication of the International Society for Heart Transplantation* 24(5): 621-623.

Abstract: Infection with West Nile virus (WNV) ranges from causing no symptoms to causing non-specific fever, meningitis, meningo-encephalitis and death. Advanced age and immunosuppression are the greatest risk factors for the development of encephalitis or other neurologic manifestations of WNV. We report the first fatal case of primary WNV infection in an orthotopic heart transplant recipient. We reviewed the literature on WNV infection in the general population and in immunocompromised hosts. As WNV becomes more common in the United States, it will become an increasing threat to our transplant population.

Descriptors: heart transplantation, immunocompromised host, immunosuppressive agents, West Nile fever immunology, fatal outcome, humans, North Carolina.

Brenner, W., G. Storch, R. Buller, R. Vij, S. Devine, and J. Dipersio (2005). **West nile virus encephalopathy in an allogeneic stem cell transplant recipient: Use of quantitative PCR for diagnosis and assessment of viral clearance**. *Bone Marrow Transplantation* 36(4): 369-370. ISSN: 0268-3369.
Descriptors: encephalopathy, quadriplegia, chronic myeloid leukemia, neoplastic disease, heart disease, magnetic resonance imaging, diagnostic techniques, stem cell transplantation, quantitative polymerase chain reaction, viral clearance.

Briese, T. and K.A. Bernard (2005). **West Nile virus--An old virus learning new tricks?** *Journal of Neurovirology* 11(5): 469-475.
Abstract: West Nile virus (WNV) has spread across the United States causing annual outbreaks since its emergence in 1999. Although severe disease develops only in about 1% of infections, WNV has claimed a total of 564 lives in the 5 years from 1999 to 2003. Observation of flaccid paralysis due to WNV infection at a higher incidence than previously documented and the devastating mortality recorded in infected American bird species triggered concerns about a potentially enhanced virulence of this virus. Here we summarize recent observations made during the American outbreaks regarding host range and transmission modes of WNV, and discuss epidemiological aspects of the emergence of this pathogen in the new habitat.
Descriptors: disease outbreaks, West Nile fever, West Nile virus, transmission of animal diseases, arthropod vectors, birds, disease reservoirs, mortality, pregnancy complications, United States.

Busch, M.P., D.J. Wright, B. Custer, L.H. Tobler, S.L. Stramer, S.H. Kleinman, H.E. Prince, C. Bianco, G. Foster, L.R. Petersen, G. Nemo, and S.A. Glynn (2006). **West Nile virus infections projected from blood donor screening data, United States, 2003.** *Emerging Infectious Diseases* 12(3): 395-402.
Abstract: National blood donor screening for West Nile virus (WNV) RNA using minipool nucleic acid amplification testing (MP-NAT) was implemented in the United States in July 2003. We compiled national NAT yield data and performed WNV immunoglobulin M (IgM) testing in 1 WNV-epidemic region (North Dakota). State-specific MP-NAT yield, antibody seroprevalence, and the average time RNA is detectable by MP-NAT were used to estimate incident infections in 2003. WNV donor screening yielded 944 confirmed viremic donors. MP-NAT yield peaked in August with >0.5% of donations positive for WNV RNA in 4 states. Peak IgM seroprevalence for North Dakota was 5.2% in late September. The average time viremia is detectable by MP-NAT was 6.9 days (95% confidence interval [CI] 3.0-10.7). An estimated 735,000 (95% CI 322,000-1,147,000) infections occurred in 2003, with 256 (95% CI 112-401) infections per neuroinvasive case. In addition to preventing transfusion-transmitted WNV infection, donor screening can serve as a tool to monitor seasonal incidence in the general population.

Descriptors: blood donors, West Nile fever, RNA, viral blood, seasons, sensitivity and specificity, time factors, United State, West Nile virus.

Cairoli, O. (2005). **The West Nile Virus and the dialysis/transplant patient.** *Nephrology News and Issues* 19(12): 73-75.
Descriptors: disease outbreaks, kidney transplantation, renal dialysis, West Nile fever, cross infection, immunocompromised host, United States.

Cannon, A.B., J.A. Luff, A.C. Brault, N.J. MacLachlan, J.B. Case, E.N. Green, and J.E. Sykes (2006). **Acute encephalitis, polyarthritis, and myocarditis associated with West Nile virus infection in a dog.** *Journal of Veterinary Internal Medicine* 20(5): 1219-1223.
Descriptors: arthritis, dog diseases, myocarditis, West Nile fever, West Nile virus, fatal outcome, myocarditis, drug therapy, encephalitis.

Cao, N.J., C. Ranganathan, W.J. Kupsky, and J. Li (2005). **Recovery and prognosticators of paralysis in West Nile virus infection.** *Journal of the Neurological Sciences* 236(1-2): 73-80.
Abstract: Previous studies have demonstrated that lesions of the anterior horn motor neurons are the primary pathology in patients with paralysis due to West Nile virus (WNV) infection. To characterize recovery and identify prognostic factors for the recovery of paralysis, we investigated 11 patients with electrophysiology testing and muscle biopsy, and one with autopsy. We found that limb weakness was markedly asymmetric and differed between upper and lower extremities, suggesting focal or segmental involvement of the spinal cord anterior horn. This was supported by segmental depletion of spinal motor neurons at autopsy. Clinical recovery was variable during a 21-month follow-up period. To explain variability, we performed motor unit number estimation (MUNE) in six patients. MUNE values and strength were correlated in tested muscles. We also detected motor nerve terminal damages in muscle biopsies, suggesting another possible mechanism for transient weakness and variable recovery. We conclude that the type of pathological lesions may vary in paralytic WNV infection, and different degrees or combinations of motor neuron loss and motor nerve terminal changes may account for the observed degrees of weakness and recovery.
Descriptors: paralysis etiology, West Nile fever complications, adult, aged, aged, 80 and over, antigens, cd metabolism, electromyography methods, extremities physiopathology, immunohistochemistry methods, laterality , microscopy, electron methods, middle aged, motor neurons physiology, muscle weakness etiology, muscle weakness metabolism, muscle weakness pathology, muscle, skeletal metabolism, skeletal pathology, skeletal ultrastructure, neural conduction, paralysis pathology, prognosis, West Nile fever pathology.

Carson, P.J., P. Konweko, K.S. Wold, P. Mariani, S. Goli, P. Bergloff, and R.D. Crosby (2006). **Long-term clinical and neuropsychological outcomes of West Nile virus infection.** *Clinical Infectious Diseases an Official Publication of the Infectious Diseases Society of America* 43(6): 723-30.

Abstract: BACKGROUND: Since its introduction in 1999, West Nile virus has rapidly become the most common arboviral infection in North America. Little is known about the long-term clinical sequelae of West Nile virus infection. METHODS: A total of 49 patients with laboratory-confirmed West Nile virus infection were identified through state-based surveillance. Stratification for disease severity was based on hospitalization during the infection episode. Assessment occurred a mean of 13 months after diagnosis. Medical records were reviewed, and a complete neurologic examination was performed. Standardized surveys for quality of life, functional ability, fatigue, and depression were performed for all subjects. An extensive battery of neuropsychological tests was performed to assess cognitive function. RESULTS: Self-reported fatigue, memory problems, extremity weakness, word-finding difficulty, and headache were common complaints. Standardized survey data confirmed an overall sense of poor physical health, fatigue, depression, and moderate-to-severe disability in 24 (49%), 24 (49%), 12 (24%), and 4 (8%) patients, respectively. New tremor was seen or reported for 10 (20%) of the patients. Neuropsychological testing showed abnormalities of motor skills, attention, and executive functions. Univariate analysis of multiple risk factors did not identify any predictors of adverse outcomes. CONCLUSIONS: Multiple somatic complaints, tremor, and abnormalities in motor skills and executive functions are common long-term problems among patients who have had West Nile virus infection. Patients with milder illness are just as likely as patients with more-severe illness to experience adverse outcomes.

Descriptors: West Nile fever diagnosis, aged, depression, fatigue , follow up studies, memory disorders, middle aged, motor skills disorders, muscle weakness, neuropsychological tests, population surveillance, risk assessment, tremor diagnosis, West Nile fever drug therapy, West Nile fever epidemiology.

Chan, C.K., S.A. Limstrom, D.G. Tarasewicz, and S.G. Lin (2006). **Ocular features of west nile virus infection in North America: a study of 14 eyes.** *Ophthalmology* 113(9): 1539-46.

Abstract: PURPOSE: To present a case series of ocular findings of West Nile virus infection (WNVI) in North America. DESIGN: Retrospective, noncomparative, observational case series. PARTICIPANTS: All patients were referred to the authors for WNVI with ocular involvement between the years 2002 and 2005. METHODS: Chart review was performed on all participants. All participants underwent complete ophthalmic evaluation during each examination, including best-corrected Snellen

visual acuity measurement, tonometry, slit-lamp biomicroscopy of the anterior and posterior segments, and dilated fundus examination with indirect ophthalmoscopy. Fundus photography and fluorescein angiography were also performed on all eyes. Relevant ocular findings associated with WNVI were recorded and tabulated. MAIN OUTCOME MEASURES: The authors studied the characteristics, frequency, and locations of ocular lesions found in participants' eyes. RESULTS: There were 14 eyes (7 patients) with ocular West Nile virus lesions from 2002 to 2005. Average patient age was 58.4 years (range, 32-85 years). Ocular findings in descending order of frequency included multifocal chorioretinal target lesions in 12 eyes (85.7%), retinal hemorrhages in 7 eyes (50.0%), vitritis in 6 eyes (42.9%), chorioretinal linear streaks in 4 eyes (28.6%), perivascular sheathing and vasculitis in 4 eyes (28.6%), narrowed retinal vessels in 4 eyes (28.6%), disc edema in 4 eyes (28.6%), optic atrophy in 2 eyes (14.3%), vascular occlusion in 2 eyes (14.3%), and VIth nerve palsy in 1 eye (7.1%). Peripheral fundus lesions were found in all 14 eyes (100%), whereas posterior fundus lesions were found in 8 eyes (57.1%). Five patients (71.4%) were diabetic. Diabetic retinopathy was present in 7 eyes (70%). CONCLUSIONS: Multifocal choroiditis is the most common ocular manifestation associated with WNVI, with a typically benign clinical course. Less frequent ocular lesions, including optic neuritis and occlusive vasculitis, frequently induce persistent and likely permanent visual deficit. Diabetic patients and those older than 50 years of age are more vulnerable to the more severe features of WNVI, including more serious ocular lesions.
Descriptors: eye infections, viral diagnosis, West Nile fever diagnosis, West Nile virus, adult, aged, 80 and over, chorioretinitis, fluorescein angiography, middle aged, ophthalmoscopy, retrospective studies, tonometry, ocular, vision disorders, visual acuity.

