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Transmission dynamics and changing epidemiology of West Nile virus

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Abstract

West Nile virus (WNV) is a flavivirus that is maintained in a bird–mosquito transmission cycle. Humans, horses and other non-avian vertebrates are usually incidental hosts, but evidence is accumulating that this might not always be the case. Historically, WNV has been associated with asymptomatic infections and sporadic disease outbreaks in humans and horses in Africa, Europe, Asia and Australia. However, since 1994, the virus has caused frequent outbreaks of severe neuroinvasive disease in humans and horses in Europe and the Mediterranean Basin. In 1999, WNV underwent a dramatic expansion of its geographic range, and was reported for the first time in the Western Hemisphere during an outbreak of human and equine encephalitis in New York City. The outbreak was accompanied by extensive and unprecedented avian mortality. Since then, WNV has dispersed across the Western Hemisphere and is now found throughout the USA, Canada, Mexico and the Caribbean, and parts of Central and South America. WNV has been responsible for >27,000 human cases, >25,000 equine cases and hundreds of thousands of avian deaths in the USA but, surprisingly, there have been only sparse reports of WNV disease in vertebrates in the Caribbean and Latin America. This review summarizes our current understanding of WNV with particular emphasis on its transmission dynamics and changing epidemiology.

Keywords: West Nile virus, flavivirus, mosquito, vector-borne disease, epidemiology

Introduction

Classification

West Nile virus (WNV) is a member of family *Flaviviridae* (ICTV, 2005). This family is comprised of three genera: *Flavivirus* (which includes WNV), *Pestivirus* and *Hepacivirus*. More than 70 viruses have been classified in the genus *Flavivirus* and the majority of these are arthropod-borne viruses (arboviruses). This genus is further divided into 12 serocomplexes, including the Japanese encephalitis virus (JEV) serocomplex which consists of WNV, Cacipacore, JEV, Koutango, Murray Valley encephalitis, St. Louis encephalitis (SLEV), Usutu and Yaounde viruses (Table 1). Kunjin virus, which is endemic in Australia, is now considered to be a subtype of WNV (Scherret *et al.*, 2001; ICTV, 2005).

Sequencing and phylogenetic studies have shown that WNV can be divided into two major genetic lineages (Berthet *et al.*, 1997; Lanciotti *et al.*, 1999, 2002; Beasley *et al.*, 2004a) (Fig. 1). WNV isolates in lineage 1 have a worldwide distribution, and include both virulent and attenuated viruses. Lineage 1 isolates have been further divided into three clades (Lanciotti *et al.*, 2002). Clade 1a contains isolates from Africa, Europe, Asia and the Americas, clade 1b consists of Kunjin viruses and clade 1c consists of isolates from India. WNV isolates in lineage 2 were once found exclusively in Africa and were usually associated with asymptomatic infections, but several virulent lineage 2 isolates were recently identified in Europe (Bakonyi *et al.*, 2006; Erdelyi *et al.*, 2007).

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Table 1. The JEV serocomplex

Virus	Principal reservoir host	Human pathogen	Animal pathogen	Geographic distribution
Cacipacore	Bird	Yes	–[1]	South America
Japanese encephalitis	Bird, pig	Yes	Pig, horse	Asia, Australia
Koutango	Rodent	Yes	–	Africa
Murray Valley encephalitis	Bird	Yes	Horse?[2]	Australia
SLEV	Bird	Yes	–	The Americas, Caribbean
Usutu	Bird	–	Bird	Africa, Europe
West Nile	Bird	Yes	Horse, bird	Africa, Asia, Europe, Australia, The Americas, Caribbean
Yaounde	Rodent, bird	–	–	Africa

[1]No.
[2]Anecdotal evidence indicates that Murray Valley encephalitis virus occasionally causes disease in horses.

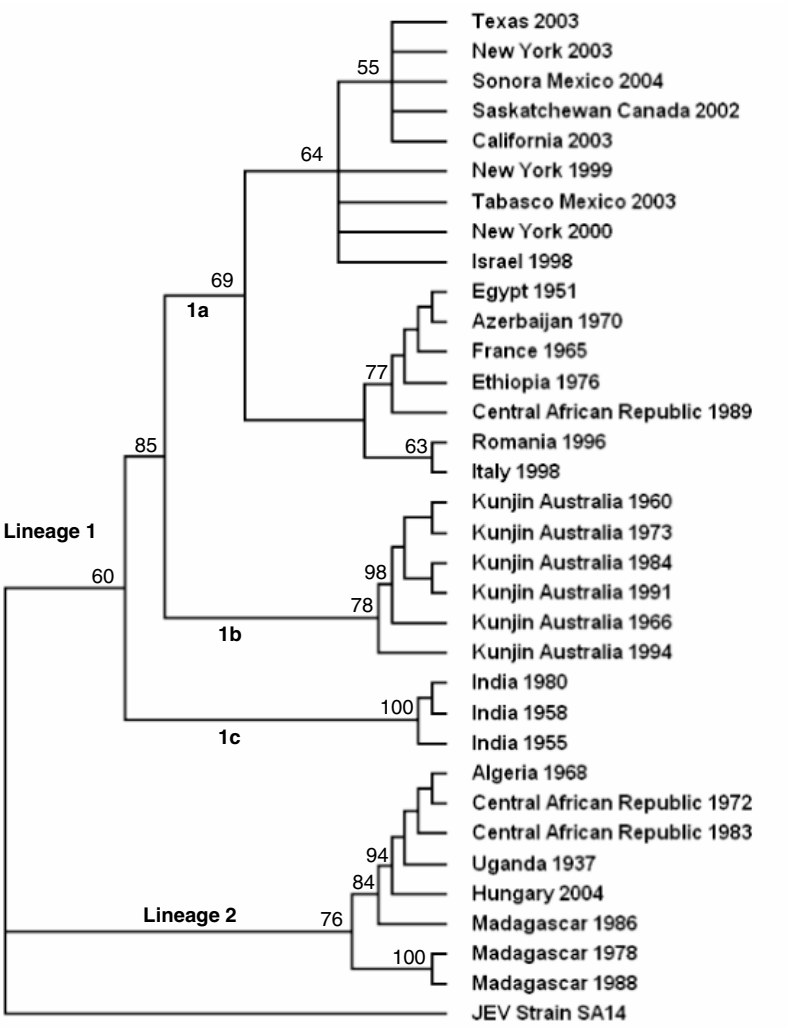

Fig. 1. Phylogenetic tree generated by parsimony analysis (PAUP) of aligned nucleotide sequences of 33 WNV strains from diverse geographic locations. The phylogenetic analysis is based on a 255-bp region of the envelope gene (positions 1402–1656). The tree is rooted using JEV (strain SA-14) as an outgroup. The two lineages (1 and 2) and three clades (1a, 1b and 1c) of WNV, as described by Lanciotti and colleagues, are denoted (Lanciotti *et al.*, 2002). Values above some branches represent the percentage support by parsimony bootstrap analysis. Bootstrap values are based on 1000 replicates.

