

WEST NILE VIRUS: EPIDEMIOLOGY AND ECOLOGY IN NORTH AMERICA

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I. INTRODUCTION

The emergence of West Nile virus (WNV) in eastern North America in 1999 was a major event in modern arbovirology, not because of its disease impact or the potential threat it represented, but because it alerted the world that pathogens may turn up anywhere at any time. Bioterrorism awareness in the United States was increasing and an expected spin-off was preparedness for emerging infectious diseases. However, New York City, the epicenter of the 1999 outbreak, had no capacity for surveillance and control of arboviral diseases. Thus, WNV exposed a great weakness in the U.S. public health system. As a result of the outbreak, and the subsequent spread of WNV across the continent, a surge of resources became available to retool the

public health system for arbovirus surveillance, prevention, and control. These resources have been used to initiate WNV surveillance in almost every state and province of the United States and Canada, and to initiate short- and long-term research projects aimed at understanding the biology of WNV in North America. This article will summarize the background information on this subject, and review the progress made in understanding WNV epidemiology and ecology in the New World.

II. HISTORY

WNV was first recognized in 1937 after it was isolated from blood of a febrile woman in the West Nile District of Uganda (Smithburn *et al.*, 1940). It became known as the etiologic agent of West Nile fever and was occasionally isolated from febrile children in North Africa and the Middle East beginning in the 1950s (Hayes, 2001). The occurrence of a dozen WNV encephalitis cases among elderly victims in Israel in 1957 was the first indication that WNV could cause serious central nervous system infections (Spigland *et al.*, 1958). Equine encephalitis caused by WNV was first noted in the early 1960s, in Egypt and France (Murgue *et al.*, 2001b; Schmidt and El Mansoury, 1963). In 1974, the largest known outbreak of WNV disease caused approximately 10,000 human fever cases in South Africa (Jupp, 2001; McIntosh *et al.*, 1976). In 1996, WNV emerged as a major cause of arboviral encephalitis in Romania, where an outbreak led to 393 recognized human cases of encephalitis, with 16 deaths (Tsai *et al.*, 1998). After 1996, outbreaks of West Nile viral encephalitis in people and horses were reported with increasing frequency in the Mediterranean Basin (Hubalek and Halouzka, 1999; Triki *et al.*, 2001), Russia (Platonov *et al.*, 2001) and Australia (Brown *et al.*, 2002). In 1997, a new strain of WNV that kills young domestic geese (*Anser* spp.) was isolated in Israel (Malkinson and Banet, 2002). An identical strain emerged in New York City in 1999 (Roehrig *et al.*, 2002).

In North America, the New York 1999 (NY99) strain of WNV was first isolated from a dead American Crow* (Lanciotti *et al.*, 1999) and subsequently from carcasses of 22 other bird species collected between August and November 1999 (Anderson *et al.*, 1999; Eidson *et al.*, 2001; Steele *et al.*, 2000). Simultaneously, WNV-specific RNA

*Latin names are provided for most bird species in Table III; otherwise, they are provided in the text.

sequences were identified from brain specimens collected from autopsies of fatal human cases (Briese *et al.*, 1999; Lanciotti *et al.*, 1999). However, the initial human cases were identified on the basis of serologic tests, which indicated that the North American St. Louis encephalitis virus (SLEV) was the likely etiologic agent (Roehrig *et al.*, 2002). SLEV is closely related to WNV, and these viruses cross-react in serologic tests. Subsequent to the identification of WNV, serologic results were reevaluated to include WNV in the testing panel, and stronger serologic reactions to WNV were observed in the patients' sera than to SLEV (Martin *et al.*, 2002).

After the initial North American outbreak in 1999, WNV overwintered in New York, with mid-winter infections discovered in hibernating mosquitoes (Nasci *et al.*, 2001b) and a fresh carcass of a Red-tailed Hawk (Garmendia *et al.*, 2000). After 1999, WNV continued to cause sporadic equine and human disease in the United States (CDC, 2002a; Marfin *et al.*, 2001), reaching Canada in 2001. In 2002, the largest outbreak of WNV encephalitis ever recorded occurred in the United States, with numerous epicenters spread across the nation's mid-section, and virus activity occurring coast-to-coast, breaching both the Canadian (Pepperell *et al.*, 2003) and Mexican borders (Blitvich *et al.*, 2003; Loroño-Pino *et al.*, 2003).

III. CLINICAL DESCRIPTION

A. Human

WNV infection in humans causes a spectrum of manifestations from subclinical infection to death (Petersen and Marfin, 2002). Most infections are subclinical but occasionally clinical manifestations will develop 2–21 days after infection. Cases lacking neurologic manifestations generally do not require hospitalization, and are termed “West Nile fever” (WNF). Neurologic cases usually involve meningoencephalitis, and have been termed “West Nile meningoencephalitis” (WNME). Asnis *et al.* (2000) published observations from a set of eight WNME patients evaluated in the 1999 New York City outbreak. The clinical picture in this group was similar to findings in Europe (Ceausu *et al.*, 1997) and the Middle East (Chowers *et al.*, 2001). The most common symptoms of cases requiring hospitalization were fever, gastrointestinal complaints, and change in mental status. Half the patients reported headache or severe muscle weakness. Two larger studies evaluated clinical characteristics of 59 and 19 hospitalized

patients, respectively (Nash *et al.*, 2001; Weiss *et al.*, 2001). No one manifestation was common to all cases, but general malaise, fatigue and flu-like gastrointestinal symptoms were common (Table I). Rare characteristics affecting less than 10% of the patients are not shown in Table I, and included tremors, shortness of breath, slurred speech, abdominal pain, focal sensory changes, pharyngitis, conjunctivitis, seizures, and lymphadenopathy. Hypertension, diabetes mellitus, and coronary artery disease were the most common underlying medical problems. Immunosuppression had been documented in 14% of the 1999 patients.

Two characteristics common in previous outbreaks of West Nile fever have been a generalized roseolar or macropapular rash and lymphadenopathy. However, these signs have occurred in proportionally fewer cases in North America (Campbell *et al.*, 2002).

Unexpected findings in North America have included a poliomyelitis-like flaccid paralysis (Asnis *et al.*, 2000; Solomon and Ravi, 2003), Guillain-Barré syndrome (Ahmed *et al.*, 2000), profound muscle weakness (Sampson and Armbrustmacher, 2001), and eye abnormalities including uveitis, vitritis, and chorioretinitis (Bains *et al.*, 2003). Neurologic sequelae caused complications in 31% of a cohort of elderly WNME survivors (Berner *et al.*, 2002), but may affect as many as 50% (Petersen and Marfin, 2002; Weiss *et al.*, 2001), and often require long-term rehabilitation. Further studies are needed to characterize these sequelae.

Recent pathologic studies in a small number of fatal human cases (Sampson *et al.*, 2000; Shieh *et al.*, 2000) have confirmed similar findings as with Japanese encephalitis (reviewed in Solomon and Vaughn, 2002), including formation of microglial nodules and perivascular cuffing in the brain parenchyma. Lesions have been observed in the brainstem, spinal cord, thalamus, cortex, and cerebellum.

B. Equine

WNV infection in horses and other domestic equids ranges from asymptomatic to fatal encephalitis. A higher proportion of infected horses develop encephalitis compared with humans. Experimental studies suggest that about 10% of infected horses develop clinical illness (Bunning *et al.*, 2002). In naturally infected horses, WNV infection typically causes attitudinal changes (somnia, listlessness, apprehension, depression, or hyperexcitability) and neurologic signs including muscle fasciculations and limb paresis or paralysis (Table II).

TABLE I
CLINICAL CHARACTERISTICS OF 78 PATIENTS HOSPITALIZED WITH
WEST NILE VIRUS INFECTION IN 1999–2000

Signs and Symptoms	No. Patients	%
Fever	70	90
Weakness	41	53
Headache	39	50
Nausea	39	50
Vomiting	38	49
Altered mental status	38	49
Diarrhea	19	24
Stiff neck	17	22
Myalgia	16	21
Rash	14	18
Cough	14	18
Photophobia	14	18
Arthralgia	9	12

From Nash *et al.*, 2001; Weiss *et al.*, 2001.

TABLE II
CLINICAL SIGNS IN HORSES WITH WEST NILE ENCEPHALITIS

Sign	%
Ataxia	85
Weakness of limbs	48
Recumbency	45
Muscle fasciculation	40
Fever	23
Paralyzed or drooping lip	18
Twitching face or muzzle	13
Teeth grinding	7
Blindness	5

From Ostlund *et al.*, 2001.

Pathologic findings of horses infected in North America have not yet been reported. They are presumably similar to pathology described for the 1998 equine outbreak in Italy, in which spinal cord was the most affected tissue (Cantile *et al.*, 2000). Histologic lesions were observed

in the brain stem and gray matter of the spinal cord in a fatal case of equine WNV in Israel in 2000 (Steinman *et al.*, 2002).

C. Avian

WNV-infected birds also suffer a spectrum of clinical outcomes ranging from no disease to death. Mortality attributable to WNV infection in North America has been reported in 198 species of birds through 2002 (Table III). Some species of birds, especially corvids* are highly susceptible to fatal outcome (Komar *et al.*, 2003a; McLean *et al.*, 2002). General signs of infection include lethargy, recumbency, and in some cases, hemorrhage (Komar *et al.*, 2003a). Swayne *et al.* (2001) documented abnormal posture in a domestic gosling (*Anser anser domesticus*). Because of the high rates of natural infection in birds during epizootics (see Section VI,C), disease in seropositive birds may be difficult to attribute to WNV infection. This is particularly a problem for captive birds such as those in zoos and wildlife rehabilitation centers, where veterinary care favors survival of birds with chronic conditions. Many of these birds are seropositive for WNV, but the etiology of their clinical signs (such as blindness in great horned owls) remains unknown.

In North America, gross and histopathologic studies have described the pathogenesis of natural, acute fatal WNV infection in birds for 14 species, representing eight orders (Steele *et al.*, 2000) and experimental infection in domestic geese (Swayne *et al.*, 2001). Brain hemorrhage, splenomegaly, meningoencephalitis, and myocarditis were the prominent findings on gross examination. Numerous cell types were damaged, in various tissues. Purkinje cells were particularly targeted except in corvids. The cause of death in most of these birds is probably multiple organ failure.

D. Other Vertebrates

Little is known of the clinical manifestation of WNV in other vertebrates, such as reptiles and amphibians and other mammals. In North America, captive alligators have died from WNV infection (Miller *et al.*, 2003), and fatal infections have been informally reported through 2002 in approximately 20 species of mammals in addition to horses and people (see Section VI,B).

*Members of the family *Corvidae*, Order Passeriformes.