Chan Tack, K.M. and G. Forrest (2006). **West nile virus meningoencephalitis and acute flaccid paralysis after infliximab treatment.** *Journal of Rheumatology* 33(1): 191-2.
Abstract: West Nile virus (WNV) can cause severe central nervous system (CNS) illnesses including meningoencephalitis (MNE) and acute flaccid paralysis (AFP). Risk factors include advanced age, immunosuppression, cancer, and diabetes. In vitro studies show that tumor necrosis factor (TNF) has anti-WNV activity and is protective against WNV infection. Anti-TNF-a monoclonal antibodies may increase susceptibility to WNV by inhibiting an adequate TNF-a response, leading to prolonged viremia, viral penetration into the CNS, and fulminant WNV-CNS disease. We describe a fatal case of WNV with MNE and AFP after infliximab therapy. During WNV outbreaks, clinicians should encourage patients receiving anti-TNF-a drugs to take appropriate preventive measures because of the risk of severe WNV-CNS disease.
Descriptors: antibodies, monoclonal adverse effects, immunocompromised host,

immunosuppressive agents adverse effects, paraplegia diagnosis, West Nile fever diagnosis, aged, viral cerebrospinal fluid, fatal outcome, paraplegia etiology, West Nile fever complications, West Nile virus, West Nile virus isolation and purification.

Chan Tack, K.M. and G. Forrest (2005). **Failure of interferon alpha-2b in a patient with West Nile virus meningoencephalitis and acute flaccid paralysis.** *Scandinavian Journal of Infectious Diseases* 37(11-12): 944-6.

Abstract: WNV infection can cause meningoencephalitis (MNE) and acute flaccid paralysis (AFP). Both syndromes are associated with high morbidity and mortality. Interferon alpha-2b (IFN-alpha-2b) inhibits WNV replication in vitro. To date, 5 patients with WNV-CNS disease have recovered neurologic function with IFN-alpha-2b. We report the first failure of IFN-alpha-2b in the treatment of WNV-MNE-AFP.

Descriptors: interferon alfa 2b therapeutic use, paraplegia drug therapy, West Nile fever drug therapy, acute disease, aged, antiviral agents therapeutic use, fatal outcome , paraplegia etiology, treatment failure, West Nile fever complications.

Cinatl, J.J., M. Michaelis, C. Fleckenstein, G. Bauer, H. Kabickova, M. Scholz, H.F. Rabenau, and H.W. Doerr (2006). **West Nile virus infection induces interferon signalling in human retinal pigment epithelial cells.** *Investigative Ophthalmology and Visual Science* 47(2): 645-51.

Abstract: PURPOSE: In addition to neuroinvasive disease, West Nile virus (WNV) infection is frequently associated with self-limiting chorioretinitis and vitritis. However, the mechanisms of ophthalmic WNV infection are rarely investigated, in part because of the lack of reliable in vitro models. The authors therefore established the first model of ocular WNV infection and investigated interaction of WNV with IFN signal-transduction mechanisms. METHODS: Human retinal pigment epithelial (RPE) cells were infected with WNV strain NY385-99 at a multiplicity of infection of 5. Virus replication was evaluated by virus titers at different times after infection. The susceptibility of RPE cells to WNV infection was confirmed by transmission electron microscopy. IFN-beta expression was assessed by quantitative real-time PCR and by measurements of antiviral activity in cell culture supernatants. IFN signaling was evaluated by phosphorylation of transducer and activator of transcription 1 and 2 (STAT1/2) proteins, with immunoblot analysis. RESULTS: RPE cells appeared to be highly sensitive to WNV infection. Maximum viral titers were found 24 hours after infection, followed by a continuous decline during the course of infection. WNV infection of RPE cells was followed by increased IFN-beta expression associated with IFN signaling and subsequent inhibition of WNV replication. CONCLUSIONS: In this study, the first cell culture model of ophthalmic WNV infection was developed and characterized in RPE cells, and the molecular mechanisms of WNV infection were studied. The data suggest that WNV induces a general

antiviral state in RPE cells. This general antiviral state correlates with WNV-induced IFN signaling in retinal cells.
Descriptors: interferon beta biosynthesis, pigment epithelium of eye virology, signal transduction physiology, West Nile virus, blotting, western, cell culture techniques, *Cercopithecus aethiops*, interferon beta genetics, microscopy, electron, transmission, models, biological, phosphorylation, pigment epithelium of eye metabolism, pigment epithelium of eye ultrastructure, reverse transcriptase polymerase chain reaction, stat1 transcription factor metabolism, stat2 transcription factor metabolism, vero cells, virus replication physiology, West Nile virus ultrastructure.

Civen, R., F. Villacorte, D.T. Robles, D.E. Dassey, C. Croker, L. Borenstein, S.M. Harvey, and L. Mascola (2006). **West Nile virus infection in the pediatric population.** *Pediatric Infectious Disease Journal* 25(1): 75-8.
Abstract: In 2004, Los Angeles County confirmed 11 cases of symptomatic West Nile virus (WNV) infections in children younger than 18 years of age. Eight had WNV fever, 2 had meningitis and 1 had encephalitis. Fever, rash, nausea and vomiting were the most prominent symptoms at presentation; median duration of illness was 7 days. Clinicians should be aware of the risk of WNV illness, confirm this diagnosis and report suspected WNV cases to their local health department.
Descriptors: West Nile fever epidemiology, adolescent, California epidemiology, child, preschool, encephalitis, viral epidemiology, exanthema, fever, meningitis, viral epidemiology, nausea, time factors, vomiting, West Nile fever physiopathology.

Cunha, B.A. (2006). **West Nile virus encephalitis: clinical diagnostic and prognostic indicators in compromised hosts.** *Clinical Infectious Diseases an Official Publication of the Infectious Diseases Society of America* 43(1): 117.
Descriptors: immunocompromised host, West Nile fever diagnosis, lymphoma, b cell complications, b cell immunology, prognosis, serologic tests, West Nile fever immunology.
Notes: Comment On: Clin Infect Dis. 2006 Mar 1;42(5):680-3.

Davis, L.E., R. DeBiasi, D.E. Goade, K.Y. Haaland, J.A. Harrington, J.B. Harnar, S.A. Pergam, M.K. King, B.K. DeMasters, and K.L. Tyler (2006). **West Nile virus neuroinvasive disease.** *Annals of Neurology* 60(3): 286-300.
Abstract: Since 1999, there have been nearly 20,000 cases of confirmed symptomatic West Nile virus (WNV) infection in the United States, and it is likely that more than 1 million people have been infected by the virus. WNV is now the most common cause of epidemic viral encephalitis in the United States, and it will likely remain an important cause of neurological disease for the foreseeable future. Clinical syndromes produced by WNV infection include asymptomatic infection, West Nile Fever, and West Nile neuroinvasive disease (WNND). WNND includes syndromes

of meningitis, encephalitis, and acute flaccid paralysis/poliomyelitis. The clinical, laboratory, and diagnostic features of these syndromes are reviewed here. Many patients with WNND have normal neuroimaging studies, but abnormalities may be present in areas including the basal ganglia, thalamus, cerebellum, and brainstem. Cerebrospinal fluid invariably shows a pleocytosis, with a predominance of neutrophils in up to half the patients. Diagnosis of WNND depends predominantly on demonstration of WNV-specific IgM antibodies in cerebrospinal fluid. Recent studies suggest that some WNV-infected patients have persistent WNV IgM serum and/or cerebrospinal fluid antibody responses, and this may require revision of current serodiagnostic criteria. Although there is no proven therapy for WNND, several vaccines and antiviral therapy with antibodies, antisense oligonucleotides, and interferon preparations are currently undergoing human clinical trials. Recovery from neurological sequelae of WNV infection including cognitive deficits and weakness may be prolonged and incomplete.

Descriptors: nervous system diseases, West Nile fever , West Nile virus.

Del Giudice, P., I. Schuffenecker, H. Zeller, M. Grelier, F. Vandenbos, P. Dellamonica, and E. Counillon (2005). **Skin manifestations of West Nile virus infection.** *Dermatology* 211(4): 348-350.

Abstract: West Nile virus (WNV) infection is a potentially lethal arbovirus infection. Many notable outbreaks have occurred during the last few years throughout the world, including Europe and the USA. The severity of the disease is mainly related to the neurological complications. A maculopapular exanthema is reported as a clinical sign of the disease. Recently an outbreak of WNV infection occurred in southern France. Three patients out of 6 had a similar skin roseola-like eruption. The cluster of 3 cases of similar febrile roseola of unexplained cause during the same week led to the diagnosis of the first WNV human outbreak in France for 40 years. Copyright 2005 S. Karger AG, Basel.