It was recently proposed that two virus isolates from the Czech Republic represent a new (third) lineage of WNV or a novel flavivirus in the JEV serocomplex (Bakonyi *et al.*, 2005). In another recent study, it was suggested that WNV can be classified into as many as five distinct lineages (Bondre *et al.*, 2007).

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Table 2. Functions of the flavivirus proteins

Protein	MW (kDa)	Function
C	11	Associates with genomic RNA in the nucleocapsid
prM	26	Protects E from undergoing an irreversible conformational change as the virion is secreted through acidified sorting compartments
M	10	Unknown
E	53	Receptor binding, host membrane fusion, viral assembly, Major antigenic determinant
NS1	46	Co-factor activity for the viral replicase
NS2A	22	Inhibits interferon responses, virion assembly and release
NS2B	14	Co-factor for NS3 protease activity
NS3	70	Serine protease, RNA capping, helicase
NS4A	16	Modulates interferon signaling
NS4B	27	Modulates interferon signaling
NS5	103	RNA-dependent RNA polymerase, methyltransferase

MW, molecular weight; C, capsid; prM, pre-membrane; M, membrane; E, envelope; NS, nonstructural.

Virion morphology

The WNV virion is a small (~50 nm in diameter), spherical, enveloped particle with icosahedral symmetry (Brinton, 2002; Mukhopadhyay *et al.*, 2003; Lindenbach *et al.*, 2007). The envelope consists of a host-derived lipid bilayer and 180 copies of both the envelope (E) and membrane (M) proteins. The E and M proteins are embedded into the lipid bilayer via their carboxy-terminal transmembrane domains. The M protein is generated by furin-mediated cleavage of the precursor membrane (prM) protein late in virus maturation (Stadler *et al.*, 1997). The prM and E proteins interact to form heterodimers that are present on the virion surface as 60 trimeric spikes. Cleavage of the prM protein enables the E protein to form head-to-tail homodimers that lie parallel to the lipid bilayer. The envelope surrounds a nucleocapsid core which is composed of multiple copies of the capsid (C) protein and a single copy of the genomic RNA.

Genomic organization

The genomic RNA of WNV, like that of other flaviviruses, is approximately 11 kb in length and consists of a 5′ untranslated region (5′ UTR), a single open reading frame (ORF) and a 3′ UTR (Brinton, 2002; Beasley, 2005; Lindenbach *et al.*, 2007). The 5′ and 3′ UTRs of the flavivirus genome are approximately 100 and 400–700 nucleotides (nt) in length, respectively, and can form highly conserved secondary and tertiary structures. The 5′ end of the genome is capped, and the 3′ end usually lacks a polyadenine tail. The ORF encodes a single large polyprotein that is co- and post-translationally processed by viral and cellular proteases to generate three structural and seven nonstructural (NS) proteins in the gene order: 5′-C-prM(M)-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3′. The flavivirus structural proteins are primarily involved in virion formation, whereas diverse functions have been assigned to the NS proteins (Table 2). RNA replication occurs in the cytoplasm in close association with the rough endoplasmic reticulum (ER). Virions are assembled in the lumen of the ER then transported within vesicles to the cell surface where they are released by exocytosis.

Clinical manifestations and pathogenesis

Humans

The majority of WNV infections in humans are asymptomatic but, during recent outbreaks in Europe, Israel and the USA, approximately 20% of infections have resulted in a mild flu-like illness known as West Nile fever (WNF) (Hayes and Gubler, 2005; Hayes *et al.*, 2005b; Davis *et al.*, 2006). The illness is characterized by a variety of nonspecific symptoms that cannot be distinguished from other infectious illnesses on clinical examination. Symptoms include an abrupt onset of fever, headache, myalgia, nausea, fatigue, weakness, vomiting and diarrhea. Symptoms usually develop 2–14 days after virus infection. The illness typically lasts for 2–5 days but, in more severe cases, fatigue can persist for over a month. Approximately 1 in 150 WNV infections lead to severe neuroinvasive disease (WNND) which is characterized by encephalitis, meningitis and/or poliomyelitis-like flaccid paralysis (Davis *et al.*, 2006; Sejvar and Marfin, 2006; Sejvar, 2007). The fatality rate in patients with WNND is approximately 10%, and long-term neurological sequelae occurs is >50% of patients. Patients presenting with encephalitis have a poorer outcome than those with meningitis. WNND is more common in elderly and immunocompromised patients and is rarely reported in patients <30 years.

The pathogenesis of WNV is similar to that of other flaviviruses. Following peripheral inoculation, initial WNV replication is believed to occur in skin Langerhans dendritic cells (Samuel and Diamond, 2006). The virus then spreads to the lymph nodes and blood stream,

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followed by peripheral tissues such as the spleen and kidney. The virus may then penetrate the central nervous system (CNS) resulting in inflammation of the medulla, brain stem and spinal cord (Guarner *et al.*, 2004; Kleinschmidt-DeMasters *et al.*, 2004; Samuel and Diamond, 2006).