TABLE III
LIST OF 198 BIRD SPECIES FATALLY AFFECTED BY WEST NILE VIRUS IN NORTH AMERICA^{a,b,c}

Common Name	Latin Name	Family	Order	Status ^d
Elegant crested tinamou	<i>Eudromia elegans</i>	Tinamidae	Tinamiformes	Exotic ^e
Emu	<i>Dromaius novaehollandiae</i>	Dromaiidae	Casuariiformes	Exotic ^e
Common loon	<i>Gavia immer</i>	Gaviidae	Gaviiformes	Native
Pied-billed grebe	<i>Podilymbus podiceps</i>	Podicipedidae	Podicipediformes	Native
Humboldt penguin	<i>Spheniscus Humboldti</i>	Spheniscadae	Sphenisciformes	Exotic ^e
Black-footed penguin	<i>Spheniscus demersus</i>	Spheniscadae	Sphenisciformes	Exotic ^e
American white pelican	<i>Pelecanus erythrorhynchos</i>	Pelecanidae	Pelecaniformes	Native
Double-crested cormorant	<i>Phalacrocorax auritus</i>	Phalacrocoracidae	Pelecaniformes	Native
Guanay cormorant	<i>Phalacrocorax bougainvillei</i>	Phalacrocoracidae	Pelecaniformes	Exotic ^e
Least bittern	<i>Ixobrychus exilis</i>	Ardeidae	Ciconiiformes	Native
Great blue heron	<i>Ardea herodias</i>	Ardeidae	Ciconiiformes	Native
Great egret	<i>Ardea alba</i>	Ardeidae	Ciconiiformes	Native
Green heron	<i>Butorides virescens</i>	Ardeidae	Ciconiiformes	Native
Black-crowned night heron	<i>Nycticorax nycticorax</i>	Ardeidae	Ciconiiformes	Native
Yellow-crowned night heron	<i>Nyctanassa violacea</i>	Ardeidae	Ciconiiformes	Native
Scarlet ibis	<i>Eudocimus ruber</i>	Threskiornithidae	Ciconiiformes	Exotic ^e
Black vulture	<i>Coragyps atratus</i>	Cathartidae	Ciconiiformes	Native
Turkey vulture	<i>Cathartes aura</i>	Cathartidae	Ciconiiformes	Native
Chilean flamingo	<i>Phoenicopterus chilensis</i>	Phoenicopteridae	Phoenicopteriformes	Exotic ^e
Greater flamingo	<i>Phoenicopterus ruber</i>	Phoenicopteridae	Phoenicopteriformes	Exotic ^e
Canada goose	<i>Branta canadensis</i>	Anatidae	Anseriformes	Native
Hawaiian goose	<i>Branta sandvicensis</i>	Anatidae	Anseriformes	Exotic ^e

(continues)

TABLE III (continued)

Common Name	Latin Name	Family	Order	Status ^d
Red-breasted goose	<i>Branta ruficollis</i>	Anatidae	Anseriformes	Exotic ^e
Emperor goose	<i>Chen canagica</i>	Anatidae	Anseriformes	Native ^e
Mute swan	<i>Cygnus olor</i>	Anatidae	Anseriformes	Introduced
Tundra swan	<i>Cygnus columbianus</i>	Anatidae	Anseriformes	Native ^e
Wood duck	<i>Aix sponsa</i>	Anatidae	Anseriformes	Native
Bronze-winged duck	<i>Anas specularis</i>	Anatidae	Anseriformes	Exotic ^e
Eurasian wigeon	<i>Anas penelope</i>	Anatidae	Anseriformes	Native ^e
Mallard	<i>Anas platyrhynchos</i>	Anatidae	Anseriformes	Native
Cinnamon teal	<i>Anas cyanoptera</i>	Anatidae	Anseriformes	Native ^e
Yellow-billed duck	<i>Anas undulata</i>	Anatidae	Anseriformes	Exotic ^e
Puna teal	<i>Anas puna</i>	Anatidae	Anseriformes	Exotic ^e
Canvasback	<i>Aythya valisineria</i>	Anatidae	Anseriformes	Native
Greater scaup	<i>Aythya marila</i>	Anatidae	Anseriformes	Native ^e
Lesser scaup	<i>Aythya affinis</i>	Anatidae	Anseriformes	Native ^e
Bufflehead	<i>Bucephala albeola</i>	Anatidae	Anseriformes	Native ^e
Common goldeneye	<i>Bucephala clangula</i>	Anatidae	Anseriformes	Native ^e
Smew	<i>Mergellus albellus</i>	Anatidae	Anseriformes	Exotic ^e
Common merganser	<i>Mergus merganser</i>	Anatidae	Anseriformes	Native ^e
Ruddy duck	<i>Oxyura jamaicensis</i>	Anatidae	Anseriformes	Native
Osprey	<i>Pandion haliaetus</i>	Accipitridae	Falconiformes	Native
Swallow-tailed kite	<i>Elanoides forficatus</i>	Accipitridae	Falconiformes	Native
Mississippi kite	<i>Ictinia mississippiensis</i>	Accipitridae	Falconiformes	Native
Bald eagle	<i>Haliaeetus leucocephalus</i>	Accipitridae	Falconiformes	Native ^e

Northern harrier	<i>Circus cyaneus</i>	Accipitridae	Falconiformes	Native
Sharp-shinned hawk	<i>Accipiter striatus</i>	Accipitridae	Falconiformes	Native
Cooper's hawk	<i>Accipiter cooperii</i>	Accipitridae	Falconiformes	Native
Northern goshawk	<i>Accipiter gentilis</i>	Accipitridae	Falconiformes	Native
Harris' hawk	<i>Parabuteo unicinctus</i>	Accipitridae	Falconiformes	Native ^e
Red-shouldered hawk	<i>Buteo lineatus</i>	Accipitridae	Falconiformes	Native
Broad-winged hawk	<i>Buteo platypterus</i>	Accipitridae	Falconiformes	Native
Swainson's hawk	<i>Buteo swainsoni</i>	Accipitridae	Falconiformes	Native
Red-tailed hawk	<i>Buteo jamaicensis</i>	Accipitridae	Falconiformes	Native
Rough-legged hawk	<i>Buteo lagopus</i>	Accipitridae	Falconiformes	Native ^e
Golden eagle	<i>Aquila chrysaetos</i>	Accipitridae	Falconiformes	Native ^e
Wedge-tailed eagle	<i>Aquila audax</i>	Accipitridae	Falconiformes	Exotic ^e
American kestrel	<i>Falco sparverius</i>	Falconidae	Falconiformes	Native
Merlin	<i>Falco columbarius</i>	Falconidae	Falconiformes	Native
Prairie falcon	<i>Falco mexicanus</i>	Falconidae	Falconiformes	Native ^e
Peregrine falcon	<i>Falco peregrinus</i>	Falconidae	Falconiformes	Native
Domestic chicken	<i>Gallus gallus</i>	Phasianidae	Galliformes	Exotic ^e
Ring-necked pheasant	<i>Phasianus colchicus</i>	Phasianidae	Galliformes	Introduced
Impeyan pheasant	<i>Lophophorus impeyanus</i>	Phasianidae	Galliformes	Exotic ^e
Monal pheasant	<i>Lophophorus ihuysii</i>	Phasianidae	Galliformes	Exotic ^e
Common peafowl	<i>Pavo cristatus</i>	Phasianidae	Galliformes	Exotic ^e
Blythe's tragopan	<i>Tragopan blythi</i>	Phasianidae	Galliformes	Exotic ^e
Satyr tragopan	<i>Tragopan satyr</i>	Phasianidae	Galliformes	Exotic ^e
Ruffed grouse	<i>Bonasa umbellus</i>	Phasianidae	Galliformes	Native
Wild turkey	<i>Meleagris gallopavo</i>	Phasianidae	Galliformes	Native
Northern bobwhite	<i>Colinus virginianus</i>	Odontophoridae	Galliformes	Native

(continues)

TABLE III (continued)

Common Name	Latin Name	Family	Order	Status ^d
Virginia rail	<i>Rallus limicola</i>	Rallidae	Gruiformes	Native
Sandhill crane	<i>Grus canadensis</i>	Gruidae	Gruiformes	Native ^e
Killdeer	<i>Charadrius vociferus</i>	Charadriidae	Charadriiformes	Native
Ruddy turnstone	<i>Arenaria interpres</i>	Scolopacidae	Charadriiformes	Native
Laughing gull	<i>Larus atricilla</i>	Laridae	Charadriiformes	Native
Ring-billed gull	<i>Larus delawarensis</i>	Laridae	Charadriiformes	Native
Herring gull	<i>Larus argentatus</i>	Laridae	Charadriiformes	Native
Great black-backed gull	<i>Larus marinus</i>	Laridae	Charadriiformes	Native
Inca tern	<i>Larosterna inca</i>	Laridae	Charadriiformes	Exotic ^e
Black skimmer	<i>Rhynchops niger</i>	Laridae	Charadriiformes	Native
Rock dove	<i>Columba livia</i>	Columbidae	Columbiformes	Introduced
White-crowned pigeon	<i>Columba leucocephala</i>	Columbidae	Columbiformes	Native
Eurasian collared-dove	<i>Streptopelia decaocto</i>	Columbidae	Columbiformes	Introduced
White-winged dove	<i>Zenaida asiatica</i>	Columbidae	Columbiformes	Native
Mourning dove	<i>Zenaida macroura</i>	Columbidae	Columbiformes	Native
Common ground-dove	<i>Columbina passerina</i>	Columbidae	Columbiformes	Native
Budgerigar	<i>Melopsittacus undulatus</i>	Psittacidae	Psittaciformes	Introduced ^e
Pacific parrotlet	<i>Forpus coelestis</i>	Psittacidae	Psittaciformes	Exotic ^e
Macaw	<i>Ara species</i>	Psittacidae	Psittaciformes	Exotic ^e
Red-crowned parrot	<i>Amazona viridigenalis</i>	Psittacidae	Psittaciformes	Exotic ^e
Thick-billed parrot	<i>Rhynchopsitta pachyrhyncha</i>	Psittacidae	Psittaciformes	Exotic ^e
Rainbow lorikeet	<i>Trichoglossus haematodus</i>	Psittacidae	Psittaciformes	Exotic ^e
Violet-necked lorikeet	<i>Eos beckstein</i>	Psittacidae	Psittaciformes	Exotic ^e

Blue-streaked lory	<i>Eos reticulate</i>	Psittacidae	Psittaciformes	Exotic ^e
Red lory	<i>Eos bornea</i>	Psittacidae	Psittaciformes	Exotic ^e
Dusky lory	<i>Pseudeos fuscata</i>	Psittacidae	Psittaciformes	Exotic ^e
Black-capped lory	<i>Lorius lory</i>	Psittacidae	Psittaciformes	Exotic ^e
Crimson rosella	<i>Platycercus elegans</i>	Psittacidae	Psittaciformes	Exotic ^e
Cockatoo	<i>Cacatua species</i>	Cacatuidae	Psittaciformes	Exotic ^e
Cockatiel	<i>Nymphicus hollandicus</i>	Cacatuidae	Psittaciformes	Exotic ^e
Yellow-billed cuckoo	<i>Coccyzus americanus</i>	Cuculidae	Cuculiformes	Native
Barn owl	<i>Tyto alba</i>	Tytonidae	Strigiformes	Native
Eastern screech-owl	<i>Otus asio</i>	Strigidae	Strigiformes	Native
Great horned owl	<i>Bubo virginianus</i>	Strigidae	Strigiformes	Native
Snowy owl	<i>Nyctea scandiaca</i>	Strigidae	Strigiformes	Native ^e
Northern hawk owl	<i>Surnia ulula</i>	Strigidae	Strigiformes	Native ^e
Spotted owl	<i>Strix occidentalis</i>	Strigidae	Strigiformes	Native ^e
Barred owl	<i>Strix varia</i>	Strigidae	Strigiformes	Native
Great gray owl	<i>Strix nebulosa</i>	Strigidae	Strigiformes	Native ^e
Long-eared owl	<i>Asio otus</i>	Strigidae	Strigiformes	Native
Tawny owl	<i>Strix aluco</i>	Strigidae	Strigiformes	Exotic ^e
Short-eared owl	<i>Asio flammeus</i>	Strigidae	Strigiformes	Native
Boreal owl	<i>Aegolius funereus</i>	Strigidae	Strigiformes	Native ^e
Northern saw-whet owl	<i>Aegolius acadicus</i>	Strigidae	Strigiformes	Native
Common nighthawk	<i>Chordeiles minor</i>	Caprimulgidae	Caprimulgiformes	Native
Chimney swift	<i>Chaetura pelagica</i>	Apodidae	Apodiformes	Native
Ruby-throated hummingbird	<i>Archilochus colubris</i>	Trochilidae	Apodiformes	Native
Belted kingfisher	<i>Ceryle alcyon</i>	Alcedinidae	Coraciiformes	Native
Micronesian kingfisher	<i>Halcyon cinnamomima</i>	Alcedinidae	Coraciiformes	Exotic ^e