Descriptors: exanthema virology, skin diseases, viral diagnosis, West Nile fever diagnosis, adult, differential diagnosis, serologic tests.

del Giudice, P., I. Schuffenecker, H. Zeller, M. Grelier, F. Vandenbos, P. Dellamonica, and E. Counillon (2005). **Skin manifestations of West Nile virus infection**. *Dermatology* 211(4): 348-350. ISSN: 1018-8665.

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Descriptors: West Nile virus, erythema, skin manifestations, maculopapular exanthema, roseola , neurological complications.

Ding, X., X. Wu, T. Duan, M. Siirin, H. Guzman, Z. Yang, R.B. Tesh, and S.Y. Xiao (2005). **Nucleotide and amino acid changes in West Nile virus strains exhibiting renal tropism in hamsters.** *American Journal of Tropical Medicine and Hygiene* 73(4): 803-807.

Abstract: Recent studies have shown that West Nile virus (WNV) can induce an asymptomatic persistent infection in the kidneys of experimentally infected hamsters. The chronically infected rodents shed virus in their urine for up to 8 months, despite the disappearance of viremia and the development of high levels of neutralizing antibodies. WNV, like most members of the Japanese encephalitis virus complex (Flavivirus; Flaviviridae), is assumed to be mainly neurotropic; little is known about the genetic basis for its renal tropism. In this study, complete sequence analyses were done to compare four WNV isolates from the urines of persistently infected hamsters with the wild-type parent virus (NY 385-99). Nucleotide changes, ranging from 0.05% to 0.09%, were identified in all of the WNV isolates from urine; most of the changes were in coding regions, causing amino acid substitutions in the E, NS1, NS2B, and NS5 proteins. The genetic changes associated with renal tropism were also accompanied by a loss of virulence for hamsters and a change in plaque morphology.

Descriptors: amino acid substitution, kidney, West Nile fever, West Nile virus, chronic disease, animal disease models, kidney pathology, molecular sequence data, urine virology, virulence.

Ferguson, D.D., K. Gershman, A. LeBailly, and L.R. Petersen (2005). **Characteristics of the rash associated with West Nile virus fever.** *Clinical Infectious Diseases* 41(8): 1204-1207.

Abstract: We characterized rash in 15 patients with West Nile virus (WNV) fever. Generalized, maculopapular rash typically occurred on days 5-12 of illness. Dysesthesia was reported by 27% of patients, and pruritus by 33% of patients. Because the rash was nonspecific and serologic test results were often negative for WNV at presentation, convalescent-phase testing was frequently required to diagnose WNV fever.

Descriptors: dermatitis diagnosis, West Nile virus fever, maculopapular rash, symptoms.

Fonseca, K., G.D. Prince, J. Bratvold, J.D. Fox, M. Pybus, J.K. Preksaitis, and P. Tilley (2005). **West Nile virus infection and conjunctival exposure.** *Emerging Infectious Diseases* 11(10): 1648-1649. ISSN: 1080-6040.

Descriptors: bird diseases, conjunctiva, crows, occupational exposure, West Nile fever transmission, West Nile virus isolation and purification.

Francisco, A.M., C. Glaser, E. Frykman, B. Cole, M. Cheung, H. Meyers, M. Ginsberg, A. Deckert, C. Jean, and B.A. Jinadu (2006). **2004 California pediatric West Nile virus case series.** *Pediatric Infectious Disease Journal* 25(1): 81-84.
Abstract: Relatively few pediatric West Nile virus cases have been recognized in the United States since the virus was first identified in 1999. We reviewed the clinical characteristics of 23 cases in pediatric patients that occurred in California in 2004 to better understand the infection in this population.
Descriptors: West Nile fever physiopathology, children California, viral encephalitis physiopathology, immunocompromised host, viral meningitis physiopathology, muscle hypotonia, paralysis, West Nile virus isolation and purification.

Frih Ayed, M., A. Boughammoura Bouatay, F. Ben Romdhane, S. Chebel, M. Chakroun, and N. Bouzouia (2005). **Acute flaccid paralysis of the upper limbs associated with West Nile virus infection.** *European Neurology* 54(3): 159-160.
Descriptors: muscle hypotonia, paralysis, West Nile fever complications, adult, electrodiagnosis, electromyography, neural conduction physiology, neurologic examination.

Gallian, P., X. De Lamballerie, P. De Micco, and G. Andreu (2005). **Le virus West Nile: Generalites et implications en transfusion sanguine. [West Nile virus (WNV): Generalities and implications for blood transfusion]**. *Transfusion* 12(1): 11-17.
Abstract: West Nile virus (WNV) is an arbovirus (genus Flavivirus, Family Flaviviridae, transmitted to humans by mosquito bite. In most cases (80%), human infection remains asymptomatic. Severe central nervous system complications (encephalitis and meningoencephalitis) are rare. In the Old World, the virus circulation has been demonstrated in Asia, Australia, Africa, Middle East and Europe. Several outbreaks in humans have been described. Following its introduction into North America in 1999, WN virus has been responsible of a large number of human cases in USA and Canada. For the first time, viral transmission by blood products was clearly demonstrated in USA in 2002. In France, the presence of virus has been reported in the Southeastern departments since 1962. In 2003, the occurrence of humans cases at specific geographical foci urged the French National Blood Agency (etablissement francais du sang) to take preventive measures for evaluating the virus transmission risks.
Descriptors: West Nile virus, arbovirus, mosquito vectors, symptoms of infection in humans, viral transmission by blood products.
Language of Text: French; Summary in English.

Garg, S. and L.M. Jampol (2005). **Systemic and intraocular manifestations of West Nile virus infection.** *Survey of Ophthalmology* 50(1): 3-13.
Abstract: Since the introduction of West Nile virus in the Western Hemisphere

in 1999, the incidence of human infection has increased dramatically. As this virus spreads westward across the United States, ophthalmologists should be aware of this presently uncommon but important condition. Systemic features of West Nile virus infection are well characterized; however, the ophthalmic presentations are not widely known. Intraocular involvement with West Nile virus infection was first described in February 2003, and a variety of ophthalmic manifestations have since been recognized. A complete survey of the systemic and intraocular manifestations of West Nile virus infection relevant to the ophthalmologist is presented.
Descriptors: chorioretinitis, eye infections, viral etiology, optic neuritis, uveitis, West Nile fever, West Nile virus isolation and purification, United States.

Garg, S., L.M. Jampol, J.F. Wilson, I.R. Batlle, and H. Buettner (2006). **Ischemic and hemorrhagic retinal vasculitis associated with West Nile virus infection.** *Retina* 26(3): 365-367.
Descriptors: eye infections, viral virology, ischemia virology, retinal hemorrhage virology, retinal vasculitis virology, retinal vessels pathology, West Nile fever, human, viral blood antibodies, viral cerebrospinal fluid, viral diagnosis, fluorescein angiography, immunoglobulin G blood, immunoglobulin M cerebrospinal fluid, West Nile virus.

Gorsche, R. and P. Tilley (2005). **The rash of West Nile virus infection.** *Canadian Medical Association Journal* 172(11): 1440.
Descriptors: exanthema, West Nile fever, West Nile virus, Alberta, rural population, time factors.

Green, M.S., M. Weinberger, J. Ben Ezer, H. Bin, E. Mendelson, D. Gandacu, Z. Kaufman, R. Dichtiar, A. Sobel, D. Cohen, and M.Y. Chowers (2005). **Long-term Death Rates, West Nile virus epidemic, Israel, 2000.** *Emerging Infectious Diseases* 11(11): 1754-1757. ISSN: 1080-6040.
Abstract: We studied the 2-year death rate of 246 adults discharged from hospital after experiencing acute West Nile Virus infection in Israel during 2000. The age- and sex-adjusted death rates were significantly higher than in the general population. This excess was greater for men. Significant adverse prognostic factors were age, male sex, diabetes mellitus, and dementia.
Descriptors: communicable diseases, emerging mortality, disease outbreaks, West Nile fever mortality, aged humans, Israel, risk factors, survival rate, West Nile virus.

Haaland, K.Y., J. Sadek, S. Pergam, L.A. Echevarria, L.E. Davis, D. Goade, J. Harnar, R.A. Nofchissey, C.M. Sewel, and P. Ettestad (2006). **Mental status after West Nile virus infection.** *Emerging Infectious Diseases* 12(8): 1260-1262. ISSN: 1080-6040 .
Abstract: Mental status after acute West Nile virus infection has not been examined

objectively. We compared Telephone Interview for Cognitive Status scores of 116 patients with West Nile fever or West Nile neuroinvasive disease. Mental status was poorer and cognitive complaints more frequent with West Nile neuroinvasive disease (p = 0.005).
Descriptors: brain diseases, virology, mental status, West Nile fever complications, virus pathogenicity, acute disease, adult human.

Hayes, E.B. (2006). **West Nile virus disease in children.** *Pediatric Infectious Disease Journal* 25(11): 1065-1066.
Descriptors: West Nile fever diagnosis, vertical disease transmission, West Nile virus physiology.