Horses

WNV has been responsible for extensive morbidity and mortality in horses in Europe, Israel and the USA (Castillo-Olivares and Wood, 2004; van der Meulen *et al.*, 2005). Experimental infection studies performed by the Centers for Disease Control and Prevention using a USA strain of WNV demonstrated that the majority of WNV infections in horses are asymptomatic; clinical signs were observed in 1 of 12 (8%) animals (Bunning *et al.*, 2002). WNV illness in horses is characterized by fever and a variety of neurologic signs (e.g. ataxia, muscular weakness and amaurosis). The illness typically lasts 3 weeks (Salazar *et al.*, 2004). In recent outbreaks, the mortality rate in clinically affected horses has ranged from 23 to 43% (Murgue *et al.*, 2001; Autorino *et al.*, 2002; Durand *et al.*, 2002; Salazar *et al.*, 2004; Ward *et al.*, 2004, 2006). Approximately 80% of clinically affected horses that survive make a full recovery (Salazar *et al.*, 2004). Vaccination can reduce the risk of death by 44% (Ward *et al.*, 2006). Although horses have been used as sentinels for human risk of infection with some mosquito-borne viruses, for example Western equine encephalitis virus (Potter *et al.*, 1977), clinical cases of WNV in horses do not usually precede human cases in the same area (Corrigan *et al.*, 2006). WNV exhibits a pronounced CNS tropism in horses; lesions are rarely detected in extraneural tissues (Cantile *et al.*, 2001; Castillo-Olivares and Wood, 2004).

Birds

In birds, WNV disease is characterized by various neurologic signs including ataxia, paralysis and incoordination, in addition to various non-neurologic signs such as depression, lethargy, ruffled feathers, weight loss and myocarditis (Komar *et al.*, 2003a; van der Meulen *et al.*, 2005). Birds that succumb to WNV infection often die in the first 24 h from the onset of clinical signs. Dead bird surveillance has provided an efficient early warning system for WNV disease in humans in the USA (Eidson *et al.*, 2001a, b). In contrast to horses, WNV infection in birds causes lesions in multiple tissues, with the most consistently infected tissues being the kidney, brain, liver, heart and spleen (Steele *et al.*, 2000; Kramer and Bernard, 2001; Panella *et al.*, 2001; Fitzgerald *et al.*, 2003; Gibbs *et al.*, 2005).

Other vertebrates

With the notable exceptions of horses and birds, WNV disease is not a common occurrence in vertebrate animals. However, neurologic disease has been observed in WNV-infected squirrels (Heinz-Taheny *et al.*, 2004; Padgett *et al.*, 2007), and hamsters and mice have been used as laboratory models for WNV-induced encephalitis (Xiao *et al.*, 2001; Beasley *et al.*, 2004a). There have been occasional reports of encephalitis in dogs following naturally-acquired WNV infection (Lichtensteiger *et al.*, 2003; Read *et al.*, 2005; Cannon *et al.*, 2006). WNV infection can also cause neurologic illness in alligators (Miller *et al.*, 2003; Klenk *et al.*, 2004; Jacobson *et al.*, 2005). Mild, non-neurologic disease has been reported in several vertebrate species, including cats, following WNV infection (Austgen *et al.*, 2004).

Geographic distribution, major outbreaks and molecular epidemiology

WNV in the Eastern Hemisphere

WNV was first isolated in 1937 from the blood of a febrile woman in the West Nile District of Uganda in East Africa (Smithburn *et al.*, 1940). The virus was later shown to be widely dispersed across Africa, Europe, Asia and Australia (Hall, 2000; Hayes, 2001; Petersen and Roehrig, 2001; Zeller and Schuffenecker, 2004; Kramer *et al.*, 2007). Between its original isolation and the mid-1990s, WNV was not considered to be a major pathogen of humans or animals; outbreaks were infrequent, associated with a low incidence of neuroinvasive disease and often took place in rural areas. The first recorded human outbreaks of WNV occurred in Israel in the 1950s (Bernkopf *et al.*, 1953; Goldblum *et al.*, 1956). Subsequent human outbreaks occurred in France in 1962–1964 and South Africa in 1974, followed by a 20-year period of relatively little WNV activity (Hannoun *et al.*, 1964; Panthier *et al.*, 1968; Jupp, 2001). However, since 1994, there has been an alarming increase in the frequency and severity of WNV outbreaks in humans and equines. In the Eastern Hemisphere, WNV outbreaks are now a regular occurrence in Europe and the Mediterranean Basin. In 1994, 50 human cases with two deaths occurred in Algeria (Le Guenno *et al.*, 1996). The next outbreak, which resulted in 393 cases with 17 deaths, took place in Romania in 1996 and represents the first recorded outbreak of WNV in an urban area (Tsai *et al.*, 1998). Additional human outbreaks occurred in Tunisia in 1997 (173 cases), Russia in 1999 (318 cases), Israel in 2000 (417 cases) and Russia in 2000–2001 (120 cases) (Zeller and Schuffenecker, 2004). Equine outbreaks of WNV have occurred in Morocco in 1996 (94 cases), Italy in 1998 (14 cases), France in 2000 (76 cases), Israel in 2000

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Table 3. Human and equine cases of WNV in the United States, 1999–2007

Year	Human cases[1]					Equine cases[1]	No. states with WNV activity[2]
	WNF	WNDD	Other[3]	Total	Fatalities		
1999	3	59	0	62	7	25	4
2000	2	19	0	21	2	60	12
2001	2	64	0	66	9	738	27
2002	1160	2946	50	4156	284	15,257	44
2003	6830	2866	166	9862	264	5181	46
2004	1269	1142	128	2539	100	1406	47
2005	1607	1294	99	3000	119	1088	48
2006	2616	1459	194	4269	177	1086	48
2007[4]	2215	1130	59	3404	98	484	46
Total	15,704	10,979	696	27,379	1060	25,325	48

[1]The case definition for WNV in humans and horses is the observation of clinical signs and at least one of the following: (1) a four-fold or greater change in WNV-specific serum antibody titer, (2) isolation of WNV from or detection of WNV antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF), or other body fluid, (3) detection of WNV-specific IgM in CSF, (4) the detection of WNV-specific IgM in serum and confirmed by demonstration of WNV-specific serum IgG in the same or a later specimen.
[2]Number of states reporting WNV infections in humans, horses, birds or mosquitoes.
[3]Includes patients with unspecified clinical manifestations.
[4]Case counts as of 14 December 2007.
WNF, West Nile fever; WNDD, West Nile neuroinvasive disease.
Data were taken from the CDC and USDA websites.

(76 cases) and France in 2004 (32 cases). In the last decade, sporadic cases of WNV disease in birds have also occurred in the Eastern Hemisphere; most notably, WNV was isolated from several birds exhibiting signs of encephalitis in Israel in 1998, and Hungary in 2003–2005 (Malkinson *et al.*, 2002; Bakonyi *et al.*, 2006; Erdelyi *et al.*, 2007).

