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Priority Contribution

West Nile virus in the New World: potential impacts on bird species

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Summary

The past five years have seen the arrival and extremely rapid expansion of West Nile virus (WNV) in the Western Hemisphere. The rapid sweep across North America has permitted little time for developing knowledge of the virus's potential impacts on wildlife in the New World. Given this information gap, we here summarize for the ornithological community what is known or can be anticipated for WNV's effect on bird communities in coming years. Our particular focus is on impacts of WNV on the conservation status of birds, the principal vertebrate reservoir for the virus.

Origins

West Nile virus (WNV) was first isolated in Uganda in 1937, and was subsequently documented as a relatively benign arbovirus across much of Africa, the Middle East and southern Europe (Hubálek and Halouzka 1999). It was viewed as a cause of mild febrile illness in humans, with no apparent negative effect on birds. WNV nevertheless caused several major human disease outbreaks, for example in South Africa in 1974 and Algeria in 1994. WNV was not fully appreciated as a serious human health concern, however, until the encephalitis outbreaks in Romania in 1996–1997, which involved hundreds of human clinical cases and a 9% case-fatality rate. Other recent outbreaks have seemed to come at shorter intervals, and with more severe effects, both on humans and on birds, suggesting to some the possible evolution of a new, more virulent strain (Petersen and Roehrig 2001).

Arrival in the Western Hemisphere

The circumstances leading to WNV's arrival in New York City in 1999 may always remain a mystery. Considering the transmission biology of the virus, the most likely means of arrival would have been via either an infected bird or an infected mosquito arriving at an airport or seaport. The suggestion of introduction via normal or accidental bird migration (Rappole *et al.* 2000) is also valid, although acute infection *and* long-distance intercontinental movement would be a surprising combination. The possibility of initial arrival in tropical America,

with subsequent transport to the United States, should not be discarded. Nevertheless, no precise answer to the question of *how* is likely to emerge.

On the other hand, a fairly clear picture of *from where* is in hand. Early analyses indicated almost complete RNA sequence identity between an isolate from New York City in 1999 and one from Israel in 1998 (Lanciotti *et al*. 1999). Subsequent analyses confirmed the close similarity of WNV sequences from the two regions (Giladi *et al*. 2001). Hence, a Middle Eastern origin of the WNV strain that arrived in New York City is indicated. The possibility that both the Israeli and New York strains originated in yet another location cannot be ruled out.

Local transmission cycles

The principal transmission cycle for WNV appears to involve ornithophilic mosquitoes as vectors, and birds as reservoir hosts (Figures 1, 2), although at least four exceptions are known. These main and alternative cycles are described in detail below.

Main cycle: birds

The principal cycle involves birds as reservoirs and mosquitoes as vectors. Among birds, the taxa that represent the most important reservoirs for maintaining