(continues)

TABLE III (continued)

Common Name	Latin Name	Family	Order	Status ^d
Abyssinian ground-hornbill	<i>Bucorvus abyssinicus</i>	Bucorvidae	Coraciiformes	Exotic ^e
Red-headed woodpecker	<i>Melanerpes erythrocephalus</i>	Picidae	Piciformes	Native
Yellow-bellied sapsucker	<i>Sphyrapicus varius</i>	Picidae	Piciformes	Native
Downy woodpecker	<i>Picoides pubescens</i>	Picidae	Piciformes	Native
Traill's flycatcher	<i>Empidonax traillii alnorum</i>	Tyrannidae	Passeriformes	Native
Eastern phoebe	<i>Sayornis phoebe</i>	Tyrannidae	Passeriformes	Native
Eastern kingbird	<i>Tyrannus tyrannus</i>	Tyrannidae	Passeriformes	Native
Scissor-tailed flycatcher	<i>Tyrannus forficatus</i>	Tyrannidae	Passeriformes	Native
Loggerhead shrike	<i>Lanius ludovicianus</i>	Laniidae	Passeriformes	Native
Warbling vireo	<i>Vireo gilvus</i>	Vireonidae	Passeriformes	Native
Red-eyed vireo	<i>Vireo olivaceus</i>	Vireonidae	Passeriformes	Native
Black-whiskered vireo	<i>Vireo altiloquus</i>	Vireonidae	Passeriformes	Native
Eurasian jay	<i>Garrulus glandarius</i>	Corvidae	Passeriformes	Exotic ^e
Steller's jay	<i>Cyanocitta stelleri</i>	Corvidae	Passeriformes	Native
Blue jay	<i>Cyanocitta cristata</i>	Corvidae	Passeriformes	Native
Western scrub-jay	<i>Aphelocoma californica</i>	Corvidae	Passeriformes	Native
Clark's nutcracker	<i>Nucifraga columbiana</i>	Corvidae	Passeriformes	Native ^e
Black-billed magpie	<i>Pica hudsonia</i>	Corvidae	Passeriformes	Native
American crow	<i>Corvus brachyrhynchos</i>	Corvidae	Passeriformes	Native
Fish crow	<i>Corvus ossifragus</i>	Corvidae	Passeriformes	Native
Hooded crow	<i>Corvus corone</i>	Corvidae	Passeriformes	Exotic ^e
Common raven	<i>Corvus corax</i>	Corvidae	Passeriformes	Native
Purple martin	<i>Progne subis</i>	Hirundinidae	Passeriformes	Native

Barn swallow	<i>Hirundo rustica</i>	Hirundinidae	Passeriformes	Native
Varied tit	<i>Parus varius</i>	Paridae	Passeriformes	Exotic ^e
Carolina chickadee	<i>Poecile carolinensis</i>	Paridae	Passeriformes	Native
Black-capped chickadee	<i>Poecile atricapillus</i>	Paridae	Passeriformes	Native
Tufted titmouse	<i>Parus bicolor</i>	Paridae	Passeriformes	Native
White-breasted nuthatch	<i>Sitta carolinensis</i>	Sittidae	Passeriformes	Native
Carolina wren	<i>Thryothaurus ludovicianus</i>	Troglodytidae	Passeriformes	Native
Winter wren	<i>Troglodytes troglodytes</i>	Troglodytidae	Passeriformes	Native
Eastern bluebird	<i>Sialia sialis</i>	Turdidae	Passeriformes	Native
Veery	<i>Catharus fuscescens</i>	Turdidae	Passeriformes	Native
Gray-cheeked thrush	<i>Catharus minimus</i>	Turdidae	Passeriformes	Native
Swainson's thrush	<i>Catharus ustulatus</i>	Turdidae	Passeriformes	Native
Hermit thrush	<i>Catharus guttatus</i>	Turdidae	Passeriformes	Native
Wood thrush	<i>Hylocichla mustelina</i>	Turdidae	Passeriformes	Native
American robin	<i>Turdus migratorius</i>	Turdidae	Passeriformes	Native
Gray catbird	<i>Dumetella carolinensis</i>	Mimidae	Passeriformes	Native
Northern mockingbird	<i>Mimus polyglottos</i>	Mimidae	Passeriformes	Native
Brown thrasher	<i>Toxostoma rufum</i>	Mimidae	Passeriformes	Native
European starling	<i>Sturnus vulgaris</i>	Sturnidae	Passeriformes	Introduced
Cedar waxwing	<i>Bombicilla cedrorum</i>	Bombycillidae	Passeriformes	Native
Nashville warbler	<i>Vermivora ruficapilla</i>	Parulidae	Passeriformes	Native
Northern parula	<i>Parula americana</i>	Parulidae	Passeriformes	Native
Yellow warbler	<i>Dendroica petechia</i>	Parulidae	Passeriformes	Native
Black-throated blue warbler	<i>Dendroica caerulescens</i>	Parulidae	Passeriformes	Native
Yellow-rumped warbler	<i>Dendroica coronata</i>	Parulidae	Passeriformes	Native
Blackpoll warbler	<i>Dendroica striata</i>	Parulidae	Passeriformes	Native

(continues)

TABLE III (continued)

Common Name	Latin Name	Family	Order	Status ^d
Ovenbird	<i>Seiurus aurocapillus</i>	Parulidae	Passeriformes	Native
Northern waterthrush	<i>Seiurus noveboracensis</i>	Parulidae	Passeriformes	Native
Kentucky warbler	<i>Oporornis formosus</i>	Parulidae	Passeriformes	Native
Common yellowthroat	<i>Geothlypis trichas</i>	Parulidae	Passeriformes	Native
Hooded warbler	<i>Wilsonia citrina</i>	Parulidae	Passeriformes	Native
Canada warbler	<i>Wilsonia canadensis</i>	Parulidae	Passeriformes	Native
American goldfinch	<i>Carduelis tristis</i>	Fringillidae	Passeriformes	Native
Eastern towhee	<i>Pipilo erythrophthalmus</i>	Emberizidae	Passeriformes	Native
Field sparrow	<i>Spizella pusilla</i>	Emberizidae	Passeriformes	Native
Savannah sparrow	<i>Passerculus sandwichensis</i>	Emberizidae	Passeriformes	Native
Fox sparrow	<i>Passerella iliaca</i>	Emberizidae	Passeriformes	Native
Song sparrow	<i>Melospiza melodia</i>	Emberizidae	Passeriformes	Native
Northern cardinal	<i>Cardinalis cardinalis</i>	Cardinalidae	Passeriformes	Native
Rose-breasted grosbeak	<i>Pheucticus ludovicianus</i>	Cardinalidae	Passeriformes	Native
Dickcissel	<i>Spiza americana</i>	Cardinalidae	Passeriformes	Native
Red-winged blackbird	<i>Agelaius phoeniceus</i>	Icteridae	Passeriformes	Native
Rusty blackbird	<i>Euphagus carolinus</i>	Icteridae	Passeriformes	Native
Brewer's blackbird	<i>Euphagus cyanocephalus</i>	Icteridae	Passeriformes	Native
Common grackle	<i>Quiscalus quiscula</i>	Icteridae	Passeriformes	Native
Boat-tailed grackle	<i>Quiscalus major</i>	Icteridae	Passeriformes	Native
Great-tailed grackle	<i>Quiscalus mexicanus</i>	Icteridae	Passeriformes	Native
Brown-headed cowbird	<i>Molothrus ater</i>	Icteridae	Passeriformes	Native
Baltimore oriole	<i>Icterus galbula</i>	Icteridae	Passeriformes	Native

Purple finch	<i>Carpodacus purpureus</i>	Fringillidae	Passeriformes	Native
House finch	<i>Carpodacus mexicanus</i>	Fringillidae	Passeriformes	Native
European goldfinch	<i>Carduelis carduelis</i>	Fringillidae	Passeriformes	Exotic ^e
Evening grosbeak	<i>Coccothraustes vespertinus</i>	Fringillidae	Passeriformes	Native
House sparrow	<i>Passer domesticus</i>	Passeridae	Passeriformes	Introduced
Zebra finch	<i>Taeniopygia guttata</i>	Estrildidae	Passeriformes	Exotic ^e

^a Compiled through 2002 from reports to Centers for Disease Control and Prevention's ArboNET surveillance databank (CDC, unpublished data), CDC's WNV-zoo surveillance program (Dominic Travis, Amy Glaser, personal communication), U.S.G.S. National Wildlife Health Center (Emi K. Saito, personal communication), Canadian Wildlife Service (Ian Barker, personal communication), and peer-reviewed publications.

^b Classification follows the A.O.U. Check-list of North American Birds, Seventh Edition (AOU, 2002).

^c The correct identification of these species is not guaranteed.

^d "Native" refers to species naturally occurring in North America; "Introduced" refers to non-native (exotic) species that have established free-ranging populations; "Exotic" indicates non-native species, without established free-ranging populations.

^e Bird(s) died in captivity only.

IV. GEOGRAPHIC DISTRIBUTION

The geographic distribution of WNV is known from human and equine outbreaks, avian epizootics (particularly in North America) and serosurveys of vertebrate hosts (Hayes, 1989; Hubalek and Halouzka, 1999; Komar, 2000). In Africa, WNV is known from most countries where arbovirus studies have been conducted, from South Africa and Madagascar in the extreme south to Morocco, Algeria, Tunisia, and Egypt in the north. Its range extends from Africa eastward through the Middle East into south Asia, where it is known from Pakistan and India. It extends northward into southern Russia, and westward through southern Europe. A variant of WN virus, Kunjin virus, is present in Australia and contiguous regions of Southeast Asia (Hall *et al.*, 2002; Scherret *et al.*, 2001).

In North America through the end of 2002, WNV had spread to every continental U.S. state except Oregon, Utah, Nevada, and Arizona (CDC, 2002k); the Canadian provinces of Saskatchewan, Manitoba, Ontario, Quebec, and Nova Scotia (P. Buck, personal communication); the Mexican states of Coahuila (Blitvich *et al.*, 2003) and Yucatan (Loroño-Pino *et al.*, 2003); the Cayman Islands (CDC, 2002a), Jamaica (DuPuis *et al.*, 2003), and the Dominican Republic (Komar *et al.*, 2003b) in the Caribbean Basin (Fig. 1).



FIG 1. Distribution of West Nile virus in North America through 2002. The single points on southern California and the Cayman Islands represent single human cases, whereas the points in Mexico, Jamaica and the Dominican Republic represent serologic evidence for local transmission in horses (Mexico) and birds (Caribbean nations).