WNV in the United States: the first 3 years

WNV was first detected in the Western Hemisphere in 1999 during an outbreak of human encephalitis in New York City (Gubler *et al.*, 2000; Nash *et al.*, 2001). There were 62 human cases with seven deaths (Table 3). Twenty-five equine cases with nine deaths occurred in the same area (Ostlund *et al.*, 2001). One striking feature of this outbreak was the extraordinarily large number of dead and dying birds which were reported. Thousands of birds, particularly American crows and other corvids, died as a result of WNV infection in New York, New Jersey and Connecticut (Anderson *et al.*, 1999; CDC, 1999). The prototype New York strain (WN-NY99), which was isolated from a flamingo that died in the Bronx zoo, was shown to be most similar to WNV isolates from Israel (Lanciotti *et al.*, 1999). Indeed, WN-NY99 and an isolate obtained from a dead domestic goose in Israel in 1998 differ by only 2 nt over a 1278 nt region (0.16% divergence) of the E gene (Lanciotti *et al.*, 1999; Malkinson *et al.*, 2002). These data suggest that WNV was introduced into New York from the Middle East, although the mode of introduction is not known.

WNV infections occurred again in 2000; there were 21 human cases with two deaths (Marfin *et al.*, 2001) (Table 3). The virus was isolated from vertebrates or mosquitoes in 12 states in the northeastern USA. The isolation of WNV from overwintering *Culex pipiens* in New York City in February 2000 suggested that the virus had persisted in mosquito vectors during the winter (Nasci *et al.*, 2001). The virus was also isolated from a red-tailed hawk that died during the winter in New York (Garmendia *et al.*, 2000). In 2001, WNV was responsible for 66 human cases with nine deaths, and extended its geographic range to encompass 27 states (O'Leary *et al.*, 2002). That same year, WNV was isolated from several dead birds found in Ontario, marking the first evidence of this virus in Canada (available at: http://www.hc-sc.gc.ca/dc-ma/wnv-vno/index_e.html). Sequencing studies revealed that WNV isolates collected in the USA in 2000 and 2001 were genetically similar to isolates from 1999, demonstrating that only limited viral evolution had taken place during this time (Anderson *et al.*, 1999, 2001; Jia *et al.*, 1999; Briese *et al.*, 2000; Ebel *et al.*, 2001; Lanciotti *et al.*, 2002). For example, the complete E gene sequences (1503 nt) of 11 WNV isolates from 2000 differed by no more than 3 nt (0.20% divergence) from the homologous region of WN-NY99 (Ebel *et al.*, 2001). Eighty-two WN virus isolates from Connecticut in 1999 and 2000 had a maximum of 3 nt differences (0.33% divergence) over a 921-nt region of the E gene when compared to the homologous region of WN-NY99 (Anderson *et al.*, 1999).

WNV in the United States: 2002 – present

In 2002, there was a dramatic and unexpected increase in the number of WNV infections in the USA; there

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were 4156 human cases with 284 deaths, and 15,257 equine cases (O'Leary *et al.*, 2004) (Table 3). Another notable feature of the 2002 transmission season was that it lasted longer than previous years with some cases occurring in December. This was presumably due to the incursion of the virus into several of the southernmost states of the USA where climatic conditions support year-round mosquito proliferation. Several previously unrecognized modes of human to human transmission were reported (CDC, 2002a, c; Iwamoto *et al.*, 2003; Mather *et al.*, 2003). The WNV outbreak in 2003 was even larger again; there were 9858 human cases with 262 deaths. This represents the largest recognized epidemic of arboviral neuroinvasive disease in the Western Hemisphere and the largest recognized epidemic of WNV in the world. Since then, WNV has continued to cause significant morbidity and mortality in the USA with >2500 human cases occurring each year. The emergence of WNV into Canada has also had disastrous consequences; there have been 2296 human cases (available at http://www.hc-sc.gc.ca/dc-ma/wnv-vno/index_e.html).

The sudden rise in WNV cases in 2002 coincided with the emergence of a new WNV genotype (WN02) (Ebel *et al.*, 2004; Davis *et al.*, 2005, 2007; Moudy *et al.*, 2007; Snapinn *et al.*, 2007). This genotype was first described by Ebel and colleagues after nucleotide sequencing was performed on the complete E genes of 67 isolates from New York in 2000–2003. Sequencing was also performed on parts of the NS5 gene and 3′ UTR of 39 isolates. WN02 represented 55% of isolates collected in 2002, and 85% of isolates collected in 2003. Shortly afterwards, it was shown that WN02 had emerged as the dominant WNV genotype across the USA and Canada (Davis *et al.*, 2005). The earlier WNV genotype (NY99) has not been detected in North America since 2004 suggesting that it has been completely displaced by WN02 (Moudy *et al.*, 2007). All viruses in the WN02 genotype contain 13 mutations when compared to the prototype WN-NY99 strain, including one non-synonymous mutation which is located at E-159 (Davis *et al.*, 2005). The extrinsic incubation time for viruses in the WN02 genotype in *Culex* species mosquitoes is up to 4 days shorter than viruses from the NY99 genotype (Ebel *et al.*, 2004; Moudy *et al.*, 2007). It is likely that the ability of WN02 to replicate in mosquitoes faster than NY99 has contributed to the dramatic increase in WNV cases in the USA since 1999–2001.

Overall, the impact that WNV has had on human, equine and avian health has been far more pronounced in the USA as compared to the Eastern Hemisphere. One recent study has provided particular insight into this phenomenon by demonstrating that WNV isolates from the USA are more virulent than their counterparts from the Eastern Hemisphere largely because of a critical amino acid difference at NS3-249 (Brault *et al.*, 2007). All WNV isolates from North America and many other recent lineage 2 strains contain a proline at NS3-249 whereas WNV isolates not associated with avian mortality usually possess an alanine, histidine or threonine at this site. American crows infected with viruses generated from infectious WN-NY99 cDNA clones exhibit 100% mortality and develop a peak mean viremia of $10^{9.4}$ pfu ml^{-1}. However, the introduction of a Pro→Thr mutation at NS3-249 generated recombinant viruses with relatively avirulent phenotypes; avian mortality decreased to 12% and the peak mean viremia was reduced to $10^{3.5}$ pfu ml^{-1}.