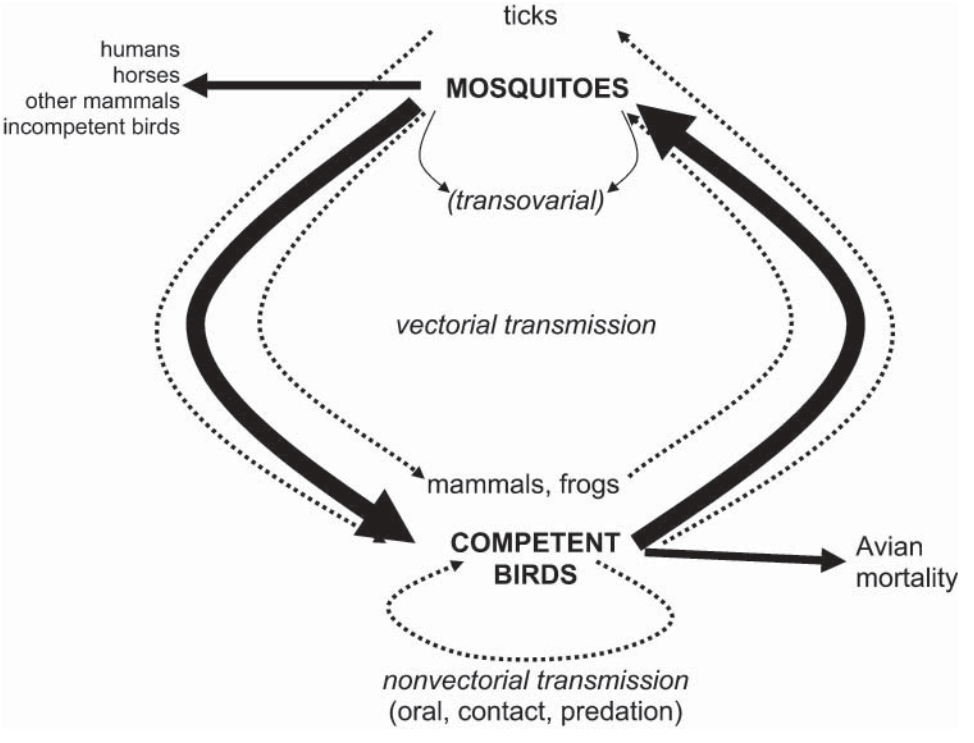

Figure 1. Summary of potential transmission cycles for WNV. Probable relative importance of different transmission cycles is indicated by the relative thickness of the arrows.

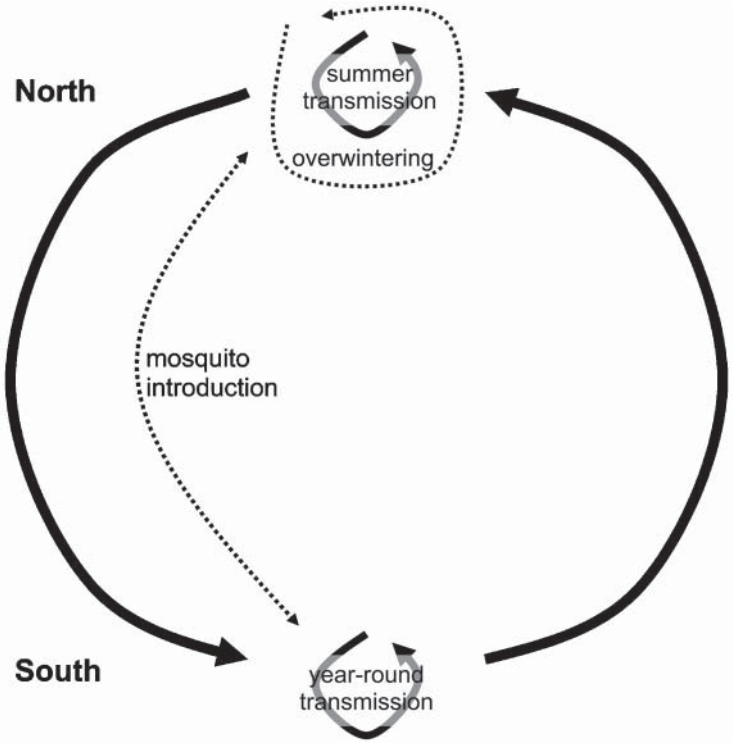

Figure 2. Summary of geographic aspects of WNV transmission cycles. See text for explanation. Thick black lines connect north and south bird migration.

local transmission remain obscure (Komar *et al*. 2003a) and are likely to vary geographically depending on the structure of local bird communities. Recent laboratory and field data, however, shed some light on the issue.

Infection resulting from mosquito bites has been demonstrated across a wide variety of birds, including 25 species of nine orders under laboratory conditions (Komar *et al*. 2003a), and many more species under field conditions (in which the role of the mosquito vector is assumed), including diverse species in Europe (Hubálek and Halouzka 1999), a wide variety of birds from across the world that were infected at New York City's Bronx Zoo in 1999 (Ludwig *et al.* 2002, Steele *et al.* 2000), hundreds of species in North America (Komar 2003) and numerous species in the Caribbean (Dupuis II *et al.* 2003, Komar *et al*. 2003b). The effects of WNV infection on these species range from benign (e.g. in many European birds) (Hubálek and Halouzka 1999, McIntosh *et al.* 1969) to almost universally fatal (Komar *et al*. 2003a, Taylor *et al*. 1956).