V. MOLECULAR EPIDEMIOLOGY

WNV is a *Flavivirus* (family *Flaviviridae*). Its structure and size are similar to other flaviviruses, including the prototype, yellow fever virus. A large body of knowledge of the molecular biology of WNV has recently been reviewed (Brinton, 2002). Numerous strains of WNV have been isolated, separated by time and space, since 1937. Phylogenetically these strains make up a grouping of closely related viruses (Fig. 2). At least two separate genetic lineages of WNV have been described (Berthet *et al.*, 1997; Burt *et al.*, 2002; Lanciotti *et al.*, 1999, 2002; Scherret *et al.*, 2001). Lanciotti *et al.* place all of the European, Middle Eastern, South Asian, Australian (Kunjin virus), and North American strains in Lineage 1. This lineage includes the strains that have caused encephalitis outbreaks in humans and horses. It also includes some African strains. Lineage 2 includes southern African strains, including some from central Africa and the Ugandan prototype strain isolated in 1937. Although Lineage 2 viruses have not been associated with outbreaks of severe disease, one South African strain was responsible for the largest WNF outbreak recorded, with over 10,000 mild fever cases in 1974. However, these strains have caused only isolated cases of human encephalitis and hepatitis, canine and equine

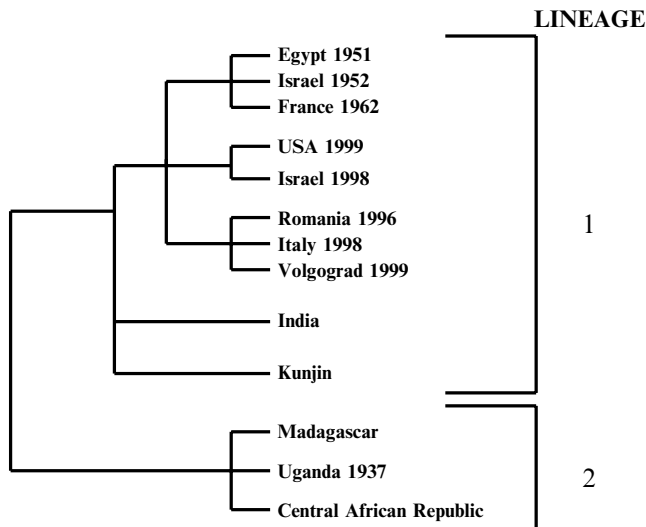


FIG 2. Simplified representation of a phylogenetic tree showing the genetic relatedness of different strains of West Nile virus, including separation into two distinct lineages.

encephalitis, and a fatality in an ostrich (*Struthio camelus*) chick (Burt *et al.*, 2002). Scherret *et al.* (2001) suggest that four or five separate WNV subgroups may be recognized. In this phylogeny, the south Asian, Malaysian, and Australian strains form three additional subgroups in addition to the Lineage 1 and 2 groups.

WNV is most closely related to other flaviviruses of the Japanese encephalitis antigenic complex, which includes Japanese encephalitis virus (JEV) in Asia; Murray Valley encephalitis (MVEV) and Alfuy viruses in Australia; Koutango and Usutu viruses in Africa; and SLEV, Rocio, Ilheus, and Cacipacore viruses in the Americas (Calisher *et al.*, 1989). Interestingly, a phylogenetic analysis determined that SLEV, although it cross-reacts with JEV serocomplex virus such as WNV, in fact falls within a separate clade that includes members of the Ntaya serocomplex (Kuno *et al.*, 1998). Serologic cross-reactions between WNV and SLEV occur at relatively low levels (Johnson *et al.*, 2003; Komar *et al.*, 2001a; Martin *et al.*, 2002). Of WNV's close relatives, a number cause encephalitis in humans (JEV, SLEV, MVEV, Rocio), and Usutu virus has been recently discovered as the etiologic agent of fatal infections of European blackbirds (*Turdus merula*) and other birds in Austria (Weissenbock *et al.*, 2002).

VI. EPIDEMIOLOGY AND EPIZOOTIOLOGY

A. Disease Incidence in Humans

In North America, human cases of WNME were first detected in New York City in August 1999 (Nash *et al.*, 2001), and continued to occur at low levels in 2000 and 2001 in a consistently growing geographic area. In 2002, case numbers increased exponentially (Table IV). Case-fatality rates (CFR) have varied over time and space, depending mainly on the local definition of a case and upon the intensity of surveillance for mild cases. Overall in the United States since 1999, there have been 217 deaths reported (through November 30, 2002) and 3536 cases, indicating a cumulative CFR of 6.1%. If non-neurologic cases (i.e., West Nile fever) are excluded, the CFR would be higher (8.5% in 2002; CDC, 2002k).

Clusters of human cases suggestive of focal outbreaks occurred in New York in 1999 (Nash *et al.*, 2001) and 2000 (Weiss *et al.*, 2001), and in the following states (greater than 100 cases) in 2002: Illinois (492 cases), Michigan (437), Ohio (277), Louisiana (202), and Texas (164) (CDC, 2002k).

TABLE IV
REPORTED NUMBER OF HUMAN AND EQUINE WEST NILE VIRUS DISEASE CASES AND CASE-FATALITY RATES, USA, 1999–2002^a

Year	Human			Equine		
	Cases	Deaths	CFR	Cases	Deaths	CFR
1999	62	7	11.3%	20	9	40.0%
2000	21	2	9.5%	60	23	38.3%
2001	64	9	14.1%	733	N.R.	N.R.
2002 ^b	3389	199	5.9%	9144	N.R.	N.R.

N.R., not reported.

CFR, Case-fatality rate.

^a Canadian cases not included in this table. In 2002, Health Canada reported 390 human cases.

^b Data for 2002 is incomplete, and includes data reported to CDC ArboNET through November 30, 2002 (Chow *et al.*, 2002).

B. Disease Incidence in Other Vertebrates

Significant natural morbidity in non-human mammals has been reported only in equids, including horses, donkeys, and mules. Recent equine epizootics have been described in Morocco in 1996 (Tber, 1996), Italy in 1998 (Autorino *et al.*, 2002), Israel in 2000 (Steinman *et al.*, 2002), and France in 2000 (Murgue *et al.*, 2001a). In North America, numbers of affected equids has increased annually since 1999 (Table IV). The initial equine outbreak was clustered on Long Island, NY, about 50 miles east of New York City, in September–October 1999 (Trock *et al.*, 2001). The subsequent increase in cases reflects the geographic spread of WNV and the increase in the equine population at risk (CDC, 2002a, 2002k; Ostlund *et al.*, 2001). The exponential increases in 2001 and 2002 probably also reflect spread of WNV into regions in which *Culex* vectors feed more frequently on horses, such as *Cx. quinquefasciatus* in the Southeast and *Cx. tarsalis* in the Great Plains states.

Whereas most other mammals appear to be susceptible to infection with WNV, few become ill or die. In North America, small numbers of disease cases and deaths attributed to WNV have occurred in squirrels (*Sciurus carolinensis* and *S. niger*) (CDC, 2002k; Heinz-Taheny *et al.*, 2004; Marfin *et al.*, 2001), an eastern chipmunk (*Tamias striatus*), a big brown bat (*Eptesicus fuscus*), a little brown bat (*Myotis lucifugas*), a striped skunk (*Mephitis mephitis*), a domestic rabbit (*Oryctolagus cuniculus*) (Marfin *et al.*, 2001), and a domestic cat (*Felis catus*) (Komar,

2000). Three dogs were reported in 2002 (CDC, 2002k). Before the North American outbreak, the only previous report of WNV illness in mammals other than people and horses had described WNV infection in a dog from Botswana (Burt *et al.*, 2002; Simpson and Kuebart, 1979).

In 1997–2000, significant avian mortality was observed in Israel, with outbreaks in young domestic geese, as well as some migrating white storks (*Ciconia ciconia*), captive white-eyed gulls (*Larus leucophthalmus*), and a lappet-faced vulture (*Torgos tracheliotus*) (Malkinson and Banet, 2002). Before 1997, the only report of natural WNV-associated morbidity in birds was a sick fledgling pigeon (a.k.a. rock dove) in Egypt in the early 1950s (Work *et al.*, 1953) and an isolate from a dead ostrich chick in South Africa in 1994 (Burt *et al.*, 2002). However, experimental infections in hooded crows (*Corvus corone sardonius*) and house sparrows resulted in 100% and 79% mortality, respectively (Work *et al.*, 1955). Experimental morbidity was also observed in black-tailed gulls (*Larus crassirostris*) and rooks (*Corvus frugilegus*) (reviewed in Hubalek and Halouzka, 1996), but not in 13 species of birds evaluated in South Africa (McIntosh *et al.*, 1969).

In North America, avian mortality has proven to be extensive. Natural fatal infections have been reported based on positive laboratory tests of over 28,000 carcasses between 1999 and 2002, representing 198 species of birds (CDC, unpublished data). Incidence of disease in birds, however, has not been well documented because most laboratory testing has been for public health surveillance purposes, and therefore effects of WNV disease on specific bird populations has generally not been reported. Anecdotal reports suggest that incidence in certain species has been extremely high, such as American crows, which may be experiencing 100% mortality in some regions. In Stillwater, Oklahoma, WNV was associated with 32% mortality in young crows in 2002 (Caffrey *et al.*, 2003). About half of the positive carcasses reported have indeed been identified as American crows. About half of the remainder is a closely related species within the Corvidae family, the blue jay. Specific mortality rates in some species of North American birds can be inferred from experimental infection studies. WNV-associated clinical signs were absent in 12 chickens inoculated by injection, although morbidity was suggested by histopathologic studies (Senne *et al.*, 2000, see Section III,B). No morbidity or mortality was observed in 21 chickens infected by mosquito bite (Langevin *et al.*, 2001). However, young chicks are known to succumb to WNV infection. Domestic geese (*Anser anser domesticus*) suffered 100% morbidity and 75% mortality in a study of four 2-week-old goslings (Swayne *et al.*, 2001).

TABLE V
MORTALITY OBSERVED IN EIGHT SPECIES OF NORTH AMERICAN BIRDS EXPOSED TO WEST NILE
VIRUS BY MOSQUITO BITE

Species	No. Exposed	No. Fatal Infections (% of exposed)	Mean No. Days to Death (range)
Ring-billed gull	2	2 (100%)	9.0 (5–13)
Blue jay	4	3 (75%)	4.7 (4–5)
Black-billed magpie	3	3 (100%)	6.0 (6–6)
American crow	8	8 (100%)	5.1 (4–6)
Fish crow	9	5 (55%)	9.6 (6–13)
Common grackle	6	2 (33%)	4.5 (4–5)
House finch	2	2 (100%)	7.0 (6–8)
House sparrow	6	3 (50%)	4.7 (3–6)

From Komar *et al.*, 2003a.

Domestic turkeys were resistant to disease (Swayne *et al.*, 2000). An assortment of 25 species of birds representing 10 different orders and 17 families suffered varying degrees of mortality after infection by mosquito bite (Table V). Mortality was observed in eight species that developed high-titered viremias, in particular among the passerine birds (Komar *et al.*, 2003a). The majority of the birds in this study survived the acute phase of WNV infection, and developed neutralizing antibodies.