WNV in Latin America and the Caribbean

Equine and avian infection surveillance has demonstrated widespread circulation of WNV in Mexico and the Caribbean since 2002 (Blitvich *et al.*, 2003; Dupuis *et al.*, 2003; Estrada-Franco *et al.*, 2003; Komar *et al.*, 2003b; Lorono-Pino *et al.*, 2003; Quirin *et al.*, 2004). The following year, WNV activity was detected for the first time in Central America (Cruz *et al.*, 2005; Morales-Betoulle *et al.*, 2006) and, in 2004, WNV seropositive horses were identified in South America (Mattar *et al.*, 2005). The first WNV isolate from Latin America was obtained from a bird that died in southern Mexico in 2003 (Estrada-Franco *et al.*, 2003; Beasley *et al.*, 2004b). This isolate is a member of the WN02 genotype and it contains an amino acid change at E-156 which abolishes a highly conserved glycosylation motif. The substitution responsible for the amino acid change was not seen in all clones, suggesting a mixed virus population. Glycosylated variants were highly lethal in mice whereas non-glycosylated variants were attenuated. Since then, another 15 WNVs (12 from Mexico, three from Argentina) have been identified and sequenced (Blitvich *et al.*, 2004; Elizondo-Quiroga *et al.*, 2005; Deardorff *et al.*, 2006; Morales *et al.*, 2006). All belong to the WN02 genotype; none contain the attenuating mutation at E-156. No WNV isolates have been obtained from the Caribbean. Indeed, one perplexing aspect of the emergence of WNV into Latin America and the Caribbean is the unexpected difficulty researchers have had isolating WNV in these areas. To date, information on the emergence and spread of WNV in Latin America and the Caribbean has mostly been derived from serologic studies. Even more perplexing is the sparse number of human cases that have occurred. The Mexican Secretary of Public Health (Secretaría de Salúd Publica) has reported seven human cases of WNV in Mexico (available at http://portal.salud.gob.mx). The cases occurred in the States of Chihuahua ($n=4$), Sonora ($n=1$) and Nuevo Leon ($n=1$) in 2003, and Sonora ($n=1$) in 2004. Three cases were classified as severe; all patients survived. These states border the USA. There have been another 5 human cases in the Caribbean (Komar and Clark, 2006). These cases occurred in the Cayman Islands in 2001 ($n=1$), the

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Bahamas in 2003 ($n=1$) and Cuba in 2003 ($n=2$) and 2004 ($n=1$). There have been only isolated reports of WNV illness in horses and birds in Latin America and the Caribbean.

It is likely that under-reporting has contributed to the low number of WNV cases in Latin America and the Caribbean particularly since the resources and funds available to the public health authorities in some regions are limited. Nevertheless, it is unlikely that a large-scale outbreak of WNV disease would go unrecognized in an urban area suggesting that other factors are contributing to the low number of WNV cases. One explanation is that pre-existing immunity to another flavivirus, such as dengue virus which is endemic in many parts of Latin America and the Caribbean, is providing partial protection to subsequent WNV infection (Tesh *et al.*, 2002; Xiao *et al.*, 2003). However, this reason does not account for the apparent lack of WNV illness in equine and avian species because dengue virus usually does not replicate in non-primate vertebrates (Thomas *et al.*, 2003). Although a number of other flaviviruses, such as SLEV, Ilheus, Bussuquara, Rocio, Cacipacore and Iguape viruses, are circulating in Latin America and/or the Caribbean, it is unlikely that they are conferring resistance to subsequent WNV infection because the prevalence of these virus infections is low (Blitvich *et al.*, 2003; Dupuis *et al.*, 2003; Fernandez-Salas *et al.*, 2003; Lorono-Pino *et al.*, 2003; Farfan-Ale *et al.*, 2004; Marlenee *et al.*, 2004; Turell *et al.*, 2005b). Another explanation is that there are critical differences in the species composition, relative abundance and susceptibility of vertebrates or vectors in Latin America and the Caribbean as compared to the USA. The emergence of attenuated WNV variants may have contributed to the lack of observed WNV illness. Indeed, as noted earlier, certain plaques obtained from a WNV isolate from southern Mexico were attenuated in mice (Beasley *et al.*, 2004b). However, the mutation responsible for this attenuation is not present in any WNV isolates from Latin America (Blitvich *et al.*, 2004; Elizondo-Quiroga *et al.*, 2005; Deardorff *et al.*, 2006; Morales *et al.*, 2006). Additional research is required to elucidate the mechanisms that have conditioned the vastly different epidemic and epizoonotic potentials of WNV in Latin America and the Caribbean as compared to the USA.

Transmission cycle

WNV is maintained in nature in an enzootic transmission cycle that primarily involves *Culex* species mosquitoes and birds (Fig. 2) (Komar, 2003; Hayes *et al.*, 2005a; Kramer *et al.*, 2007). Mosquito species that participate in this cycle are referred to as amplification vectors, and are strongly ornithophilic (feed almost exclusively on avian blood). Mosquito species with more general feeding habits can transmit WNV to humans, horses and other non-avian vertebrates after feeding upon viremic birds. These mosquito species are known as bridging vectors. Humans, horses and other non-avian vertebrates are incidental (dead-end) hosts because they usually, although not always, produce viremias of insufficient magnitude to infect susceptible mosquitoes. Generally, an infected vertebrate must produce a viremia of $\geq 10^5$ pfu ml^{-1} to serve as a reservoir (amplification) host (Turell *et al.*, 2000; Sardelis *et al.*, 2001). WNV can infect a remarkably large number of vertebrate and arthropod species and this is a major reason why the virus has successfully spread over such a large geographic region. Evidence of WNV infection has been detected in >300 species of birds, >30 species of mammals, several reptilian and amphibian species, >60 species of mosquitoes and several other arthropod species.

Birds

Birds are the natural reservoir hosts for WNV (McLean *et al.*, 2001; Komar, 2003; Hayes *et al.*, 2005a). Passeriformes (song birds) are considered to be the principal reservoir hosts, although competent birds have also been identified in several other orders, including Charadriiformes (shorebirds), Falconiformes (hawks) and Strigiformes (owls). In contrast, Anseriformes (ducks), Columbiformes (pigeons) and Piciformes (woodpeckers) usually generate viremias insufficient to infect mosquitoes. One species that plays a particularly important role in the primary WNV transmission cycle is the house sparrow (Komar *et al.*, 2003a; Langevin *et al.*, 2005). This species is considered to be a major reservoir host in both North America and Europe because it is highly abundant in those regions, frequently seropositive for WNV in field studies and develops high and prolonged WNV viremias in laboratory studies. Antibodies to WNV were detected in 60% of house sparrows sampled in New York City in 1999 (Komar *et al.*, 2001). Experimental infection studies have shown that house sparrows develop WNV viremias that exceed 10^{10} pfu ml^{-1}, and maintain viremias above 10^5 pfu ml^{-1} for five days (Komar *et al.*, 2003a; Langevin *et al.*, 2005). Other avian species that develop exceptionally high viremias (>10^{10} pfu ml^{-1}) include the blue jay, American crow and common grackle (Komar *et al.*, 2003a; Reisen *et al.*, 2005).