Hayes, E.B., J.J. Sejvar, S.R. Zaki, R.S. Lanciotti, A.V. Bode, and G.L. Campbell (2005). **Virology, pathology, and clinical manifestations of West Nile virus disease.** *Emerging Infectious Diseases* 11(8): 1174-1179. ISSN: 1080-6040.
Abstract: West Nile virus (WNV) causes epidemics of febrile illness, meningitis, encephalitis, and flaccid paralysis. Since it was first detected in New York City in 1999, and through 2004, >16,000 WNV disease cases have been reported in the United States. Over the past 5 years, research on WNV disease has expanded rapidly. This review highlights new information regarding the virology, clinical manifestations, and pathology of WNV disease, which will provide a new platform for further research into diagnosis, treatment, and possible prevention of WNV through vaccination.
Descriptors: West Nile fever pathology, West Nile virus growth and development, antiviral agents, viral vaccines.

Hermanowska Szpakowicz, T., S. Grygorczuk, M. Kondrusik, J. Zajkowska, and S. Pancewicz (2006). **Zakazenie Wirusem Zachodniego Nilu. [Infections caused by West Nile virus]**. *Przeglad Epidemiologiczny* 60(1): 93-98.
Abstract: West Nile virus (WNV) is a flavivirus related to tick-borne encephalitis virus endemic in northeast of Poland. WNV circulates among wild birds, transmitted by wide range of haematophagous arthropods. Several mosquito species, especially Culex spp., can transmit WNV to humans. WNV is responsible for a broad range of clinical symptoms, from asymptomatic infection to severe encephalitis and polio-like myelitis. Elderly persons are at especially high risk of death and severe neurological complications, with mortality among patients over 65 years old with WNV-related encephalitis being as high as 35%. Warm and humid climate favors the spread of WNV, however, it can also be effectively transmitted in moderate climate zones. During the last decade a worldwide spread of WNV was observed, with establishment of new enzootic foci and growing number of human cases. WNV seems to be already present in several European countries, including Poland. We describe detec-

tion of anti-WNV IgM antibodies in serum of patient with acute febrile infection, which may constitute the first case of confirmed WNV infection in Poland.
Descriptors: viral antibodies, birds, climate, disease outbreaks, disease vectors, immunoglobulin M immunology, Poland, seasons, West Nile fever transmission, world health.
Language of Text: Polish.

Hochman, J.A. (2005). **Misinterpretation of liver-function tests and West Nile virus infection in children.** *Pediatrics* 115(5): 1445; Author Reply 1445-1446.
Descriptors: diagnostic errors, liver failure, acute diagnosis, transaminases blood, West Nile fever complications, child, ischemia complications, acute etiology, liver function tests.
Notes: Comment On: Pediatrics. 2004 Dec;114(6):1673-5.

Hoekstra, C. (2005). **West Nile virus: a challenge for transplant programs.** *Progress in Transplantation* 15(4): 397-400.
Abstract: West Nile virus is a new challenge for transplant programs worldwide. It is a mosquito-borne disease, which has become increasingly prevalent in North America since it was first recognized in New York in 1999. A review of a case study and the literature shows that the morbidity and mortality associated with West Nile virus infection and transplant recipients are alarmingly high. Treatment options are limited because of transplant programs' limited experience in working with this virus. Transplant programs must develop action plans for education on West Nile virus and its prevention to decrease the risk of infection among their transplant recipients.
Descriptors: infection control, organ transplantation adverse effects, West Nile fever prevention and control, immunocompromised host, lung transplantation, humans, North America, transplant recipients.

Huhn, G.D. and M.S. Dworkin (2006). **Rash as a prognostic factor in West Nile virus disease.** *Clinical Infectious Diseases an Official Publication of the Infectious Diseases Society of America* 43(3): 388-389.
Descriptors: exanthema etiology, West Nile fever complications, prognosis, rash, infections.
Notes: Comment On: Clin Infect Dis. 2006 May 1;42(9):1234-40.

Julander, J.G., Q.A. Winger, L.F. Rickords, P.Y. Shi, M. Tilgner, I. Binduga Gajewska, R.W. Sidwell, and J.D. Morrey (2006). **West Nile virus infection of the placenta.** *Virology* 347(1): 175-182. ISSN: 0042-6822.
Abstract: Intrauterine infection of fetuses with West Nile virus (WNV) has been implicated in cases of women infected during pregnancy. Infection of timed-pregnant mice on 5.5, 7.5, and 9.5 days post-coitus (dpc) resulted in fetal infection. Infection

of dams on 11.5 and 14.5 dpc resulted in little and no fetal infection, respectively. Pre-implantation embryos in culture were also infected with WNV after the blastocyst stage and the formation of trophectoderm. Green fluorescent protein (GFP) expression was observed in a trophoblast stem (TS) cell line after infection with a GFP-expressing WNV construct. However, no fluorescence was observed in differentiated trophoblast giant cell (TGC) cultures. GFP fluorescence was present in TGC cultures if infected TS cells were induced to differentiate. These results suggest that embryos are susceptible to WNV infection after the formation of the trophectoderm around 3.5 dpc through the formation of the functional placenta around 10.5 dpc.
Descriptors: placenta diseases, West Nile fever, West Nile virus, blastocyst, cell line, green fluorescent proteins, mice, recombinant proteins, stem cells, trophoblasts.

Khairallah, M., S. Ben Yahia, S. Attia, B. Jelliti, S. Zaouali, and A. Ladjimi (2006). **Severe ischemic maculopathy in a patient with West Nile virus infection.** *Ophthalmic Surgery, Lasers and Imaging* 37(3): 240-242.
Abstract: A 60-year-old man with diabetes mellitus had a sudden decrease in vision in his right eye 3 weeks after confirmed West Nile virus infection. Visual acuity in the right eye was 20/400. Fundus examination showed bilateral multifocal chorioretinitis, which was associated with proliferative diabetic retinopathy in the right eye and severe nonproliferative diabetic retinopathy in the left eye. There were deep, dense retinal hemorrhages, retinal opacification, and retinal arterial sheathing in the macula of the right eye. Fluorescein angiography revealed extensive capillary nonperfusion in the macular area of the right eye. Six months later, vision remained unchanged and a choroidal neovascularization developed over a chorioretinal scar in the same eye.
Descriptors: chorioretinitis, eye infections, retinal vessels, pathology, West Nile fever complications, chorioretinitis diagnosis, diabetic retinopathy diagnosis, fluorescein angiography, ischemia diagnosis, laser coagulation, vision disorders.

Khairallah, M., S. Ben Yahia, S. Attia, S. Zaouali, B. Jelliti, S. Jenzri, A. Ladjimi, and R. Messaoud (2006). **Indocyanine green angiographic features in multifocal chorioretinitis associated with West Nile virus infection.** *Retina* 26(3): 358-359.
Descriptors: chorioretinitis, diagnostic use of coloring agents, eye infections, indocyanine green, West Nile fever diagnosis, viral blood antibodies, chorioretinitis, viral virology, fluorescein angiography, immunoglobulin M blood.

Khosla, J.S., M.J. Edelman, N. Kennedy, and S.G. Reich (2005). **West Nile virus presenting as opsoclonus-myoclonus cerebellar ataxia.** *Neurology* 64(6): 1095.
Descriptors: non-small cell lung carcinoma complications, cerebellar ataxia, paraneoplastic syndromes, nervous system, West Nile fever, fatal outcome, immunoglobulin M blood, paraneoplastic syndromes, nervous system diagnosis.

Koevary, S.B. (2005). **Ocular involvement in patients infected by the West Nile virus.** *Optometry St. Louis, Mo.* 76(10): 609-612.

Abstract: West Nile virus (WNV), a mosquito-borne RNA virus for which there is no treatment, began emerging as a threat to health in the United States in 1999. Since then, its frequency and apparent clinical severity have increased. Patients with severe disease may experience ocular complications that include pain, vitreous inflammation, nonrelapsing chorioretinitis, retinal vasculitis, chorioretinal scarring, optic neuritis, and retinal hemorrhages. Age appears to be a risk factor for the development of ocular symptoms. Most patients with ocular involvement report floaters or decreased vision. Many ocular inflammatory conditions with an associated underlying systemic cause often present with chorioretinitis. However, the characteristic distribution and linear array or scattered pattern of the chorioretinal lesions seen in WNV is distinctive. The foregoing notwithstanding, other multifocal choroidopathies must be considered including syphilis, ocular histoplasmosis, multifocal choroiditis, tuberculosis, and sarcoid. Although topical steroids were reported to resolve WNV-induced uveitis and its associated keratic precipitates, most cases resolved irrespective of treatment, and relapses were uncommon. It is important for the eye care professional to be alert to the possible presence of WNV, particularly in older patients who present with ocular symptoms during mosquito season. Thus, a thorough ocular evaluation should include a dilated fundus examination and, when indicated, fluorescein angiography should be performed in patients suspected of being infected with WNV.

Descriptors: chorioretinitis, eye infections, viral etiology, optic neuritis, retinal hemorrhage, retinal vasculitis, West Nile fever, complications, age factors, risk factors.

Kraushaar, G., R. Patel, and G.W. Stoneham (2005). **West Nile Virus: A case report with flaccid paralysis and cervical spinal cord: MR imaging findings.** *American Journal of Neuroradiology* 26(1): 26-29.

Abstract: We present a case of serologically proved West Nile virus (WNV) flaccid paralysis of the right upper extremity. Radiologic correlation revealed striking T2 hyperintensities in the anterior horns of the cervical spinal cord, similar to those seen in cases of poliomyelitis. Recognition of the MR imaging findings in cases of WNV flaccid paralysis can provide early evidence of infection.

Descriptors: computer assisted image processing, magnetic resonance imaging, spinal cord paralysis, West Nile fever, West Nile virus, anterior horn cells, arm innervation, follow-up studies, motor neuron disease, muscle hypotonia, muscle weakness.

LaBeaud, A.D., M.V. Lisgaris, C.H. King, and A.M. Mandalakas (2006). **Pediatric West Nile virus infection: Neurologic disease presentations during the 2002 epidemic in Cuyahoga County, Ohio.** *Pediatric Infectious Disease Journal* 25(8): 751-753.