C. Seroprevalence

A seroprevalence study after the 1999 WNV epidemic in northeast Queens, New York City, found 2.6% of the resident human population to be positive. The number of cases reported from the same neighborhoods was used to estimate the ratio of cases to infections, which was 140:1. WNF symptoms were recalled by 21% of the seropositive respondents (Mostashari *et al.*, 2001). The ratio of cases to subclinical infections was similar to that determined for the Romania WNV outbreak of 1996 (Tsai *et al.*, 1998). Additional serosurveys of humans in the metropolitan NYC region after the 2000 epidemic in Staten Island confirmed the low infection rates in the general population (CDC, 2001; McCarthy *et al.*, 2001).

Seroprevalence studies in equines after WNV epizootics have also been reported. Apparently healthy stablemates of 1999 horse cases in

Long Island, New York, were seropositive at a rate of 29%, indicating a high rate of subclinical infections (Trock *et al.*, 2001). Infection of horses was also detected in Queens at the epicenter of the human outbreak, where one of 18 police horses was seropositive. In other boroughs of New York City, the infection rates of horses were even lower. The seroprevalence study of New York City horses in October 1999 was part of a study that included pet and stray dogs (and small number of pet cats as well) to evaluate whether infections in domestic mammals might be useful for surveillance purposes. No seropositive cats were detected, but nine of 80 dogs in Queens and the Bronx were seropositive (Komar *et al.*, 2001b). An equine serosurvey on Staten Island (the epicenter of a human outbreak) in 2000 detected seven seropositive horses of 91 surveyed (Trock *et al.*, 2001).

High seroprevalence has been found in birds in epizootic transmission foci. Most of these studies have been aimed at understanding the ecology of WNV proliferation, and are described in greater detail later (see Section VII,B). In summary, seroprevalences in resident birds was 50% and 23% in the epicenters of the 1999 and 2000 outbreaks, respectively (Komar *et al.*, 2001a, 2001c). In October 1999, the seroprevalence was evaluated for resident and migratory birds at the outskirts of New York City. Overall, 0.8% of 1018 birds sampled were seropositive for WNV (McLean *et al.*, 2002).

D. Risk Factors

Hayes (1989) reviewed risk factors in the Old World. The principal risk factor for infection was geographic location because WNV was noted to be active in certain well-defined locations within specific countries. It was also noted that advanced age was the principal risk factor for severe human disease. Han *et al.* (1999) found that time spent outdoors and in flooded basements were risk factors for infection during the 1996 outbreak in Bucharest, Romania. Bin *et al.* (2001) reported that close contact with sick geese was a risk factor for human infection in Israel in 1999, but not residence in areas along bird migration routes. The study of human risk factors for WNV infection and disease in North America is in its infancy.

In New York City in 1999, the initial series of eight patients had clustered residences within a 2-mile radius in Queens, and all had outdoor exposure (Asnis *et al.*, 2000). An analysis of the full series of 59 hospitalized cases determined that all had disease onsets between early August and late September (Nash *et al.*, 2001). Thus, risk is greatest in the New York City region during the third quarter of

the year coincident with the seasonal blood-feeding by mosquitoes. This seasonality of risk would be less restricted where mosquito blood-feeding is extended or occurs year-round.

The median age of human cases was 71 (range, 5–95) and the attack rate was 20 times greater in persons older than 50 years of age than in younger persons (Nash *et al.*, 2001). Age ≥ 75 years was a risk factor for death (relative risk 8.5), as was diabetes mellitus (age-adjusted relative risk 5.1). Another study observed that the ratio of WNME to infection was 1:50 in persons aged ≥ 65 years, and 1:300 in persons aged < 65 (Mostashari *et al.*, 2001). Advanced age was also associated with disease severity among 19 hospitalized patients in 2000 (Weiss *et al.*, 2001).

The large number of cases in 2002 provided a better understanding of the effect of age on the risk of development of both WNME and WNF. Whereas 36% of WNME cases were less than 50 years old, 55% of WNF cases met this criterion. The mean age of WNF cases was 48 years compared with 59 years for WNME. Youth seems to protect from development of severe disease after infection with WNV (CDC, 2002k).

Mostashari *et al.* (2001) attempted to identify risk factors of human infection (not disease) through questions that were administered to healthy subjects living in Queens in October 1999. From these questions, and seroprevalence status, the risk factors that emerged were: time outdoors when mosquitoes were biting, and presence of dead birds in the neighborhood. For those who spent time outdoors, use of mosquito repellent had a protective effect. Another study implicated vegetation cover as linked with WNV risk in humans during the 1999 outbreak (Brownstein *et al.*, 2002).

Because WNV is known to cause viremia in humans, blood transfusion was considered a potential risk factor for WNV infection after the 1999 epidemic in New York City. The theoretical risk of transmission from donors was estimated at 1.8:10,000 (Biggerstaff and Petersen, 2002). In 2002 the first cases of transfusion transmission were documented. These and other cases that occurred due to transmission by means other than mosquito bite are discussed later (see Section VII,C). Other risks that emerged in 2002 besides infection by mosquito bite included organ transplantation, pregnancy (risk to developing fetus), breastfeeding (risk to infant), and occupation (laboratory workers that contact WNV directly). No risk of WNV infection has been described for the following potentially risky behaviors: caring for human cases, sexual contact, bird feeding, handling live birds or other vertebrates, eating bird-derived foods, and handling of infected animal carcasses (outside the laboratory). Presumably risk of WNV infection through these behaviors is exceedingly low, or possibly overlooked.

Risk factors for WNV infection in North American horses were evaluated by a case control study conducted at 150 horse premises in 2000 (USDA, 2001). Proximity to communal bird roosts or waterfowl congregations, and dead birds noted on premises were more frequent in case premises relative to controls, but these associations were not statistically significant. Age and gender were not risk factors for either infection or disease in horses.

Risk of local transmission was evaluated early in 2000 when the significance of finding a WNV-positive dead crow was not yet understood (Nasci *et al.*, 2002). In three locations around New York City in May–July, 2000, where single dead WNV-positive crows were detected, other indicators of local transmission were also present, including WNV-infected *Culex* mosquitoes (in all three locations), and seropositive immature house sparrows (in one of the three locations).

The significance of the finding of WNV-positive dead birds as a risk factor for human disease has been the subject of much debate. The initial observation that many counties with WNV-positive dead birds did not report human cases suggested that this finding was a poor predictor for human infections (but clearly an indicator that the primary bird-mosquito WNV transmission cycle was active) (Eidson *et al.*, 2001a, 2001b). However, a recent analysis of data from 2001 found that a single WNV-infected dead crow early in the transmission season (before August 5) indeed indicated elevated risk (relative risk 6.4) of human cases (Guptill *et al.*, 2003). Preliminary analysis of the 2002 data determined a relative risk of 2.4. However, this risk varied regionally. Density of reported dead crows was evaluated as a potential risk factor for human cases in 2000 in the northeast United States (Eidson *et al.*, 2001c; Hadler *et al.*, 2001; Julian *et al.*, 2002). Weekly dead crow densities above 0.6 per square mile predicted the appearance of human cases in the four New York state counties where cases occurred. These observations need further corroboration. In 2001, complex GIS software programs designed to detect clusters of dead birds in space and time successfully predicted locations of future human WNME cases in New York City (Mostashari *et al.*, 2003; Theophilides *et al.*, 2003).

VII. ECOLOGY

A. *Invertebrate Hosts (Vectors)*

Important mosquito vectors for WNV in Europe, Africa, the Middle East and Asia are various ornithophilic members of the *Culex* genus, including *Cx. tritaeniorhynchus* in south Asia, *Cx. annulirostris*

in Australia, *Cx. perexiguus* (formerly *Cx. univittatus*) in North Africa and the Middle East, *Cx. univittatus* in sub-Saharan Africa, and Old World forms of *Cx. pipiens* and *Cx. quinquefasciatus* throughout the regions where their ranges overlap with the distribution of WNV activity (Komar, 2000). The 1996 Bucharest outbreak was driven by *Cx. pipiens* (Savage *et al.*, 1999). Numerous other mosquito species have been found infected, but in general the infection rates in these species have been low. Similarly, WNV isolates have been made from several species of ticks belonging to the families Ixodidae and Argasidae, but none of these other mosquitoes or ticks are thought to be vectors of important consequences to public health (Hayes, 1989). In North America, data on vectors have come from both field and laboratory studies.

1. Field Studies

In New York City, the outbreak investigation of September 1999 yielded 15 isolates from *Culex* species mosquitoes, including *Cx. pipiens*, *Cx. salinarius*, and *Cx. restuans* (Nasci *et al.*, 2001a). Minimum infection rates (MIRs) in each of these species could not be derived because many of the pools of *Culex* mosquitoes that were tested carried more than one species of *Culex*. The overall MIR derived for all *Culex* was 3.1 per 1000 mosquitoes tested. In contrast, no isolates were made from 3274 *Ochlerotatus triseriatus* or from 7956 *Aedes vexans*, two abundant mammalophilic species. Similarly, higher infection rates were observed in *Culex* species relative to other species in Connecticut in 2000 (Andreadis *et al.*, 2001), New York State in 2000 (White *et al.*, 2001), and New York City in 2000 (Kulasekera *et al.*, 2001), although in 2000 locally high MIRs in *O. triseriatus* (5.0) and *O. japonicus* (0.7) occurred in Staten Island (Kulasekera *et al.*, 2001) and Orange County, NY (White *et al.*, 2001), respectively. *O. triseriatus* feeds primarily on small mammals, such as squirrels, suggesting that small mammals may be involved in a WNV transmission cycle. Although the Asian species, *O. japonicus*, is known to feed on mammals (including people), the identity of its preferred host in North America is unknown. This exotic species was recognized in the New York City region 1 year before the emergence of WNV, in 1998, and like WNV, has also spread rapidly from New York City (Fonseca *et al.*, 2001; Peyton *et al.*, 1999). Also in 2000, numerous WNV-infected *Cx. restuans* and *Cx. salinarius* pools were reported (Marfin *et al.*, 2001). As WNV spread southward and westward in the United States, additional *Culex* species mosquitoes became infected, including *Cx. nigripalpus* and *Cx. quinquefasciatus* in southeastern U.S. states (CDC, 2002a) and *Cx. tarsalis* in west-central states (CDC, 2002k). These three species are also important vectors for SLEV

(Tsai and Mitchell, 1988). Preliminary surveillance data for the United States in 2002 reported 4943 WNV-positive mosquito pools (representing 1.3 million mosquitoes tested), 55% of which were *Culex* mosquitoes (CDC, 2002k). Through 2002, 36 WNV-infected mosquito species had been reported in the United States. The role of most of these species in WNV transmission cycles has not yet been confirmed.

Culex spp. are also important in their potential role for overwintering WNV in temperate climates, where they hibernate as adult mosquitoes. Field evidence of this phenomenon was observed in the cold months of early 2000 when three WNV-infected hibernating adult *Cx. pipiens* mosquitoes were collected in Queens, New York City, near the epicenter of the 1999 outbreak (Nasci *et al.*, 2001b). In the fall, *Cx. pipiens* mosquitoes destined for hibernation undergo a developmental arrest (diapause) determined by the effect on the pupal stages of shortening day-length. The mosquitoes entering diapause feed only on plant sugars and do not blood-feed, so presumably the overwintering mosquitoes acquired their infection by vertical transmission, which is discussed later (see Section VII,C).