In general, species of the order Passeriformes appear to represent the most competent reservoirs for WNV transmission, although other orders may also contribute. For example, members of all nine orders tested in the most comprehensive study (Komar *et al*. 2003a) were susceptible to infection (variable *s*); however, seven species (all non-passeriforms) never reached infectious levels of viraemia (mean infectiousness = *i*). Also, duration of infectiousness (variable *d*) varied from 0 to 5.5 days, with the 10 lowest values all pertaining to

non-passeriforms. In terms of a composite reservoir competence index ($C_i = s{\cdot}i{\cdot}d$), the "top 10" included only one non-passeriform, and the "bottom 10" included only non-passeriforms, suggesting strongly that passeriforms act as especially important reservoirs for WNV (Komar *et al*. 2003a).

Main cycle: mosquitoes

WNV infection has been documented in a wide variety of mosquito species, including at least 43 species in Europe (Hubálek and Halouzka 1999). In the Western Hemisphere, although dozens of mosquito species have already been documented as carrying the virus (Andreadis *et al*. 2001a, b, Bernard *et al*. 2001, Sardelis and Turell 2001, Sardelis *et al*. 2001, Turell *et al*. 2001), the genus *Culex* appears to be key and quite dominant in maintaining local transmission cycles. For example, of 32,814 mosquitoes tested from New York and New Jersey in 1999, including 14,798 mosquitoes of genera other than *Culex*, only *Culex* were found infected with WNV (Petersen and Roehrig 2001). Other genera, however, could be more important in other geographic regions. For example, *Ochlerotatus japonicus* and *Aedes albopictus* have both had higher vector competence than seven *Culex* species evaluated in recent studies (reviewed in Komar 2003).

An additional consideration, and one that may prove to be key in understanding WNV transmission dynamics, is the role of ornithophilic mosquitoes in maintaining local transmission cycles. Other mosquito species — so-called bridge vectors — that feed on birds and other classes of vertebrate hosts such as mammals and reptiles may be responsible for most transmission to humans, horses and other taxa (alligators, bats, chipmunks, squirrels, rabbits, skunks, etc.).

Alternative cycles

Although the bird–mosquito–bird cycle described above is probably responsible for most WNV transmission, several alternative cycles have been documented, and serve to augment the complexity of the system (Figure 1). First, the potential for a bird–tick–bird transmission cycle has been documented in Europe (Hubálek and Halouzka 1999). WNV has been isolated from both the bird-feeding "soft" ticks (argasid ticks) and from the more generalist "hard" ticks (amblyommine ticks). Under experimental conditions, ticks of the genera *Argas*, *Ornithodoros* and *Dermacenter* have been demonstrated capable of WNV transmission to birds, although tests of vector competence in North American ticks were negative (Anderson *et al*. 2003). Other possible vectors of WNV may include louseflies (Hippoboscidae), from which WNV has been isolated in North America (Komar 2003).

Second, many species of mosquito may be able to transmit WNV vertically to offspring. Experimental studies have demonstrated low rates of vertical transmission in *Aedes albopictus*, *Ae. aegypti*, *Culex tritaeniorhynchus* and *Cx. pipiens*. Natural transovarial transmission is presumed to be the cause of infection in overwintering *Culex* spp. and a male *Cx. univittatus* mosquito (reviewed in Komar 2003).

Third, under restricted circumstances, taxa other than birds are able to harbour infections of WNV that reach levels sufficient to infect mosquitoes. For example,

frogs *Rana ridibunda* have been documented as competent hosts (i.e. able to infect mosquitoes with WNV) in Russia (Hubálek and Halouzka 1999). However, several North American reptile and amphibian taxa were found to be incompetent as reservoirs for the New York strain of WNV (Klenk and Komar 2003). Although WNV infections have been observed in a wide variety of mammal taxa, only the lemur *Lemur fulvus* is suspected of supporting local transmission cycles (Rodhain *et al*. 1985).

Finally, and perhaps most intriguing, WNV is an arthropod-borne virus (arbovirus) that can be transmitted without the participation of a vector species. Several means of direct transfer (non-vectorial) from bird to bird have now been documented. For example, in laboratory experiments (Komar *et al*. 2003a), oral exposure was documented to occur in at least five of 11 species tested — this means of infection provides a double peril for corvids, raptors, and other carrion-feeding birds or predators, which frequently feed on dead or sick animals, and may become infected by that means as well. Transmission can also occur by direct contact (exact means remain unclear), as unexposed individuals housed in the same cages as exposed individuals became infected in four of 18 species tested (Komar *et al*. 2003a). The possibility of transovarial transmission in birds remains an open question, and the discovery of viral infections concentrated in ovarian tissues is suggestive (Komar *et al*. 2003a).