It is often assumed that an avian species that is susceptible to WNV infection must be resistant to WNV illness or death to be considered an important reservoir host. However, under some conditions, avian die-off may actually enhance WNV transmission because it reduces the likelihood of infected mosquitoes feeding on immune hosts (Foppa and Spielman, 2007). Furthermore, dying birds are relatively immobile and therefore provide an easy source of blood for mosquitoes. The susceptibility of a bird to WNV disease is dependent upon various factors including its species and age, and the strain of

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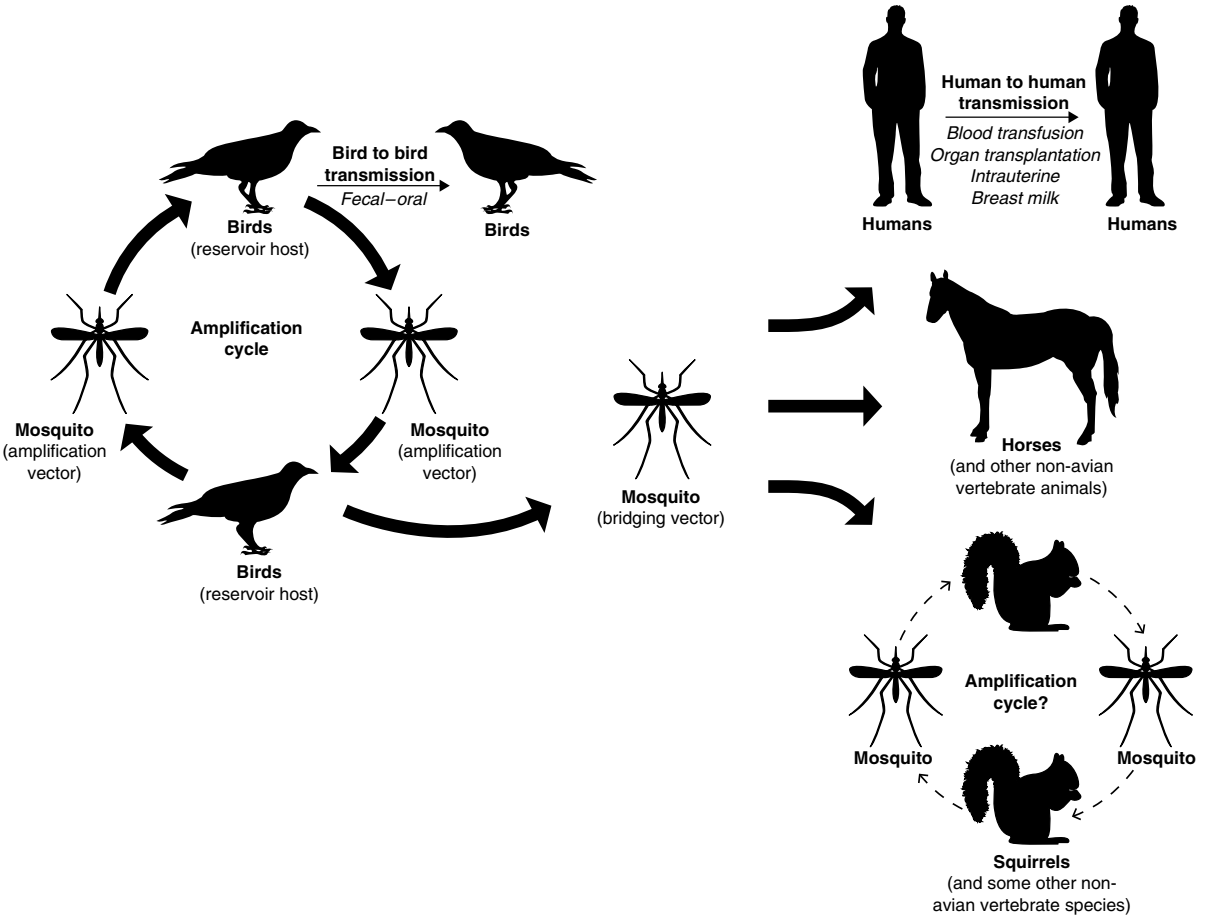

Fig. 2. Overview of the WNV transmission cycle. Thick, solid arrows denote common routes of transmission. Thin, solid arrows denote routes of transmission that occur infrequently. Thin, broken arrows denote proposed routes of transmission that have not been confirmed in nature.

the virus. Crows, jays and other members of the family *Corvidae* suffer extremely high mortality rates following WNV infection. Several studies have demonstrated 100% mortality in American crows experimentally infected with New York strains of WNV (Komar *et al.*, 2003a; Brault *et al.*, 2004, 2007; Bunning *et al.*, 2007). The overall American crow population in the USA has declined by an estimated 45% since the introduction of the virus in 1999 (LaDeau *et al.*, 2007). However, the detection of antibodies to WNV in a subset (16.5%) of American crows in Georgia in 2002–2004 suggests that resistant populations have emerged (Wilcox *et al.*, 2007). House sparrows exhibit mortality rates of approximately 16% (Reisen *et al.*, 2005). In contrast, WNV infection has no apparent adverse effect on many other avian species; for example, no signs of illness were observed in 17 of 25 North American bird species experimentally infected with a New York strain of WNV (Komar *et al.*, 2003a).

Viremic migratory birds are widely regarded to be the major long-distance dispersal agents of WNV (Peterson *et al.*, 2003), although the non-directional movement of viremic resident birds also contributes to the spread of the virus (Rappole *et al.*, 2006). The movement of migratory birds is also a critical determinant of the timing and severity of WNV outbreaks. For example, the migratory behavior of the American robin influences the transmission dynamics of WNV in the northeastern USA (Kilpatrick *et al.*, 2006). American robins are the preferred source of blood for *Cx. pipiens* in this region; more than half of the engorged *Cx. pipiens* collected in May and June had fed on this avian species. However, by September, there is a 7-fold increase in the proportion of *Cx. pipiens* that have fed on humans. This shift in feeding behavior coincides with a sharp decrease in the abundance of American robins in the northeastern USA as a result of long-distance migration, as well as a dramatic increase in the number of human cases in the same area. American robins are also a common source of blood for *Culex* spp. mosquitoes in other regions of the USA (Molaei *et al.*, 2006; Savage *et al.*, 2007).