Bloodfeeding patterns of mosquitoes are important for understanding the vector potential of different species. Apperson *et al.* (2002) analyzed 256 engorged mosquitoes (including 185 *Culex* species mosquitoes) collected from parks around northeast Queens, NYC, during the summer following the 1999 outbreak. *Cx. pipiens* and *Cx. restuans* were predominantly ornithophilic (bird:mammal ratios 23:1 and 6:1, respectively) while *Cx. salinarius* was predominantly mammalophilic (ratio 1:4). These observations favored a bridge (bird-to-mammal) vector role for *Cx. salinarius*, but indicate that the ornithophilic *Culex* species may be responsible for many mammalian infections as well. The avian blood meals identified to species from *Cx. pipiens* ($n = 38$) were mostly from American robins (16%), northern cardinals (13%), and northern mockingbirds (13%). The absence of identified blood meals from corvids, pigeons, house sparrows, and waterfowl was surprising, as the avian mortality surveillance (Eidson *et al.*, 2001a) and the seroprevalence studies (Komar *et al.*, 2001a) from the same locations indicated high WNV infection rates in these species. Corvids may have been locally extirpated by WNV during the period of the study. More blood meal analyses from a wider range of habitats are needed.

2. Laboratory Studies

Initial vector competence studies on field-collected mosquitoes from New York revealed that *Cx. pipiens* and *A. vexans* were moderately efficient vectors, although this efficiency was dependent on the dose

of virus imbibed (Turell *et al.*, 2000). When *Cx. pipiens* mosquitoes fed on viremic blood containing $10^{5.2}$ pfu/mL, only 2% of these mosquitoes were able to transmit after a period of extrinsic incubation. However, if the infecting dose concentration were increased to $10^{7.0}$, 20% of mosquitoes transmitted. Expanded studies by Turell and colleagues evaluated 13 additional species for vector competence (Sardelis and Turell, 2001; Sardelis *et al.*, 2001, 2002; Turell *et al.*, 2001a). *Culex* species were moderately competent (when exposed to a viremia of 10^7 pfu/mL), whereas certain container-breeding species (e.g., *A. albopictus*, *O. japonicus*) were most competent, and floodwater-breeding species (e.g., *A. vexans*, *A. taeniorhynchus*) were least competent (Table VI). However, competence is only one factor that contributes to the importance of mosquitoes as vectors. When other factors are considered, such as mosquito

TABLE VI
ESTIMATED VECTOR COMPETENCE OF SELECTED NORTH AMERICAN MOSQUITO SPECIES FOR WEST NILE VIRUS, BASED ON INGESTION OF A BLOODMEAL CONTAINING APPROXIMATELY 10^7 PFU/ML AND 12–15 DAYS EXTRINSIC INCUBATION

Species	<i>n</i>	Infection Rate ^a	Transmission Rate ^b
<i>Culex erythrothorax</i>	25	100	64
<i>Cx. nigripalpus</i>	127	84	10
<i>Cx. pipiens</i>	209	84	25
<i>Cx. quinquefasciatus</i>	236	63	30
<i>Cx. salinarius</i>	20	95	35
<i>Cx. stigmatosoma</i>	48	77	19
<i>Cx. tarsalis</i>	91	81	62
<i>Ochlerotatus dorsalis</i>	29	41	34
<i>O. japonicus</i>	119	76	71
<i>O. melanimon</i>	60	48	20
<i>O. sierrensis</i>	50	14	6
<i>O. taeniorhynchus</i>	75	12	3
<i>Aedes albopictus</i>	241	81	66
<i>A. sollicitans</i>	50	70	12
<i>A. vexans</i>	35	37	17
<i>Culiseta inornata</i>	28	75	21

^a Percentage of mosquitoes exposed per os that become infected.

^b Estimated percentage of mosquitoes exposed per os that are competent to transmit by bite.

From Goddard *et al.*, 2002; Sardelis and Turell, 2001; Sardelis *et al.*, 2001, 2002; Turell *et al.*, 2000, 2001a.

densities, host-feeding preferences, feeding behavior, and seasonal activity, the ornithophilic *Culex* species are implicated as most important enzootic vectors, whereas *A. albopictus*, *O. japonicus*, and *O. triseriatus* are suspected bridge vectors (Turell *et al.*, 2001b, 2002). *Cx. salinarius* is likely an important bridge vector as well, due to its catholic feeding behavior (Apperson *et al.*, 2002; Kulasekera *et al.*, 2001).

In preparation for WNV's arrival in California, researchers there evaluated the vector competence of several California mosquito populations, including 10 species (Goddard *et al.*, 2002). In accordance with previous studies, they found that all mosquitoes tested were competent to some degree, and that competence varied widely. Interestingly a significant difference was found in susceptibility to infection among *Cx. quinquefasciatus* derived from extreme southern California compared with the same species derived from Bakersfield in the Central Valley (see also Sardelis *et al.* [2002] for a description of competence variation among strains of *A. albopictus*). Transmission rates increased among all species when the extrinsic incubation period (the interval between feeding on a blood meal and the test for transmission) was increased from 7 to 14 days. Consistent with other studies, infection rates were reduced when the initial dose was decreased to about 10^5 pfu/mL. However, *Cx. tarsalis*, *Cx. pipiens*, *Cx. stigmatosoma*, and *Cx. erythrothorax* transmission rates were 82%, 60%, 34%, and 30%, respectively, after feeding on this dose, suggesting that thresholds for infection of these populations are significantly less than 10^5 pfu/mL. These transmission rates were determined by detecting virus particles in mosquito saliva after 14 days of incubation.

The differences found in the transmission rates for *Cx. pipiens* mentioned previously could be related to environmental temperature, since this variable is well known to affect flavivirus replication in mosquitoes during the extrinsic incubation period (Hess *et al.*, 1963; Whitman, 1937). The New York mosquitoes were incubated at 26 °C, compared with 28 °C for the California mosquitoes. Increased temperature does increase the vector competence of *Cx. pipiens* (Dohm *et al.*, 2002) and *Cx. univittatus* (Cornel *et al.*, 1993).

The role of non-culicine arthropods, such as ticks, lice, mites, fleas, tabanids, etc., in the transmission cycle of WNV in North America is currently unknown. Hayes (1989) considered ticks as potential vectors. Several isolates were reported from *Argas*, *Hyalomma*, and *Ornithodoros* species ticks, but ecologic data did not suggest an important role for ticks. However, experimental infection studies demonstrated vector competence of four species of *Ornithodoros* soft ticks. In experimental studies, four North America tick species were found incompetent,

including *Amblyomma americanum*, *Dermacentor variabilis*, *D. andersoni*, and *Ixodes scapularis*. Transtadial transmission occurred from larvae to nymphs in the latter three species, but they failed to transmit to mice or hamsters (Anderson *et al.*, 2003). The only other evidence for non-culicine vectors in North America was the isolation of WNV from a pool of blood-engorged ectoparasitic louseflies (Diptera: *Hippoboscidae*) collected from a WNV-positive great horned owl in Pennsylvania (M. Hutchinson, personal communication). The vector competence of this fly is unknown.

B. Vertebrate Hosts (Reservoirs)

Birds are the primary vertebrate hosts for WNV in the Old World (Hayes, 1989). This was determined by extensive serosurveys of birds and mammals in several locations, including Egypt (Taylor *et al.*, 1956), Israel (Akov and Goldwasser, 1966), South Africa (McIntosh *et al.*, 1968), Pakistan (Hayes *et al.*, 1982), Romania (Savage *et al.*, 1999), and others. In these endemic/enzootic regions, birds were frequently infected (as determined by presence of antibodies) and experimental infection studies confirmed that some birds developed high levels of viremia. Essentially all vertebrate hosts that were exposed, whether by inoculation or by infectious mosquito bite, developed viremia and/or raised antibodies. However, birds stand out from other vertebrates as being important WNV amplification hosts due to the development of viremias of sufficient duration and magnitude to infect vector mosquitoes (Fig. 3). Other vertebrates are rarely involved in transmission cycles.

New information on vertebrate hosts of WNV in North America has mostly derived from experimental infection studies with the New York 1999 strain of WNV and field studies in New York State.

1. Field Studies

Komar *et al.* (2001a, 2001b) evaluated WNV exposure of domestic and peridomestic birds and mammals after the 1999 epidemic in New York City. A total of 430 live birds of 18 species were sampled and 33% had neutralizing antibody to WNV. Birds were sampled in four boroughs of New York City and two adjacent counties. The seroprevalence rates in these locations radiated outward from the epicenter in the borough of Queens, where the most human cases were recorded. Here the seroprevalence in birds reached 50% (compared with 2.6% in people). The relative importance of different species was analyzed for Queens by combining seroprevalence and relative abundance data (Table VII).

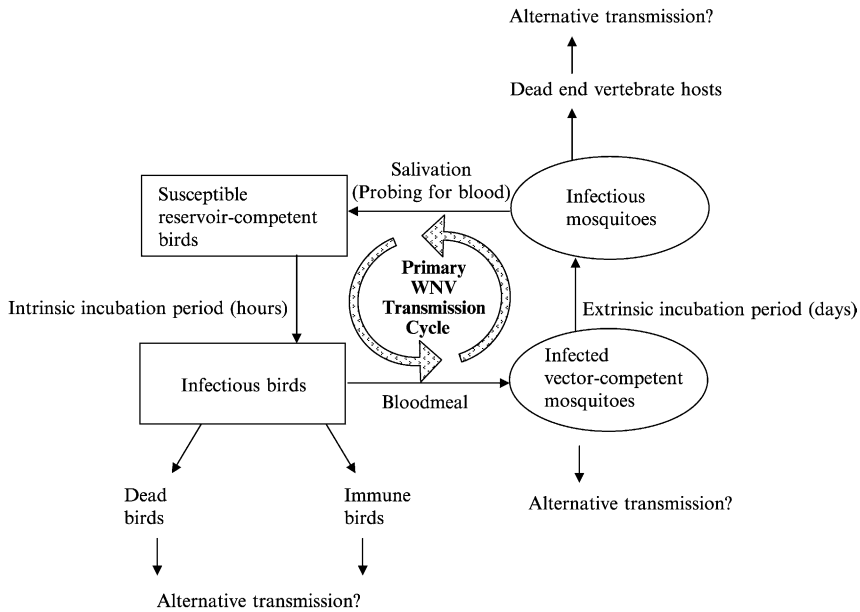


FIG 3. The primary West Nile virus transmission cycle involves certain reservoir-competent birds that transmit virus to feeding mosquitoes during a brief period of elevated viremia that follows infection. Viremia is rapidly neutralized by development of antibodies. A portion of vector-competent mosquitoes that survive the extrinsic incubation period may transmit to susceptible birds to keep the cycle going. Alternative modes of transmission may exist at multiple points along the cycle.

TABLE VII
RELATIVE ABUNDANCE, SEROPREVALENCE ("INFECTION RATE"), AND RELATIVE NUMBER OF
WEST NILE VIRUS INFECTIONS AMONG BIRDS SAMPLED IN QUEENS, NEW YORK CITY,
SEPTEMBER 1999

Species	Relative Abundance	Infection Rate	Rel. No. Infections ^a
House sparrow	6000	0.60	4186
Pigeon	1000	0.27	314
Canada goose	60	0.29	20
Mallard	60	0.06	4
Chicken	3	0.63	2
Domestic goose	1	0.86	1

^a Relative number of infections = relative abundance × infection rate.
From Komar *et al.*, 2001a.

Infections in house sparrows occurred far more frequently than any other type of bird tested.