Abstract: Knowledge is currently limited about West Nile virus (WNV) infection

and its sequelae among children. Available evidence suggests that when compared with adults, children less than 18 years old can be at high risk for WNV exposure and infection yet manifest a lower risk for WNV-related morbidity and mortality. We detail clinical features of pediatric West Nile-associated neurologic disease (WNND) epidemic cases in Cuyahoga County during 2002. We present a structured review of pediatric and adult WNND cases hospitalized in Cuyahoga County, Ohio. During the epidemic, 5 children were hospitalized with confirmed WNND (estimated incidence = 1.4/100,000 children 5-17 years old at risk). Compared with adults, children had shorter hospitalization (mean, 4.6 versus 12.3 days), fewer neurologic symptoms, better neurologic outcomes, and lower mortality (0% versus 5.3%). Cerebrospinal fluid results were similar. When compared with adults, children had significantly lower rates of WNND. Children are at a decreased risk for severe WNV and less likely to present with neurologic signs or suffer neurologic sequelae.
Descriptors: viral encephalitis epidemiology, viral meningitis virology, West Nile fever epidemiology, Ohio, children.

Leis, A.A. and D.S. Stokic (2005). **Long-term assessment of motor function after severe West Nile virus infection**. *Neurology* 64(6, Suppl. 1): A248. ISSN: 0028-3878.
Descriptors: West Nile virus infection, long term assessment of motor function, muscle weakness, nervous system disease, myelitis, diagnostic techniques.
Notes: Meeting Information: 57th Annual Meeting of the American Academy of Neurology, Miami Beach, Florida, USA; April 9 -19, 2005.

Marciniak, C. and E.L. Rosenfeld (2005). **Serial electrodiagnostic studies in West Nile virus-associated acute flaccid paralysis.** *American Journal of Physical Medicine and Rehabilitation* 84(11): 904-910.
Abstract: A man in his 70s presented for acute rehabilitation with severe acute flaccid asymmetric weakness in both lower limbs. Cerebrospinal fluid and serum immunoglobulin M titers were positive for West Nile virus. Electrodiagnostic studies demonstrated severe diffuse motor axonopathy consistent with an anterior myelitis. Electrodiagnostic and clinical improvements were monitored. Electrodiagnostic testing at 6 and 18 mos demonstrated continuing reinnervation; nascent voluntary motor unit action potentials were first noted proximally and, at 18 mos, distally in the left lower limb, including muscles in which motor unit potentials were not initially noted. Corresponding clinical improvements, though slow, were demonstrated even at 1(1/2) yrs after onset. Thus, motoric changes after West Nile virus-associated anterior myelitis need to be monitored over a prolonged time period to allow accurate assessment of prognosis for recovery in rehabilitation programs.
Descriptors: myelitis, West Nile fever complications , West Nile virus isolation and purification, acute disease, viral cerebrospinal fluid, muscle weakness, skeletal muscle, paraplegia rehabilitation, time factors.

Mawhorter, S.D., A. Sierk, S.M. Staugaitis, R.K. Avery, R. Sobecks, R.A. Prayson, G.W. Procop, and B. Yen Lieberman (2005). **Fatal West Nile Virus infection after rituximab/fludarabine--induced remission for non-Hodgkin's lymphoma.** *Clinical Lymphoma and Myeloma* 6(3): 248-250.

Abstract: West Nile virus (WNV) infections are potentially life threatening in immunocompromised hosts. Currently, the best diagnostic test is serology. Reverse-transcriptase polymerase chain reaction (RT-PCR) testing has a role, but, because WNV is a cell-associated neurotropic virus, RT-PCR results are frequently negative even in cases of active infection. We present a case in which serology results were persistently negative because the patient was immunocompromised following lymphoma treatment. The role of humoral immunity in resolution of WNV is also discussed.

Descriptors: monoclonal antibodies, administration and dosage, antineoplastic agents, follicular lymphoma, West Nile fever, West Nile virus fatal outcome , lymphoma, follicular drug therapy, remission , vidarabine administration and dosage.

McBride, W., K.R. Gill, and L. Wiviott (2006). **West Nile Virus infection with hearing loss.** *Journal of Infection* 53(5): E203-E205.

Abstract: Since the New York outbreak in 1999, West Nile Virus (WNV) has spread across the United States and North America. The spectrum of disease may range from asymptomatic to debilitating neuroinvasive. We present a case of a woman with myasthenia gravis on immunosuppressive therapy who developed WNV meningoencephalitis presenting as flaccid paralysis and severe bilateral hearing loss. To our knowledge this is the first reported case of WNV-associated hearing loss, and the first reported in a patient with previously diagnosed myasthenia gravis. Of epidemiologic interest was that her mother, 81 years old and in previous good health, developed a WNV infection shortly before the patient's infection.

Descriptors: hearing loss, immunocompromised host, West Nile fever, disease transmission, immunosuppressive agents, therapeutic use, myasthenia gravis, WNV meningoencephalitis.

Michaelson, P.G. and E.A. Mair (2005). **West Nile virus: A primer for the otolaryngologist.** *Otolaryngology - Head and Neck Surgery* 132(3): 347-352.

Abstract: BACKGROUND: Since recognition in the United States with a 1999 New York City epidemic, West Nile virus has enduringly migrated westward, leaving few states unaffected. Infection rates are rising at an alarming rate, doubling every year since introduction, with more than 9800 cases in 2003 alone and more than 260 deaths. Patients may present with myriad symptoms including a maculopapular rash that affects the face and trunk and diffuse lymphadenopathy, both of which may result in the initial consultation of the otolaryngologist. We review the clinical history of West Nile virus and its epidemiology, laboratory findings, and variable clinical presentation, with an emphasis on otolaryngologic manifestations. STUDY

DESIGN AND SETTING COMPREHENSIVE: review of the literature over the past 50 years with an emphasis on what the present-day otolaryngologist needs to know concerning West Nile virus. Clinical manifestations of the head and neck such as encephalitis, meningitis, maculopapular rash, lymphadenopathy and dysphagia are discussed. RESULTS: To date, there are no articles in the otolaryngology literature discussing West Nile virus. These patients may present initially to multiple providers in diverse specialties because of multifarious initial signs and symptoms. The otolaryngologist must be educated on this quickly growing affliction and practice with a high index of suspicion. CONCLUSIONS: In this article we describe the clinical manifestations of West Nile virus, with an emphasis on the otolaryngologic manifestations. The otolaryngologist must become educated about this entity to facilitate preventative measures, adequately treat, and assist other providers in hopeful control and potential eradication of this infectious threat.

Descriptors: West Nile fever diagnosis, West Nile fever epidemiology, otolaryngology.

Montgomery, S.P., J.A. Brown, M. Kuehnert, T.L. Smith, N. Crall, R.S. Lanciotti, A. Macedo de Oliveira, T. Boo, and A.A. Marfin (2006). **Transfusion-associated transmission of West Nile virus, United States 2003 through 2005.** *Transfusion* 46(12): 2038-2046.

Abstract: BACKGROUND: National blood donation screening for West Nile virus (WNV) started in June 2003, after the documentation of WNV transfusion-associated transmission (TAT) in 2002. STUDY DESIGN AND METHODS: Blood donations were screened with investigational nucleic acid amplification assays in minipool formats. Blood collection agencies (BCAs) reported screening results to state and local public health authorities. Donor test results and demographic information were forwarded to CDC via ArboNET, the national electronic arbovirus surveillance system. State health departments and BCAs also reported suspect WNV TATs to CDC, which investigated these reports to confirm WNV infection in blood transfusion recipients in the absence of likely mosquito exposure. RESULTS: During 2003 to 2005, a total of 1,425 presumptive viremic donors were reported to CDC from 41 states. Of 36 investigations of suspected WNV TAT in 2003, 6 cases were documented. Estimated viremia levels were available for donations implicated in four TAT cases; the median estimated viremia was 0.1 plaque-forming units (PFUs) per mL (range, 0.06-0.50 PFU/mL; 1 PFU equals approximately 400 copies/mL). CONCLUSIONS: National blood screening for WNV identified and removed more than 1,400 potentially infectious blood donations in 2003 through 2005. Despite the success of screening in 2003, some residual WNV TAT risk remained due to donations containing very low levels of virus. Screening algorithms employing selected individual-donation testing were designed to address this residual risk and were fully

implemented in 2004 and 2005. Continued vigilance for TAT will evaluate the effectiveness of these strategies.
Descriptors: blood donation, West Nile virus, transfusion transmission, blood collection, viremic donors, surveillance system , screening.

Moon, T.D., A. Nadimpalli, E.B. Martin, M.A. Ortiz, and R.B. Van Dyke (2005). **Balance and gait abnormalities of a child with West Nile virus infection.** *Pediatric Infectious Disease Journal* 24(6): 568-570.
Abstract: We describe a case of a previously healthy 2-year-old female patient with rash, fever and vomiting for 10 days who presented for medical attention with acute profound balance and gait disturbances and intentional movement dysmetria. West Nile virus-specific IgM and IgG antibodies were detected in the patient's cerebrospinal fluid.
Descriptors: movement disorders, West Nile fever physiopathology, West Nile virus, children, gait, immunoglobulin G cerebrospinal fluid, immunoglobulin M cerebrospinal fluid, musculoskeletal equilibrium.