Mammals

Naturally-acquired WNV infections have been reported in a diverse range of mammalian species (Hayes, 1988;

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van der Meulen *et al.*, 2005). Mammalian species susceptible to WNV infection, as indicated by the detection of WNV antibody, antigen or nucleic acid in serum or tissues, include the alpaca, baboon, bat, black bear, brown bear, camel, cat, cow, coyote, dog, goat, horse, human, jaguar, lemur, pig, pigtail macaque, mouse, opossum, rabbit, raccoon, rhesus macaque, rat, reindeer, sheep, skunk, squirrel, white-tailed deer and wolf. Most mammalian species do not contribute to the WNV amplification cycle because they develop low WNV viremias of short duration. The highest viremia detected in horses experimentally infected with a USA strain of WNV was $10^{3.0}$ pfu ml^{-1} (Bunning *et al.*, 2002). The peak WNV viremias reported in experimentally-infected cats, pigs and dogs were $10^{4.0}$, $10^{3.1}$ and $10^{2.2}$ pfu ml^{-1}, respectively (Austgen *et al.*, 2004; Teehee *et al.*, 2005). However, golden hamsters, eastern cottontail rabbits, eastern chipmunks and fox squirrels can develop WNV viremias sufficient to infect mosquitoes (Tesh *et al.*, 2005; Tiawsirisup *et al.*, 2005; Tonry *et al.*, 2005; Root *et al.*, 2006; Platt *et al.*, 2007). For example, Platt and colleagues reported viremias as high as $10^{7.8}$ pfu ml^{-1} in eastern chipmunks experimentally-infected with WNV and demonstrated that these viremias were sufficient to infect *Culex* and *Aedes* spp. mosquitoes. Root and colleagues reported maximum viremias of $10^{5.0}$ pfu ml^{-1} in fox squirrels experimentally-infected with WNV. Surveillance studies have demonstrated that fox squirrels exhibit high seroprevalence rates to WNV; for example, 49% of tree squirrels sampled in a WNV serosurvey in the central and eastern USA in 2003 had antibodies to WNV (Root *et al.*, 2005). Golden hamsters experimentally-infected with WNV develop viremias up to $10^{5.0}$ pfu ml^{-1} and can persistently shed WNV in urine for months (Tesh *et al.*, 2005; Tonry *et al.*, 2005). Taken together, these experimental infection data suggest that several mammalian species contribute to the WNV amplification cycle, although this has not been confirmed in nature.

Reptiles and amphibians

Experimental infection studies have provided evidence that some species of ectothermic vertebrates contribute to the WNV amplification cycle. For instance, American alligators develop viremias that exceed $10^{5.0}$ pfu ml^{-1} (Klenk *et al.*, 2004; Jacobson *et al.*, 2005). Lake frogs have also been demonstrated to develop WNV viremias capable of infecting mosquitoes (Kostiukov *et al.*, 1986). WNV viremias were also detected in green iguanas and North American bullfrogs following WNV inoculation, although the maximum titers were $10^{3.2}$ and $10^{2.2}$ pfu ml^{-1}, respectively, suggesting that these species are incompetent reservoir hosts (Klenk and Komar, 2003). Red-ear sliders and garter snakes failed to develop detectable WNV viremias. Antibodies to WNV have been detected in healthy farmed crocodiles in Israel and Mexico, but their role in WNV transmission has not been studied (Steinman *et al.*, 2003; Farfan-Ale *et al.*, 2006).

Mosquitoes

Several criteria must be fulfilled for a mosquito species to be considered an important vector of WNV, including (1) demonstration that WNV efficiently infects and is transmitted by the mosquito species after receiving an infectious blood meal under laboratory conditions, (2) a high relative abundance of the species in the field and (3) frequent isolation of WNV from the species in the field (Turell *et al.*, 2001). The frequency at which WNV is detected in mosquitoes in the field, known as the minimal infection rate (MIR), is expressed as the number of positive mosquito pools per 1000 mosquitoes tested. The MIR of WNV in mosquitoes provides an indicator of the intensity of virus transmission in a given area and often is related to the risk of human disease (Hayes *et al.*, 2005a). In surveillance studies, mosquitoes are usually assayed for the presence of WNV by RT-PCR or virus isolation in cell culture; MIRs calculated by the former approach are usually 2–6-fold greater (Nasci *et al.*, 2001, 2002; Shi *et al.*, 2001; White *et al.*, 2001). As already noted, the feeding preference of a mosquito species is also a critical determinant of its role in WNV transmission. Mosquito species that feed readily on reservoir hosts are involved in the primary amplification cycle, whereas those that feed primarily on incidental hosts are involved in the secondary transmission cycle.

Culex species mosquitoes are the major amplification vectors of WNV, although the virus has been isolated from mosquitoes belonging to at least 11 other genera: *Aedes*, *Aedemomyia*, *Anopheles*, *Coquilletidia*, *Culiseta*, *Deinocerites*, *Mansonia*, *Mimomyia*, *Orthopodomyia*, *Psorophora* and *Uranoteania* (Hayes, 1988; Zeller and Schuffenecker, 2004; Hayes *et al.*, 2005a). The principal amplification vectors of WNV in Europe and Africa are *Cx. pipiens*, *Culex univittatus* and *Culex antennatus*. In Asia, members of the *Culex vishnui* complex, such as *Culex tritaeniorhynchus*, *Cx. vishnui* and *Culex pseudovishnui*, are major vectors. In Australia, the principal vector is *Culex annulirostris*. In the USA, the major amplification vectors are *Cx. pipiens* and *Culex restuans* in the northeast, *Culex tarsalis* in the west and *Culex quinquefasciatus* in the south.