The seroprevalence of house sparrows in northeastern Queens was 60% in September 1999. In July 2000, seroprevalence in adult ("after hatch-year") house sparrows at the same location was still 60%, and approximately 1% of hatch-year birds were seropositive, suggesting that transmission was continuing in house sparrows in 2000 in spite of the high levels of background immunity in the adult population (Nasci *et al.*, 2002).

Mammals sampled in 1999 included horses, dogs and cats. None of 12 pet cats circulated antibodies. However, some dogs and horses were seropositive, with most of the infections detected from Queens, where one of 18 (5.6%) horses and six of 55 dogs (10.9%) were positive. The investigators concluded that because the infection rates in peridomestic mammals and birds were greater than that in humans in the epicenter of the outbreak, these animals may serve as useful sentinels.

A similar evaluation of birds (but not mammals) was conducted in the New York City borough of Staten Island after the epidemic there in 2000 (Komar *et al.*, 2001c). Transmission was focal throughout the island, with seroprevalence rates in house sparrows ranging from 0% to 25% among the nine study sites. Overall seroprevalence in house sparrows was low (9%), but these were still considered important hosts because of their abundance. Captive pigeons had a very high seroprevalence (54%), leading the researchers to speculate that pigeons in particular would make good sentinels. Free-ranging species with high seroprevalence included northern cardinal (69%), house finch (40%), and gray catbird (35%), all of which were residents on the island during the period of the study.

The seroprevalence in 257 resident birds in Staten Island was compared with that in 96 transient migrants. No seropositive migrants were detected, as expected, because migrants had arrived in Staten Island after the period of epizootic activity, and were unlikely to have come from other areas with intense WNV transmission. The exposure rate in resident species was 23%.

A role for migratory birds in the transmission cycle of WNV in North America has not been established. However, a recent Israeli study may shed some light on birds as dispersal vehicles for WNV. A mortality event in white storks was investigated near the city of Eilat on the Red Sea (Malkinson *et al.*, 2002). A flock of 1200 migrating storks arrived outside Eilat in August 1998. These storks do not usually pass through Eilat on their southward migration, but rather migrate across the Rift Valley either further north or further south. Their arrival in

Eilat was attributed to unusually strong westerly winds, which grounded the birds. Two days after arrival, 13 of these birds were observed ill and dead, and four WNV isolates were made from brain samples from these birds. Four days later, three of 11 healthy storks sampled in Eilat circulated antibodies indicating previous exposure of the flock to WNV. Because most of these birds were less than 1 year old, it was determined that exposure to WNV occurred earlier in the 1998 season, either in Europe or along the migration route. Sequence data from the stork isolates matched isolates made from other dead birds collected further north in Israel in 1998. Although the authors suggest that these storks present evidence that migrating birds, such as storks, disseminate WNV, the importance of this event is uncertain. For migrating storks to disseminate the virus, the virus would have to pass to other hosts along their migration route. However, no data are presented to indicate that the storks were sufficiently viremic to infect vector mosquitoes. Furthermore, although unlikely, it is possible that many of the dead storks, if not all of them, became infected in or near Eilat, where WNV may be endemic (Bin *et al.*, 2001). Because experimental pathogenesis studies in storks are lacking, the incubation period for WNV in storks is unknown. An unpublished report of a WNV isolate from 25 white storks sampled in 1998 further south in the Sinai Peninsula of Egypt, if corroborated, would strengthen the hypothesis that these storks carried the infection south on their migration (Malkinson and Banet, 2002).

Migrating birds have often been speculated as dispersal hosts for WNV (Malkinson and Banet, 2002; Malkinson *et al.*, 2002; Rappole *et al.*, 2000; Tsai *et al.*, 1998). While epizootiologic data collected in the field have not disproven this hypothesis, they have not definitively proven it either (Murgue *et al.*, 2002).

2. Laboratory Studies

Serologic studies in the field indicate which species are exposed to WNV infection (Komar, 2001). However, quantitative experimental data on viremia are needed to better understand which species are important reservoir hosts* from which blood-feeding mosquitoes may become infected. Such data from Egypt and South Africa confirmed the role of passerine birds as important reservoirs in those regions

* The term "reservoir host" as used herein refers to vertebrate hosts that are infectious to vector mosquitoes. The concept of reservoir host can be confusing because mosquitoes may harbor arbovirus infections for a longer time than the vertebrate hosts that infected them.

(McIntosh *et al.*, 1969; Work *et al.*, 1955). Similarly, numerous experimental infection studies of mammals using Old World WNV strains have indicated that most species, albeit susceptible, are incompetent as reservoir hosts (reviewed in Hayes, 1989; Komar, 2000).

Experimental infection studies with the New York 1999 strain of WNV have now been published for 28 species of birds and three species of mammals. Senne *et al.* (2000) and Langevin *et al.* (2001) evaluated chickens as hosts. Both studies found ephemeral, low level viremias, with maximum viremia not exceeding 10^5 pfu/mL, indicating that chickens are relatively ineffective sources of infection for most mosquito vectors. Both studies found that chickens shed low amounts of WNV per cloaca, and Langevin *et al.* found that low-level shedding also occurred per os. Langevin *et al.* concluded that chickens were generally safe to use in arbovirus surveillance programs as sentinels, although biosafety precautions were recommended for handlers.

Swayne *et al.* (2000, 2001) evaluated four domestic geese and eight turkeys as hosts for WNV. Young goslings and poults were used, and in both cases viremias lasted longer and reached higher maxima compared with chickens. However, maximum viremias ($10^{7.5}$ TCID₅₀/mL in goslings; $10^{5.5}$ TCID₅₀/mL in poults) were only weakly infectious for *Cx. pipiens* mosquitoes. Low-level cloacal shedding (but not oral) was observed in poults, and low-level oral shedding (but not cloacal) was observed in goslings. Fatal pathology occurred in three of four goslings. The authors concluded that goslings but not poults were competent reservoir hosts.

Komar *et al.* (2003a) evaluated 25 species of birds, including domestic and free-ranging species, as WNV hosts. Reservoir competence index values (Table VIII) were calculated for each of the species, based on a formula that was developed for work with eastern equine encephalitis virus (Komar *et al.*, 1999). The values were derived from viremia profiles. The competence index indicates the relative number of infectious *Cx. pipiens* mosquitoes that would derive from feeding on an average infected vertebrate host, assuming each received the same number of bites. Passerine species scored highest, although fish crow and European starling had relatively low competence values. Ring-billed gull and killdeer, both charadriiforms, had high competence scores, mainly due to long-lasting viremias, making these long-distance migrants candidates for important WNV dispersal hosts. Great horned owl and American kestrel also scored high.

Several orders of birds were incompetent in this study including Piciformes (a woodpecker), Psittaciformes (parakeets), and Galliformes (quail, pheasant). Anseriformes (ducks and geese), Gruiformes

TABLE VIII
COMPUTATION OF RESERVOIR COMPETENCE INDEX VALUES FOR SELECTED BIRD SPECIES FOR
TRANSMISSION OF WEST NILE VIRUS TO MOSQUITOES^a

Species (n)	<i>s</i>	<i>i</i>	<i>d</i>	<i>c_i</i>
Blue jay (4)	1.0	0.68	3.75	2.55
Common grackle (6)	1.0	0.68	3.0	2.04
American crow (8)	1.0	0.50	3.25	1.62
House sparrow (6)	1.0	0.53	3.0	1.59
Ring-billed gull (2)	1.0	0.28	4.5	1.26
American kestrel (2)	1.0	0.31	3.0	0.93
Great horned owl (1)	1.0	0.22	4.0	0.88
Killdeer (2)	1.0	0.29	3.0	0.87
Fish crow (9)	1.0	0.26	2.8	0.73
European starling (6)	1.0	0.12	1.8	0.22
Canada goose (3)	1.0	0.10	0.3	0.03
Rock dove [pigeon] (6)	1.0	0.00	0.0	0.00
Budgerigar [parakeet] (3)	0.7	0.00	0.0	0.00

^a Each value (*c_i*) was derived by taking the product of *s*, the proportion of hosts that were susceptible to infection by mosquito bite, *i*, the mean infectiousness to *Culex pipiens* mosquitoes (a value derived from viremia titers), and *d*, the mean duration (in days) of infectious-level viremias (Komar *et al.*, 2003a).

(a coot), and Columbiformes (doves) were weakly competent, although pigeons were incompetent. These groups of birds are unlikely to be important reservoir hosts.

Viral shedding was evaluated in most of these bird species, and both oral and cloacal shedding was confirmed in the majority. In general, shedding was low and considered inconsequential. However, several species, particularly passerines and owls, shed large quantities of virus (up to 10^{6.4} pfu/swab), suggesting that shedding could be a source of infection for contacts, even human handlers. Furthermore, swabs of oral and cloacal cavities of corvids that died were consistently high titered, indicating oral and cloacal swabs as a source of diagnostic specimens easily obtained from carcasses (Komar *et al.*, 2002).

Interestingly, infectious WNV was detected in tissues of some surviving birds up to 13 days after viremia was no longer detectable (Table IX), suggesting possible long-term persistence in birds. Chronic WNV infections, as well as other flavivirus infections, have been documented previously (reviewed in Kuno, 2001a). Birds that no longer have viremias but contain viable virus in tissue may be the source of

TABLE IX
WEST NILE VIRUS QUANTITIES DETECTED IN TISSUES FROM SELECTED BIRDS AT 14 DAYS
POST-INFECTION BY MOSQUITO BITE^a

Bird	Tissue	Viral Load	Days Post-viremia
Killdeer 1	Skin	110 pfu/0.5 cm ³	9
Killdeer 2	Spleen	550	10
Killdeer 2	Skin	20,000	10
Mourning dove	Kidney	100	11
Budgerigar	Heart	130	13
Blue jay	Eye	360	9
Common grackle	Skin	380	11
Common grackle	Eye	150	11
House sparrow 1	Skin	370	8
House sparrow 2	Spleen	120	10
House sparrow 2	Lung	590	10
House sparrow 3	Brain	300	8

^a Only tissues with viral loads of ≥ 100 pfu/0.5 cm³ are presented. Negative results are not shown.

From Komar *et al.*, 2003a.

oral infection of predators but would not be expected to transmit to mosquitoes through bloodmeals (see Section VII,C).

A study of horses was conducted to better understand the pathogenesis of WNV in this susceptible host (Bunning *et al.*, 2002). Horses were incompetent reservoir hosts, with a maximum viremia of $10^{3.0}$ pfu/mL, well below the threshold for infecting vector mosquitoes. To be sure, 652 *A. albopictus* mosquitoes were fed on horses circulating as much as $10^{2.7}$ pfu/mL and incubated for 7 days before testing for infection. None of the mosquitoes became infected.

Two studies of small rodents found that laboratory mice and hamsters may indeed develop infectious level viremias. Kramer and Bernard (2001) showed that detectable viremia endured 3–4 days in Balb/C mice (*Mus musculus*) after intraperitoneal inoculation, reaching a maximum of $10^{5.4}$ pfu/mL. Golden hamsters (*Mesocricetus auratus*) developed peak viremias between 10^5 and $10^{5.8}$ TCID₅₀/mL (Xiao *et al.*, 2001). However, viremia profiles of naturally occurring North American rodent species have not been studied.