Murray, K., S. Baraniuk, M. Resnick, R. Arafat, C. Kilborn, K. Cain, R. Shallenberger, T.L. York, D. Martinez, J.S. Hellums, D. Hellums, M. Malkoff, N. Elgawley, W. McNeely, S.A. Khuwaja, and R.B. Tesh (2006). **Risk factors for encephalitis and death from West Nile virus infection**. *Epidemiology and Infection* 134(6): 1325-1332.
Abstract: We conducted a nested case-control study to determine potential risk factors for developing encephalitis from West Nile virus (WNV) infection. Retrospective medical chart reviews were completed for 172 confirmed WNV cases hospitalized in Houston between 2002 and 2004. Of these cases, 113 had encephalitis, including 17 deaths, 47 had meningitis, and 12 were fever cases; 67% were male. Homeless patients were more likely to be hospitalized from WNV compared to the general population. A multiple logistic regression model identified age [odds ratio (OR) 1.1, P<0.001], history of hypertension, including those cases taking hypertension-inducing drugs (OR 2.9, P=0.012), and history of cardiovascular disease (OR 3.5, P=0.061) as independent risk factors for developing encephalitis from WNV infection. After adjusting for age, race/ethnicity (being black) (OR 12.0, P<0.001), chronic renal disease (OR 10.6, P<0.001), hepatitis C virus (OR 23.1, P=0.0013), and immunosuppression (OR 3.9, P=0.033) were identified as risk factors for death from WNV infection.
Descriptors: West Nile virus, risk factors, encephalitis control study, fever, meningitis, age, immunosuppression.

Murtagh, B., Y. Wadia, G. Messner, P. Allison, Y. Harati, and R. Delgado (2005). **West Nile virus infection after cardiac transplantation.** *Journal of Heart and Lung Transplantation* 24(6): 774-776.

Abstract: West Nile virus is a mosquito-borne RNA Flavivirus infection transmitted to humans and other vertebrates, mainly by the Culex species of mosquito. Since the mid-1990s, the frequency and apparent clinical severity of West Nile virus outbreaks have increased. We report the case of a patient who developed West Nile virus encephalitis shortly after undergoing cardiac transplantation. Clinicians should be aware of the possibility of West Nile virus infection in transplant recipients and in patients receiving blood transfusions.

Descriptors: blood transfusion, adverse effects, heart transplantation, West Nile fever etiology, middle aged humans.

Myers, J.P., T.K. Leveque, and M.W. Johnson (2005). **Extensive chorioretinitis and severe vision loss associated with West Nile virus meningoencephalitis.** *Archives of Ophthalmology* 123(12): 1754-1756.

Descriptors: blindness, chorioretinitis, viral eye infections, West Nile fever complications, West Nile virus isolation and purification, electroretinography, fluorescein angiography, meningoencephalitis.

O'Leary, D.R., S. Kuhn, K.L. Kniss, A.F. Hinckley, S.A. Rasmussen, W.J. Pape, L.K. Kightlinger, B.D. Beecham, T.K. Miller, D.F. Neitzel, S.R. Michaels, G.L. Campbell, R.S. Lanciotti, and E.B. Hayes (2006). **Birth outcomes following West Nile Virus infection of pregnant women in the United States: 2003-2004.** *Pediatrics* 117(3): E537-E545.

Abstract: BACKGROUND: Congenital West Nile virus (WNV) infection was first described in a single case in 2002. The proportion of maternal WNV infections resulting in congenital infection and clinical consequences of such infections are unknown. METHODS: In 2003 and 2004, women in the United States who acquired WNV infection during pregnancy were reported to the Centers for Disease Control and Prevention by state health departments. Data on pregnancy outcomes were collected. One of the maternal WNV infections was identified retrospectively after the infant was born. Maternal sera, placenta, umbilical cord tissue, and cord serum were tested for WNV infection by using serologic assays and reverse-transcription polymerase chain reaction. Infant health was assessed at delivery and through 12 months of age. RESULTS: Seventy-seven women infected with WNV during pregnancy were clinically followed in 16 states. A total of 71 women delivered 72 live infants; 4 women had miscarriages, and 2 had elective abortions. Of the 72 live infants, 67 were born at term, and 4 were preterm; gestational age was unknown for 1. Of 55 live infants from whom cord serum was available, 54 tested negative for anti-WNV IgM. One infant born with umbilical hernia and skin tags had anti-WNV

IgM in cord serum but not in peripheral serum at age 1 month. An infant who had no anti-WNV IgM in cord blood, but whose mother had WNV illness 6 days prepartum, developed WNV meningitis at age 10 days. Another infant, whose mother had acute WNV illness at delivery, was born with a rash and coarctation of the aorta and had anti-WNV IgM in serum at 1 month of age; cord serum was not available. A fourth infant, whose mother had onset of WNV illness 3 weeks prepartum that was not diagnosed until after delivery, had WNV encephalitis and underlying lissencephaly detected at age 17 days and subsequently died; cord serum was not available. The following major malformations were noted among live-born infants: aortic coarctation (n = 1); cleft palate (n = 1); Down syndrome (n = 1); lissencephaly (n = 1); microcephaly (n = 2); and polydactyly (n = 1). One infant had glycogen storage disease type 1. Abnormal growth was noted in 8 infants. CONCLUSIONS: Of 72 infants followed to date in 2003 and 2004, almost all seemed normal, and none had conclusive laboratory evidence of congenital WNV infection. Three infants had WNV infection that could have been congenitally acquired. Seven infants had major malformations, but only 3 of these had defects that could have been caused by maternal WNV infection based on the timing of the infections and the sensitive developmental period for the specific malformations, and none had any conclusive evidence of WNV etiology. However, the sensitivity and specificity of IgM testing of cord blood to detect congenital WNV infection are currently unknown, and congenital WNV infection among newborns with IgM-negative serology cannot be ruled out. Prospective studies comparing pregnancy outcomes of WNV-infected and -uninfected women are needed to better define the outcomes of WNV infection during pregnancy.

Descriptors: pregnancy complications, West Nile fever, children, vertical disease transmission, fetal blood, immunoglobulin M analysis , human milk, viral RNA analysis, West Nile virus isolation and purification.

Notes: Comment In: Pediatrics. 2006 Mar;117(3):936-9.

Orton, S.L., S.L. Stramer, and R.Y. Dodd (2006). **Self-reported symptoms associated with West Nile virus infection in RNA-positive blood donors.** *Transfusion* 46(2): 272-277.

Abstract: BACKGROUND: In 2003, West Nile virus (WNV) nucleic acid amplification testing (NAT) was implemented to detect potentially infected donors. Of more than 5.3 million donations screened prospectively by the American Red Cross during the epidemic periods of 2003 and 2004, 974 were NAT-reactive and 519 confirmed-positive. A subset of both the confirmed-positive and the false-positive groups was assessed for demographic characteristics, symptoms, and symptom reporting relative to date of donation. STUDY DESIGN AND METHODS: All donors with initial WNV NAT-reactive results were invited to participate in a study that included

a demographic, symptom, and date-of-symptom questionnaire. WNV confirmed-positive cases were compared to false-positive controls for comparison of frequency of symptom reporting before, on the day of, and after donation. RESULTS: Enrolled cases and controls were similar in all characteristics except cases were more likely to live in rural areas. Symptoms were reported by 61 percent of cases versus 20 percent of controls, with 74 percent of symptoms reported by cases within the 14 days after donation. The frequency of headache and fever reported together in the 7 days before donation was not significantly different between cases and controls; only the individual frequencies of headache, eye pain, and new rash during this time were significantly different. The most commonly reported symptoms, after adjustment for symptom reporting by controls, were headache, new rash, and generalized weakness; these symptoms were reported by 25 percent of cases. CONCLUSIONS: The demographic characteristics of infected donors reflected the rural nature of the 2003 to 2004 WNV epidemics. This study suggests that asking donors about predonation headache and fever had no detectable contribution to blood safety.
Descriptors: blood donors, West Nile fever diagnosis, West Nile virus, fever, headache, logistic models, mass screening, middle aged humans, multivariate analysis, viral RNA.

Ou, A.C. and R.C. Ratard (2005). **One-year sequelae in patients with West Nile Virus encephalitis and meningitis in Louisiana.** *Journal of the Louisiana State Medical Society* 157(1): 42-46.

Abstract: West Nile Virus (WNV) infection can be severe and complicated by neuroinvasive disease, such as meningitis and encephalitis. Not much is known about the one-year sequelae following a complicated WNV infection. From July to December 2002, the first large outbreak of WNV in the Southern United States occurred in Louisiana. This epidemic resulted in 329 cases of WNV infection including 125 cases of West Nile fever and 204 cases complicated by meningitis and encephalitis. One year later, during the fall of 2003, a telephone survey was conducted to understand the one-year effects of severe WNV infections. WNV case fatality rate was 19.6%. Death certificates of all the deceased were reviewed to verify the mortality rate from WNV infection. The telephone survey revealed that one-year sequelae from severe WNV infections are common and can affect the body and mind. In addition, it can change a person's perception of the state of their health.
Descriptors: meningitis, West Nile fever, West Nile virus, viral antibodies, disease outbreaks, enzyme linked immunosorbent assay, Louisiana, retrospective studies, survival rate.

Paisley, J.E., A.F. Hinckley, D.R. O'Leary, W.C. Kramer, R.S. Lanciotti, G.L. Campbell, and E.B. Hayes (2006). **West Nile virus infection among pregnant women in a northern Colorado community, 2003 to 2004.** *Pediatrics* 117(3): 814-820.