Cx. pipiens and *Cx. restuans* populations in the northeastern USA are highly competent laboratory vectors of WNV and are strongly ornithophilic; the ratio of blood meals taken from birds and mammals by *Cx. pipiens* and *Cx. restuans* in New York City was 23 : 1 and 6 : 1, respectively (Turell *et al.*, 2000; Sardelis *et al.*, 2001; Apperson *et al.*, 2002). Because of their high abundance, both species have also been implicated as major bridging vectors of WNV in this region (Kilpatrick *et al.*, 2005). *Culex salinarius* is an important bridging vector of

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WNV in the northeastern USA; it is a highly competent laboratory vector and an opportunistic feeder (Sardelis *et al*., 2001; Apperson *et al*., 2004). Seventy-two percent of engorged *Cx. salinarius* from New Jersey had fed on mammals; 25% had fed on birds (Apperson *et al*., 2004). The WNV MIR in *Cx. salinarius* in New York State in 2000 was 1.6 (calculated using RT-PCR data), exceeded only by *Cx. pipiens* which had a MIR of 3.5 (Bernard *et al*., 2001). In this study, the authors considered a county or borough to be in the epicenter of the WNV outbreak if the MIR for any mosquito species in that region was $\geq$1.0. In Connecticut in 2000, the overall MIR in *Culex* spp. mosquitoes was 0.7 (cell culture data), and the MIRs in *Cx. restuans*, *Cx. pipiens* and *Cx. salinarius* were 1.8, 1.4 and 0.5, respectively (Andreadis *et al*., 2001).

As WNV spread across the USA, its geographic distribution overlapped with that of other mosquito species. In 2003, almost one-third of the reported WNV-infected mosquito pools were composed of *Cx. tarsalis* (Hayes *et al*., 2005a). This species is considered to be the principal amplification vector of WNV in the western USA because it is highly abundant in that region and is one of the most efficient laboratory vectors of WNV (Goddard *et al*., 2002; Turell *et al*., 2002, 2005a). *Cx. tarsalis* is ornithophilic, but it also feeds on mammals, particularly in the latter part of the transmission season, and therefore it may also be an important bridging vector (Reisen and Reeves, 1990). The WNV MIR in *Cx. tarsalis* in Colorado in 2003 and 2004 was 34.5 and 8.7, respectively (RT-PCR data) (Bolling *et al*., 2007).

Cx. quinquefasciatus accounted for 51% of the WNV-infected mosquito pools reported in 2004 (Hayes *et al*., 2005a). This species, which is common in the southern USA, is a moderately competent laboratory vector of WNV (Sardelis *et al*., 2001; Goddard *et al*., 2002; Turell *et al*., 2005a). *Cx. quinquefasciatus* populations in the southern USA feed readily on both birds and mammals. For instance, 50% of engorged *Cx. quinquefasciatus* in Arizona had fed on humans, 32% had fed on birds and 12% had fed on dogs (Zinser *et al*., 2004). *Cx. quinquefasciatus* populations in Louisiana fed most frequently (69%) on dogs, followed by birds (16%) and humans (11%) (Niebylski and Meek, 1992). The MIR in *Cx. quinquefasciatus* in Florida in 2001 was 4.8 (cell culture data) (Blackmore *et al*., 2003). *Culex nigripalpus* is also an important vector in the southern USA (Turell *et al*., 2001).

The efficiency with which mosquitoes transmit WNV is dependent upon various environmental factors, particularly temperature and rainfall (Epstein, 2001). Laboratory studies have shown that *Cx. pipiens* and *Cx. tarsalis* held at high (28–30°C) temperatures are more vector competent for WNV than those held at lower (14–22°C) temperatures (Dohm *et al*., 2002; Reisen *et al*., 2006). In this regard, the outbreaks of WNV that took place in Romania in 1996, Russia in 1999 and the USA in 2002–2004 occurred during periods of above-average summer temperatures (Han *et al*., 1999; Platonov *et al*., 2001; Reisen *et al*., 2006). Shaman and colleagues reported that extreme drought, which brings avian hosts and mosquito vectors into close contact, can also increase the intensity of WNV transmission (Shaman *et al*., 2005). Drought-induced transmission has also been described for SLEV (Shaman *et al*., 2002, 2003). Additionally, above-average rainfall can also lead to increased transmission of WNV and other mosquito-borne pathogens, particularly if the availability of larval habitat is limited (Takeda *et al*., 2003; Landesman *et al*., 2007).

Other arthropods

WNV has occasionally been isolated from various species of field-collected argasid (soft) and ixodid (hard) ticks (Hubalek and Halouzka, 1999; Mumcuoglu *et al*., 2005). Laboratory transmission of WNV has been demonstrated for soft ticks but not hard ticks (Anderson *et al*., 2003; Lawrie *et al*., 2004; Hutcheson *et al*., 2005). WNV has also been isolated from mites, and WNV RNA has been detected in hippoboscid flies, but the roles of these arthropods in WNV transmission have not been studied (Farajollahi *et al*., 2005; Mumcuoglu *et al*., 2005).

Non-vector-borne transmission

Although WNV is primarily transmitted to vertebrates by arthropod vectors, various non-vector-borne modes of transmission have been documented (Fig. 2). For example, WNV has been transmitted to humans as a result of organ transplantation, blood transfusion, breast feeding, intrauterine transmission and needle-stick injury (CDC, 2002a, b, c; Iwamoto *et al*., 2003; Mather *et al*., 2003). Oral transmission of WNV has been reported for the American crow, common grackle, great horned owl, house finch and house sparrow (Komar *et al*., 2003a). Direct transmission has been documented with the American crow, blue jay, black-billed magpie and ring-billed gull. American crows can shed $>10^{8.8}$ pfu g^{-1} of WNV in their feces which suggests that exposure to contaminated fecal material is a potential source for direct WNV transmission (Kipp *et al*., 2006). The consumption of WNV-infected horse meat was implicated as the source of the WNV outbreaks in the alligator farm in Georgia in 2001–2002 (Miller *et al*., 2003). Alligators can also become infected via direct contact with infected tank-mates (Klenk *et al*., 2004). Cats have become infected via the ingestion of infected mice (Austgen *et al*., 2004). Taken together, these data suggest that WNV transmission can occur in the absence of mosquito vectors.

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Conclusions

In the last decade, WNV has undergone a dramatic expansion of its geographic range, and has emerged as a major pathogen of humans, horses and birds. This should serve as a reminder of the ongoing threat that vector-borne pathogens present to human and animal health, and highlights the need for continual and improved surveillance, diagnosis and treatment for WNV.

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