There are rare occasions in which non-avian hosts may function as reservoir hosts. For example, Malagasy lemurs (*Lemur fulvus*) infected with a lineage 2 Madagascar strain of WNV transmitted the infection

to *A. aegypti* mosquitoes and were suspected as reservoir hosts in Madagascar (Rodhain *et al.*, 1985). Lake frogs (*Rana ridibunda*) inoculated with a Russian strain of WNV developed viremia as high as $10^{5.7}$ SMLD₅₀/mL and transmitted the virus to *Cx. pipiens* (Kostiukov *et al.*, 1986). Isolation of WNV from free-ranging frogs suggested involvement in a transmission cycle in Russia (Kostiukov *et al.*, 1985). Late in 2002, WNV was isolated from captive American Alligators (*Alligator mississippiensis*) in Georgia (Miller *et al.*, 2003). Post-mortem serum samples from captive alligators in Florida revealed high-titered viremias suggesting that this species may be a competent vertebrate reservoir for WNV (CDC, unpublished data). The importance of non-avian vertebrates for WNV in North America requires more study.

C. Alternative Modes of Transmission

Potential alternative modes of transmission for WNV include vertical transmission and direct contact transmission between vertebrate hosts in the absence of arthropod vectors. The latter category would include sexual, fecal-oral, bloodborne, oral, and aerosol transmission. Kuno (2001b) reviewed potential alternatives to mosquito-borne transmission for WNV and other arboviruses.

Vertical transmission of WNV has been reported for both vertebrates and invertebrates. In 2002, one case of WNV infection in an infant in the United States was attributed to transplacental transmission that occurred subsequent to a mosquito-borne infection in August in a 20-year-old pregnant woman (CDC, 2002m). The infant was born with severe brain damage approximately 11 weeks later (at full term) in November. Anti-WNV IgM was present both in blood and CSF, and placental tissue was positive for WNV RNA. Vertical transmission in non-human vertebrates has not been reported. However, the report of a low-level persistent infection in the ovary of a common grackle 11 days after termination of detectable viremia raises the question whether transovarial transmission in birds may be possible (Komar *et al.*, 2003a). Transplacental transmission in mice (Mathur *et al.*, 1982), pigs (Burns *et al.*, 1950), and humans (Chaturvedi *et al.*, 1980) has been reported for JEV.

Transovarial transmission of WNV is known to occur in mosquitoes. Transmission to adult F1 progeny occurred at a low rate (1:325) in *Cx. tritaeniorhynchus* mosquitoes that were inoculated intrathoracically, and at moderate rates in *A. albopictus* (1:124) and *A. aegypti* (1:62) (Baqar *et al.*, 1993). Low rates of transovarial transmission were observed in intrathoracically inoculated *Cx. pipiens*, with minimum filial

infection rate of 0.6 per 1000 (Turell *et al.*, 2001a) and 2.1 per 1000 (Dohm *et al.*, 2002). In the latter study, none of over 13,000 adult progeny of infected *A. albopictus* mosquitoes were infected. These investigators speculated that vertically infected mosquitoes that hibernate during winter reinitiate primary transmission cycles in the spring. Natural evidence for vertical transmission was obtained from the isolation of WNV from a pool of male *Cx. univittatus* in Kenya (Miller *et al.*, 2000) and from hibernating female *Culex* spp. mosquitoes in New York City (Nasci *et al.*, 2001b).

The apparent die-offs observed in domestic geese in Israel and free-ranging corvids in the United States, both of which began in the late 1990s, suggested that alternative modes of transmission other than mosquitoes might exist among birds for these new aviopathogenic strains of WNV. Six separate experimental infection studies in the United States evaluated direct transmission among cage contacts. Langevin *et al.* (2001) and Senne *et al.* (2000) placed uninfected chickens together in cages with infected chickens. One cage mate (of 16 exposed) in Langevin's study became infected in the absence of mosquitoes. The mode of this "cage mate transmission" was not determined, although low-level shedding of WNV per cloaca (as well as per os) was observed in some of the chickens, suggesting the possibility of fecal-oral transmission. Orally challenged chickens did not become infected (Langevin *et al.*, 2001). One cage mate domestic gosling also became infected (of two exposed), and low-level shedding per os (but not per cloaca) in three of four infected goslings was also detected (Swayne *et al.*, 2001). Direct transmission was observed among needle-inoculated American crows held together in an indoor aviary (McLean *et al.*, 2002). The same observation was made among mosquito-inoculated American crows held in cages with contact controls (Komar *et al.*, 2003a). In this study, four cages each held two infected and one uninfected crow, except for one cage that held two uninfected crows. Viremias were monitored daily in these birds, and both the mosquito-inoculated birds and their contacts developed similar viremia profiles, with onsets in the contacts typically occurring about 1 day after the infected cagemates died from the infection (Fig. 4). In the cage with two contacts, the onset of the second contact occurred shortly after the death of the first contact control bird, suggesting that in this cage, transmission occurred from crow to crow to crow. Komar *et al.* (2003a) evaluated 17 other species of birds for direct contact transmission in the laboratory, and observed transmissions in three of these species, including blue jay, black-billed magpie, and ring-billed gull (Table X). The occurrence of direct contact transmission among birds in nature

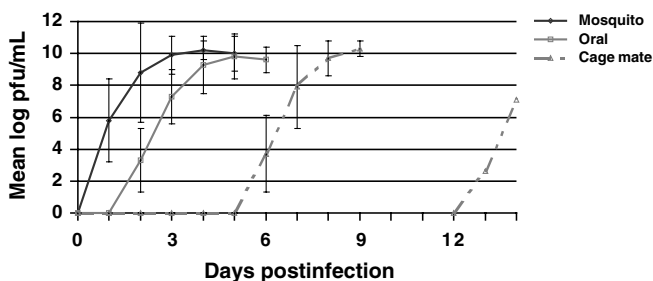


FIG 4. Viremia profiles in American crows infected by mosquito ($n = 8$) or by ingestion of infected bird carcasses ($n = 5$) or by direct contact with other infected crows ($n = 4$). Error bars show standard deviation of log-transformed viremia titers (Komar *et al.*, 2003a).

TABLE X
DIRECT TRANSMISSION OF WEST NILE VIRUS IN CAPTIVE BIRDS^a

Species	No. Cages	No. Mosquito-Exposed Birds	No. Contact-Exposed Cage Mates	No. Transmissions
American crow	4	8	5	5
Blue jay	2	2	2	2
Black-billed magpie	3	3	3	2
Ring-billed gull	1	2	1	1

^a Uninfected birds (contact-exposed group) were placed within cages containing birds (of the same species) that were infected by mosquito bite (mosquito-exposed group). Transmission to uninfected cage mates was determined by development of viremia or seroconversion (Komar *et al.*, 2003a). Negative results are not shown.

has not been documented. However, the case of a fatal WNV infection in a red-tailed hawk in early February 2000, in Westchester County, New York, was speculated to have occurred in the absence of mosquito-borne transmission (Garmendia *et al.*, 2000). One proposed method was ingestion of infected prey.

Oral transmission in birds using the NY99 strain of WNV was evaluated in 16 species of birds (Komar *et al.*, 2003a; Langevin *et al.*, 2001; McLean *et al.*, 2002). Of these, five species were susceptible to infection through ingestion of WNV-contaminated material, including water, dead birds and mice, and infected mosquitoes (Table XI). These findings suggest the possibility of naturally acquired infections through ingestion of invertebrate or vertebrate prey items, or even contaminated

TABLE XI
ORAL TRANSMISSION OF WEST NILE VIRUS ACHIEVED UNDER EXPERIMENTAL CONDITIONS IN FIVE SPECIES OF BIRDS

Species	<i>n</i>	Dose ^a	No. Viremic
Common grackle	4	1000 pfu	4
House finch	1	mosquito	1
House sparrow	6	10 ⁷ pfu	6
American crow	6	sparrow	5
American crow	3	10 ⁷ pfu	3
Great horned owl	1	mice	1

^a Oral doses of were given in liquid suspensions, in dead infected mosquitoes, or in dead infected carcasses (sparrows or mice). Negative results are not shown.

From Komar *et al.*, 2003a.

water (e.g., by fecal material). Although natural transmission per os has not been reported in birds, such transmission would be very difficult to distinguish from other modes of infection. However, a report of WNV infection in a suckling infant human being strongly suggests that natural oral transmission occurred in humans through the ingestion of breast milk containing virus (CDC, 2002f). The infection in the mother occurred after childbirth by transfusion of contaminated blood products, and subsequently breast milk tested positive for WNV RNA. The infant had minimal outdoor exposure, indicating the breast milk as the most likely source of infection. A second infant with similar exposure remained healthy (CDC, 2002g). Flaviviruses appear to have tropism for exocrine glands, such as salivary gland, mammary gland, mucus secreting cells, and pancreas (Harrison *et al.*, 1980; Monath *et al.*, 1983).

Blood-borne transmission has also been reported. A series of seven reports published by the Centers for Disease Control and Prevention and collaborators documented a complex web of WNV infections apparently contracted through the blood supply (CDC, 2002b, 2002c, 2002e, 2002g-j; Pealer *et al.*, 2003). At least six infections were confirmed in the United States in 2002. Transfused blood products that resulted in transmissions included fresh-frozen plasma and packed red blood cells. This was the first evidence of bloodborne transmission for WNV. The initial discovery of transfusion transmission came as a result of investigating four cases of WNV infection in humans, all of whom received organ transplants from the same viremic donor. The transplanted organs included liver, heart, and kidneys (Iwamoto *et al.*, 2002).

These were the first cases of WNV in humans resulting from organ transplantation. The donor had been infected by blood transfusion.

Additional human WNV infections occurred as a result of percutaneous exposure in two US laboratory workers (CDC, 2002l), serving as a reminder that exposure to high concentrations of WNV particles requires enhanced biosafety practices for laboratorians. Both of these incidents followed laceration of skin with contaminated sharp instruments. Historically, WNV infection of laboratory workers was one of the criteria that led to its designation as a BSL-3 agent (Anonymous, 1980).

Although not well documented, aerosol transmission may be another mode of infection of concern to laboratory workers and other potential vertebrate hosts of WNV. The finding of shedding of WNV in high concentrations from passerine and some other types of birds raises the possibility that aerosol transmission may in fact occur in nature or among handlers of infected birds, such as zoo keepers or wildlife rehabilitators.

Taken together, these observations of alternative modes of transmission that do not include arthropod vectors suggest that vectors are not the only means of transmission in nature, and that some of these alternative transmission routes may in fact have contributed to the rapid spread of WNV in North America.

VIII. FUTURE DIRECTIONS

WNV has become endemic in North America, causing disease in vertebrates annually since its arrival in 1999. Between 1999 and 2002, it flared up in numerous local epidemic/epizootic hot spots. Presumably it will continue to cause local epidemics in its continued spread within North America. Tropical regions of the Americas are presumably the next frontier, and WNV might eventually spread to tropical, subtropical and temperate regions of Central and South America. The public and veterinary health impacts in regions where WNV already exists in a quiet equilibrium, or where other closely related flaviviruses have already generated genetic resistance in vertebrate populations, remain to be determined.

Many questions yet exist on the basic ecology of WNV in North America, such as the epidemiologic significance of alternative transmission cycles, and the precise mode of geographic dissemination (and especially the role of migrating birds as dispersal vehicles). The early years of WNV's establishment on this continent are the time to attack these questions, as once new equilibria are reached, the presence of the virus may become cryptic like its cousin SLEV. The lessons learned during the next several years of study of WNV will have

far-reaching impacts on our preparations for defending against WNV in the coming years and other future arboviral invaders.

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