What are the effects of WNV on birds?

Mortality is especially frequent among North American birds exposed to WNV, probably owing to a combination of lack of previous exposure, lack of immunity, and to possible evolution of increased virulence in the WNV strain circulating there (Petersen and Roehrig 2001). At least 198 bird species have died as a result of WNV infections in North America (Komar 2003). In laboratory experiments, clinical signs of illness (e.g. lethargy, ruffled feathers, unusual posture, lack of motor control and ataxia) were followed by death within 24 hrs (Komar *et al*. 2003a); mortality in nature is likely to be even more certain and swift than in laboratory experiments, as predators and additional stresses are present (although birds recently brought into captivity may be more susceptible owing to high cortisone levels as a result of stress). Curiously, few Eastern Hemisphere WNV infections produce any symptoms in birds (Hubálek and Halouzka 1999).

No clear picture of effects on birds has yet been developed in tropical areas of the Western Hemisphere, where WNV has nonetheless spread through local bird communities, such as in Jamaica (Dupuis II *et al*. 2003), Mexico (Comité Intersectorial para la Vigilancia, Prevención y Control del Virus del Oeste del Nilo, http://www.cenave.gob.mx/von/archivos/Ornitologia.pdf) and the Dominican Republic (Komar *et al*. 2003b). In Mexico during 2003, for example, 10 individuals of nine species were documented to have died with WNV infections, and antibodies to WNV were found in 233 individuals of 56 species, in states ranging from the northern to the southern extremes of the country.

Several possible factors may lead to reduced effects of WNV on tropical birds in the Western Hemisphere. Theoretically, prior exposure to related viruses of the family Flaviviridae may have allowed whole communities of birds to develop resistance to severe infections (Male 2003), although such effects are not documented. At least 10 Neotropical species are already known to have died as

a result of exposure to WNV in North American zoos and avicultural collections, as well as many Neotropical migratory species during their summer residency in North America (Komar 2003). A dilution effect caused by greater biodiversity (compared with temperate regions) could in theory reduce the likelihood of an epizootic in tropical areas; the idea is that a greater diversity of species that are poor reservoir hosts dilutes potential negative effects of vector-borne pathogens (Ostfeld and Keesing 2000, Schmidt and Ostfeld 2001, LoGiudice *et al*. 2003).

It is also possible that bird mortality in tropical areas has simply gone unnoticed or unreported. In the Caribbean sites mentioned, an abundant introduced predator, the Indian grey mongoose *Herpestes edwardsi*, could dramatically reduce evidence of sick or dying birds; research on WNV transmission in mongoose populations would be useful. Greater diversity and abundance of scavengers, such as mongooses, ants and others, in tropical areas compared with temperate areas, reduce opportunities for bird mortality to be detected.

Avian survivors of WNV infections generally show detectable levels of WNV-neutralizing antibodies. For instance, in serological surveys in the New York City area in 1999, 33% of 430 individual birds of 18 species tested positive for WNV antibodies (Komar *et al*. 2001b). In laboratory tests (Komar *et al*. 2003a), infected birds generally developed antibodies; however, one Budgerigar *Melopsittacus undulatus* that did not produce detectable antibodies indeed proved to have a WNV infection in heart tissue, so antibody detection is not an absolute indicator. Individual Rock Pigeons *Columba livia* for which antibody responses were tracked over 4–9 weeks showed detectable antibody levels throughout the study period (Komar *et al*. 2003a). Particularly intriguing is the discovery that birds surviving WNV infections frequently harbour ongoing infections sequestered in particular organs much past the disappearance of circulating virus particles in blood ("viraemia") (Komar *et al*. 2003a).