Abstract: OBJECTIVE: Since West Nile virus (WNV) was first detected in New York in 1999, it has spread across North America and become a major public health concern. In 2002, the first documented case of intrauterine WNV infection was reported, involving an infant with severe brain abnormalities. To determine the frequencies of WNV infections during pregnancy and of intrauterine WNV infections, we measured WNV-specific antibodies in cord blood from infant deliveries after a community-wide epidemic of WNV disease. METHODS: Five hundred sixty-six pregnant women who presented to Poudre Valley Hospital (Fort Collins, CO) for delivery between September 2003 and May 2004 provided demographic and health history data through self-administered questionnaires and hospital admission records. Umbilical cord blood was collected from 549 infants and screened for WNV-specific IgM and IgG antibodies with enzyme-linked immunosorbent assays, with confirmation by plaque-reduction neutralization tests. Newborn growth parameters, Apgar scores, and hearing test results were recorded. RESULTS: Four percent (95% confidence interval: 2.4-5.7%) of cord blood samples tested positive for WNV-specific IgG antibodies. No cord blood samples were positive for WNV-specific IgM antibodies. There were no significant differences between infants of seropositive and seronegative mothers with respect to any of the growth parameters or outcomes measured. CONCLUSIONS: Intrauterine WNV infections seemed to be infrequent. In our study, WNV infection during pregnancy did not seem to affect adversely infant health at birth. Larger prospective studies are necessary to measure more completely the effects of maternal WNV infection on pregnancy and infant health outcomes.
Descriptors: pregnancy complications, infectious epidemiology, West Nile fever, Colorado, fetal blood, immunoglobulin A blood, immunoglobulin G blood, children, seroepidemiologic studies, West Nile virus isolation and purification.

Pergam, S.A., C.E. Delong, L. Echevarria, G. Scully, and D.E. Goade (2006). **Myocarditis in west nile virus infection.** *American Journal of Tropical Medicine and Hygiene* 75(6): 1232-1233. ISSN: 0002-9637.

Abstract: West Nile virus (WNV) myocarditis has been documented pathologically in birds and mammals but has rarely been reported in human clinical syndromes. We describe myocarditis associated with WNV.
Descriptors: West Nile virus, myocarditis, birds, mammals, clinical syndromes, humans.

Petropoulou, K.A., S.M. Gordon, R.A. Prayson, and P.M. Ruggierri (2005). **West Nile virus meningoencephalitis: MR imaging findings.** *American Journal of Neuroradiology* 26(8): 1986-1995.

Abstract: BACKGROUND AND PURPOSE: Reports of MR imaging in West Nile virus (WNV) meningoencephalomyelitis are few and the described findings limited. The purpose of this study was to review the spectrum of MR imaging find-

ings for WNV meningoencephalomyelitis and investigate whether any of the findings correlates with clinical presentation of flaccid paralysis. METHODS: We reviewed the MR imaging findings of 17 patients with confirmed WNV encephalitis and/or myelitis. MR imaging brain studies were evaluated for location of signal intensity abnormalities, edema, hydrocephalus, or abnormal enhancement. MR imaging spine studies were evaluated for signal intensity abnormalities in cord and/or enhancement. RESULTS: Retrospective review of the MR imaging studies of 17 patients was performed by 2 neuroradiologists. Eleven of 16 brain MR images demonstrated abnormalities. Eight (50%) patients had abnormal studies related to meningoencephalitis. All 8 patients had abnormal findings in the deep gray matter and/or brain stem; 2 had additional white matter abnormalities. Three patients with abnormal MR studies of the spine had extremity weakness on examination. The imaging findings included abnormal signal intensity more pronounced in the ventral horns and/or enhancement around the conus medullaris and cauda equina. One patient had additional abnormalities in the pons. CONCLUSION: Abnormal MR imaging findings in patients with WNV meningoencephalomyelitis are nonspecific but not uncommon. Anatomic areas commonly affected are basal ganglia, thalami, mesial temporal structures, brain stem, and cerebellum. Extremity weakness or flaccid paralysis corresponds to spinal cord/cauda equina abnormalities.
Descriptors: magnetic resonance imaging, meningoencephalitis, West Nile fever, brain pathology, extremities, muscle weakness, paraplegia, retrospective studies, spinal cord, humans.

Prasad, S., M.J. Brown, and S.L. Galetta (2006). **Transient downbeat nystagmus from West Nile virus encephalomyelitis.** *Neurology* 66(10): 1599-1600 .
Descriptors: pathologic nystagmus etiology, West Nile fever, brain stem, electromyography, ocular motility disorders, quadriplegia, abnormal reflex.

Saad, M., S. Youssef, D. Kirschke, M. Shubair, D. Haddadin, J. Myers, and J. Moorman (2005). **Acute flaccid paralysis: The spectrum of a newly recognized complication of West Nile virus infection.** *Journal of Infection* 51(2): 120-127.
Abstract: OBJECTIVES: Acute flaccid paralysis (AFP) has recently emerged as a major central nervous system complication associated with West Nile virus (WNV) infection. The spectrum of clinical presentations of AFP in WNV infection and its sequelae have not been well-studied. METHODS: We describe three patients with AFP due to WNV infection and review the clinical presentations of 56 patients with this complication derived from published studies. RESULTS: Patients with AFP and WNV presented with a spectrum of illness ranging from single extremity paralysis to quadriparalysis with cranial nerve involvement. Patients commonly developed respiratory failure (54%) and bladder dysfunction (22%). While fever was nearly universal (92%), signs of meningismus were less common (17%). Cerebrospinal fluid

(CSF) analysis generally revealed a modest pleocytosis, and imaging studies were not diagnositic. Persistent neurologic impairment occurred in all survivors; overall mortality rate was high (22%) and was associated with both the extent of paralysis and advanced age. CONCLUSION: AFP in the setting of WNV is associated with significant mortality and long-term morbidity.

Descriptors: acute flaccid paralysis, West Nile fever complications, viral blood antibodies, muscle hypotonia, quadriplegia, West Nile virus.

Schafernak, K.T. and E.H. Bigio (2006). **West Nile virus encephalomyelitis with polio-like paralysis & nigral degeneration.** *Canadian Journal of Neurological Sciences* 33(4): 407-410.

Abstract: BACKGROUND: Patients infected with West Nile virus (WNV) may develop acute neurologic disease, which can be severe or even fatal, including WNV meningitis, encephalitis, and an irreversible acute flaccid paralysis or poliomyelitis-like syndrome. Movement disorders have also been described. REPORT: We report combined neuronal loss, gliosis, and neurofibrillary tangle formation in the substantia nigra of a 41-year-old man with a history of WNV encephalomyelitis and poliomyelitis-like paralysis. CONCLUSIONS: Clinically our patient did not display parkinsonism, however, it is interesting to speculate whether, in the absence of the residual subacute poliomyelitis-like syndrome, the neuropathologic findings could have eventually evolved clinically into WNV-associated postencephalitic parkinsonism.

Descriptors: West Nile virus infection, neurologic disease, meningitis, encephalitis, flaccid paralysis, movement disorders, poliomyelitis like paralysis.

Sejvar, J.J., A.V. Bode, A.A. Marfin, G.L. Campbell, D. Ewing, M. Mazowiecki, P.V. Pavot, J. Schmitt, J. Pape, B.J. Biggerstaff, and L.R. Petersen (2005). **West Nile virus-associated flaccid paralysis.** *Emerging Infectious Diseases* 11(7): 1021-1027. ISSN: 1080-6040.

Abstract: The causes and frequency of acute paralysis and respiratory failure with West Nile virus (WNV) infection are incompletely understood. During the summer and fall of 2003, we conducted a prospective, population-based study among residents of a 3-county area in Colorado, United States, with developing WNV-associated paralysis. Thirty-two patients with developing paralysis and acute WNV infection were identified. Causes included a poliomyelitislike syndrome in 27 (84%) patients and a Guillain-Barre-like syndrome in 4 (13%); 1 had brachial plexus involvement alone. The incidence of poliomyelitislike syndrome was 3.7/100,000. Twelve patients (38%), including 1 with Guillain-Barre-like syndrome, had acute respiratory failure that required endotracheal intubation. At 4 months, 3 patients with respiratory failure died, 2 remained intubated, 25 showed various degrees of improvement, and 2 were lost to followup. A poliomyelitislike syndrome likely

involving spinal anterior horn cells is the most common mechanism of WNV-associated paralysis and is associated with significant short- and long-term illness and death.
Descriptors: muscle hypotonia, paralysis, West Nile fever complications, humans, guillain barre syndrome, respiratory insufficiency virology, cerebrospinal fluid.

Sejvar, J.J., A.V. Bode, A.A. Marfin, G.L. Campbell, J. Pape, B.J. Biggerstaff, and L.R. Petersen (2006). **West Nile Virus-associated flaccid paralysis outcome.** *Emerging Infectious Diseases* 12(3): 514-516. ISSN: 1080-6040 .
Abstract: We report 1-year follow-up data from a longitudinal prospective cohort study of patients with West Nile virus-associated paralysis. As in the 4-month follow-up, a variety of recovery patterns were observed, but persistent weakness was frequent. Respiratory involvement was associated with considerable illness and death.
Descriptors: muscle hypotonia, paralysis, West Nile fever, follow up studies, artificial respiration, respiratory insufficiency, mortality.