Few studies of WNV impacts on North American bird populations have been completed. Most of these studies have focused on American Crows *Corvus brachyrhynchos* because of observations that natural mortality observed in this species was frequently due to WNV infection (Eidson *et al*. 2001a). In Stillwater, Oklahoma, a marked crow population suffered a 40% reduction after WNV's initial introduction in 2002 (Caffrey *et al.* 2003), followed by a 60% reduction the following year (C. Caffrey *et al*. pers. comm.). A similar study in Champaign-Urbana, Illinois, in 2002 documented a 68% WNV-attributed decline among marked crows (Yaremych *et al.* 2004). Monitoring of American Crow populations throughout the northeastern United States by Christmas Bird Counts revealed an 11% decline for this species, but no significant declines for other species (Caffrey and Peterson 2003). An analysis of Project Feeder-Watch data revealed significant declines in American Crows, two species of chickadee *Poecile* spp. and Blue Jays *Cyanocitta cristata* in many locations in the eastern United States (Bonter and Hochachka 2003).

Where is WNV now?

Since its arrival in North America, WNV has spread extremely quickly. The year-to-year pattern is summarized in Figure 3: from an initially minute area

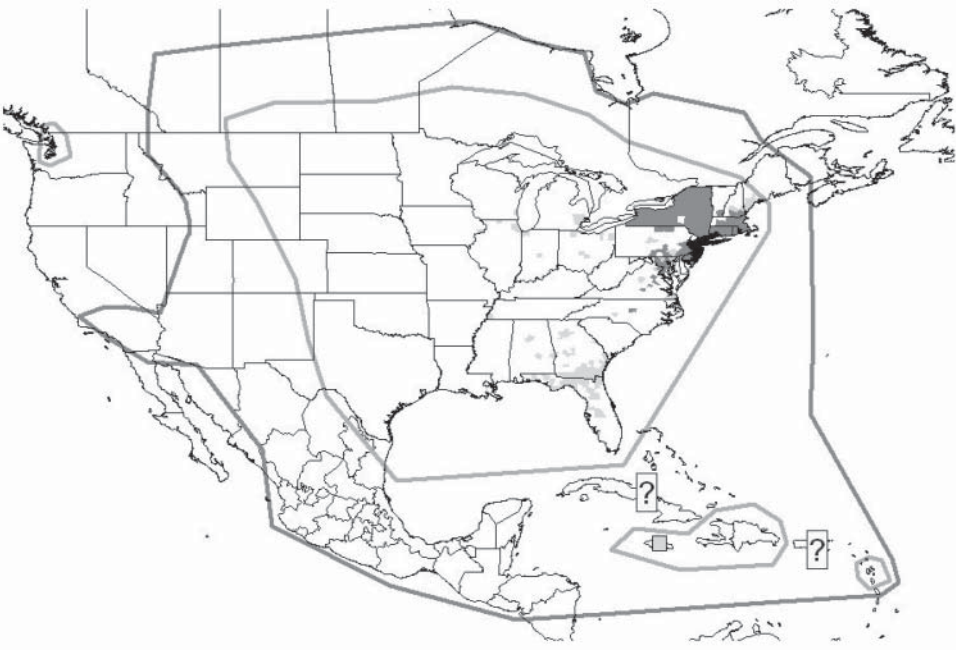

Figure 3. Summary of the pattern of expansion of WNV in the New World. Black, 1999; medium grey, 2000; light grey, 2001; inner (light) polygon, 2002; outer (darker) polygon, 2003.

surrounding New York City in 1999, the virus was essentially continental just 4 years later. As of early 2004, the only parts of North America from which WNV has not been documented are the extreme north, the Great Basin, and the Pacific coast from central California northwards to Alaska (except Puget Sound, Washington, where WNV activity occurred in 2002).

South of the United States, however, the pattern of expansion has been less well documented. As of early 2004, occurrences were documented in northern Mexico (Blitvich *et al*. 2003a), Tabasco (southern Mexico) (Estrada-Franco *et al*. 2003), Yucatan (Loroño-Pino *et al*. 2003) and possibly Chiapas (Ulloa *et al*. 2003). In the Caribbean, 2001–2002 found WNV arriving in Jamaica (Dupuis II *et al*. 2003), Dominican Republic (Komar *et al*. 2003b), Guadalupe (Quirin *et al*. 2004) and the Cayman Islands (CDC 2002a). No documentation places WNV farther south, but at the same time few or no detailed studies have sought the virus farther south, and many of the usual indicators (e.g. avian mortality) may not be noted in tropical regions. Hence, its distribution may prove to be more extensive than is presently thought, likely to now (mid-2004) include all of the Caribbean and Central America, and potentially much of South America.

Where is it going and what will it do?