Skupski, D.W., G.S. Eglinton, A.D. Fine, E.B. Hayes, and D.R. O'Leary (2006). **West Nile virus during pregnancy: A case study of early second trimester maternal infection.** *Fetal Diagnosis and Therapy* 21(3): 293-295.
Abstract: A woman who contracted West Nile virus (WNV) neuroinvasive illness during her second trimester subsequently elected to terminate her pregnancy due to concerns of possible adverse effects of WNV on her developing fetus. Consent was obtained to test maternal and post-mortem fetal tissues for WNV infection. Fetal blood, liver, kidneys, spleen, umbilicus and amniotic fluid were negative for WNV RNA by polymerase chain reaction and negative for WNV IgM antibodies by ELISA, indicating that in this case there was no evidence of WNV transmission to the fetus. Until further information regarding outcomes of WNV infection during pregnancy is available, pregnant women in areas where WNV is transmitted should take precautions to avoid mosquito bites. Women with WNV illness during pregnancy should undergo regular prenatal checkups including ultrasound examinations to assess fetal development, and healthcare providers should promptly report cases of WNV in pregnant women to their state or local health department or to CDC. Copyright 2006 S. Karger AG, Basel.
Descriptors: pregnancy complications, West Nile fever, induced abortion, amniotic fluid, vertical disease transmission, fetal blood, gestational age, kidney, liver.

Steele, N.P. and D. Myssiorek (2006). **West Nile virus induced vocal fold paralysis.** *Laryngoscope* 116(3): 494-496.
Abstract: OBJECTIVE: West Nile virus has recently become a public health concern in the United States, after an outbreak in New York City in 1999. It is a mosquito-borne virus that causes a spectrum of disease from flu-like symptoms to

encephalopathy, muscle weakness, and, in some cases, death. STUDY DESIGN: Case Report. METHODS: A patient infected with West Nile virus presented with progressive muscle weakness, and later developed bilateral vocal fold paresis. His clinical presentation, physical and laboratory examination findings, and course of illness will be discussed. RESULTS: After a prolonged hospital stay, and presumptive treatment for Guillain-Barre, repeat CSF analysis revealed infection with the West Nile virus. The patient developed bilateral vocal fold paralysis during his hospital course. At long-term follow-up, the patient's left vocal fold paralysis persisted, while the right vocal fold paralysis had resolved. CONCLUSIONS: Although several viruses have been associated with recurrent laryngeal nerve injury, this is the first report of West Nile virus induced vocal fold paralysis.
Descriptors: vocal cord paralysis, West Nile fever complications, West Nile virus, disease diagnosis, fiber optics endoscopy, follow-up studies, stroboscopy.

Teperman, L.W., T. Diflo, A. Fahmy, G.R. Morgan, R.E. Wetherbee, L. Ratner, D. Cohen, J. Ackelsberg, E. Debernardo, A. Fine, E. Lumeng, N.P. Tavakoli, B. Dixon, A. Weltman, and B. Tsoi (2005). **West Nile virus infections in organ transplant recipients New York and Pennsylvania, August-September, 2005**. *Morbidity and Mortality Weekly Report* 54(40): 1021-1023. ISSN: 0149-2195.
Descriptors: West Nile virus infection, organ transplant recipients, neuroinvasive disease , liver transplantation, kidney transplantation, clinical techniques, lung transplantation.

Ward, M.P., J.A. Schuermann, L.D. Highfield, and K.O. Murray (2006). **Characteristics of an outbreak of West Nile virus encephalomyelitis in a previously uninfected population of horses.** *Veterinary Microbiology* 118(3-4): 255-259.
Abstract: Equine West Nile virus (WNV) encephalomyelitis cases - based on clinical signs and ELISA serology test results - reported to Texas disease control authorities during 2002 were analyzed to provide insights into the epidemiology of the disease within a previously disease-free population. The epidemic occurred between June 27 and December 17 (peaking in early October) and 1,698 cases were reported. Three distinct epidemic phases were identified, occurring mostly in southeast, northwest and then central Texas. Significant ($P<0.05$) disease clusters were identified in northwest and northern Texas. Most (91.1%) cases had no recent travel history, and most (68.9%) cases had not been vaccinated within the previous 12 months. One-third of cases did not survive, 71.2% of which were euthanatized. The most commonly reported presenting signs included ataxia (69%), abnormal gait (52%), muscle fasciculations (49%), depression (32%) and recumbency (28%). Vaccination status, ataxia, falling down, recumbency and lip droop best explained the risk of not surviving WNV disease. Results suggest that the peak risk period for encephalomyelitis caused by WNV may vary substantially among regions within Texas. Recumbent horses

have a poor prognosis for survival. Vaccines, even if not administered sufficiently in advance of WNV infection within a district, may reduce the risk of death by at least 44%.
Descriptors: West Nile virus, horses, infection, clinical signs, Texas, prognosis, vaccines, death, depression, ataxia, recumbancy.

Watson, N.K., R.E. Bartt, S.A. Houff, S.E. Leurgans, and M.J. Schneck (2005). **Focal neurological deficits and West Nile virus infection.** *Clinical Infectious Diseases* 40(7): E59-E62.
Abstract: Our experience with West Nile virus infection revealed that 54% of 28 patients had a focal neurological deficit at presentation. A meningitis or encephalitis syndrome was absent in 47% of patients with focal deficits. Details of the variety of deficits, the time to development of deficits, and the associated radiological and laboratory findings are also discussed in the present report.
Descriptors: nervous system diseases, West Nile fever complications, humans.

Wesson, D.M. (2006). **Is West Nile virus a teratogen?** *Birth Defects Research* 76(5): 357. ISSN: 1542-0752.
Descriptors: West Nile virus, obstetrics, teratogen, nervous system disease, viral disease etiology, pregnancy, immunosuppression.
Notes: Meeting Information: 46th Annual Meeting of the Teratology Society, Tucson, Arizona, USA; June 24 -29, 2006.

Whitney, E.A., K.L. Heilpern, C.W. Woods, C.C. Bahn, E.A. Franko, C. Del Rio, B.J. Silk, J.J. Ratcliff, K.A. Bryant, M.M. Park, S.J. Watkins, L.B. Caram, H.M. Blumberg, and R.L. Berkelman (2006). **West Nile virus among hospitalized, febrile patients: A case for expanding diagnostic testing.** *Vector Borne and Zoonotic Diseases* 6(1): 42-49. ISSN: 1530-3667.
Abstract: In Georgia, most individuals reported with West Nile virus (WNV) disease have been diagnosed with West Nile neuroinvasive disease (WNND). Relatively few cases of West Nile Fever (WNF) are reported, and the burden of illness due to WNV is likely underestimated. From July through October 2003, WNV serologic testing was performed on enrolled patients>or=18 years of age with fever admitted to a large, urban hospital in Atlanta, Georgia through the emergency department (ED). Patients' history, clinical, and laboratory data were recorded. Residual blood drawn in the ED was tested to determine the presence of WNV IgG and IgM antibodies. Of 254 patients tested for WNV, four (1.6%) patients were positive for WNV IgM and IgG antibodies, and had a clinical illness compatible with WNV. None of the four positive patients were clinically suspected of having WNV infection; discharge diagnoses included pneumonia, migraine, stroke, and gout. These four patients accounted for 80% of all WNV diagnosed in this hospital, 44% of all cases in Fulton County,

and 7% of all cases reported in Georgia in 2003. The occurrence of WNV disease may be substantially greater than currently reflected in disease statistics in Georgia and many other states. When indicators of WNV activity are present and patients are likely to have had intensive mosquito exposure, WNV should be considered in the differential diagnosis of seriously ill, febrile patients.
Descriptors: West Nile virus, febrile patients, diagnostic testing, neuroinvasive disease, mosquitoe exposure, differential diagnosis.

Wu, J.J., D.B. Huang, and S.K. Tyring (2006). **West Nile virus rash on the palms and soles of the feet.** *Journal of the European Academy of Dermatology and Venereology* 20(10): 1393-1394.
Descriptors: West Nile virus, rash, palms, soles, feet.

Selected Web Sites

West Nile Virus - West Nile Fever.
Online: www.westnilefever.com/

West Nile Virus. *Audubon.*
Online: www.audubon.org/bird/wnv/

West Nile Virus Information. *Bayer Advanced*.
Online: www.bayeradvanced.com/garden/west-nile-virus/index.cfm?source=ga

West Nile Virus. *CDC*.
Online: www.cdc.gov/ncidod/dvbid/westnile

West Nile Virus: Reducung the Risk. *Food & Drug Administration*.
Online: www.fda.gov/FDAC/features/2003/103_virus.html

West Nile Virus. *Health Canada. Diseases & Conditions*.
Online: www.hc-sc.gc.ca/dc-ma//wnv-vno/index_e.html

Medline Plus - West Nile Virus. *NIH. National Library of Medicine*.
Online: www.nlm.nih.gov/medlineplus/westnilevirus.html

MedlinePlus Medical Encyclopedia - West Nile Virus. *NIH. National Library of Medicine*.
Online: www.nlm.nih.gov/medlineplus/ency/article/007186.htm

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Online: www.phac-aspc.gc.ca/wn-no/index_e.html

Emerging Disease Issues - West Nile Virus. *State of Michigan*.
Online: www.michigan.gov/emergingdiseases/0,1607,7-186-25805---,00.html

West Nile Virus. *U.S. food and Drug Administration*.
Online: www.fda.gov/oc/opacom/hottopics/westnile/html

West Nile virus Maps - 2002. *U.S. Geological Service*.
Online: http://westnilemaps.usgs.gov/202/

Wildlife Disease Information Node - West Nile Virus. *U.S. Geological Survey's National Wildlife Health Center*.
Online: http://westnilevirus.nbii.gov/

Animal Health Monitoring & Surveillance - West Nile Virus. *USDA. Animal and Plant Health Inspection Service*.
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West Nile Virus Information. *USDA. NAL. Animal Welfare Information Center*.
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West Nile Bibleography, 1965-2004. *USDA. NAL. Animal Welfare Information Center.*
Online: www.nal.usda.gov/awic/pubs/westnile/westnilebib.htm

West Nile Virus. *Wikipedia*.
Online: http://en.wikipedia.org/wiki/West_Nile_virus