Geography

Linkage of local transmission cycles of WNV in southern tropical areas and northern temperate areas by migratory birds (Figure 2) has long been suspected

and discussed (Hubálek and Halouzka 1999, Rappole *et al*. 2000). The idea is that tropical bird and mosquito communities may maintain permanent local transmission cycles. However, spring migrations serve to transport the virus northward, infecting temperate areas, and providing the possibility for sporadic disease outbreaks in such areas. The WNV outbreaks in southern Europe have been attributed to this effect (Hubálek and Halouzka 1999). Under this view, temperate zone virus concentrations may be transitory in nature, although the possibility of transmission cycles being maintained via infected overwintering mosquitoes does exist (Hubálek and Halouzka 1999, Nasci *et al*. 2001).

Although considerable circumstantial evidence has pointed to a role of migratory birds in transporting WNV to new regions (Rappole *et al*. 2000), the apparent equilibrium nature of WNV in the Eastern Hemisphere prevented detailed hypothesis tests. WNV's non-equilibrium status in the Western Hemisphere provided an opportunity, however: the virus's spread south into the Neotropics should follow predictable patterns that mimic avian migratory patterns (Rappole *et al*. 2000). If, on the other hand, WNV's spread southward is via movements or introductions of mosquitoes by human transportation, the resulting pattern would be quite different. Extensive searches for WNV in migrating birds in North America have to date not revealed any concrete indications (Rappole and Hubálek 2003).

A recent set of tests based on geographic and ecological modelling (Peterson *et al*. 2003) addressed the hypothesis of WNV's spread being mediated by migratory birds. Based on the ecological niches and geographic distributions of three *Culex* mosquito species in the eastern United States, scenarios of spread were developed with and without the participation of 41 migratory bird species as means of broad-scale dispersal. Only the scenario involving migratory birds simulated the observed pattern of spread.

Given the potential spread of WNV by migratory birds, understanding migratory routes and interconnections becomes key in anticipating the spatial dynamics of WNV. The generalities of bird migration are clear — birds from the eastern United States migrate in large part to the Caribbean, South America and the Atlantic lowlands of Mesoamerica. In contrast, birds of the western United States generally migrate south into Mexico and Central America. In effect, Mexico and Central America will constitute a key transfer point between eastern and western North America, because few east–west movements of birds occur in the central-western United States. Mexico provides east–west connectivity given its rich migratory populations from across North America. Migratory patterns within the tropics (Powell and Bjork 1995) and within South America (Chesser and Levey 1998) are much less well understood.

Considering global patterns of bird migration, an intriguing phenomenon can be noted: western Alaska and the Aleutian Islands are inhabited by bird species that overwinter in the Americas but also by species that overwinter in Australasia (Figure 4). That is to say, a suite of species (e.g. Arctic Warbler *Phylloscopus borealis*) considers western Alaska to be effectively an extension of Asia, and as such migrates through Asia to the Old World tropics. This pattern raises the possibility that WNV, upon spreading into western Alaska, could be transported in autumnal migration south-west into Asia and eventually Australia. In this way, WNV could become essentially cosmopolitan in a

Figure 4. Schematic of key features of bird migration in the Americas, Asia and Australia, showing the potential role of western Alaska in transferring West Nile Virus from North America into eastern Asia.

relatively short time following its "jump" across the Atlantic Ocean. Its possible interactions with Asian and Australian strains of WNV (e.g. Kunjin virus) or other Asian flaviviruses such as Japanese encephalitis virus are unknown. Given that several shorebird species breed in Alaska and pass through or winter in the Hawaiian islands, the Pacific islands are another area of potential concern. Many Hawaiian island endemic bird species could be seriously threatened by the arrival of WNV.

Rare and endangered species

The broad-spectrum mortality patterns associated with WNV in North America are a potentially serious concern for wild bird species (Male 2003). Although the effects are particularly strong among Corvidae, mortality is quite diverse in terms of species, families and orders affected. WNV appears to have caused, at least temporarily, local extirpations of some species (e.g. American Crow) from areas of the eastern United States (Komar *et al*. 2001a).

As WNV spreads across the Western Hemisphere, a number of impacts are possible. Corvids, of course, would be a first consideration. This family includes many microendemic and range-restricted species (see Figure 5 for a summary of distributions of endemic species of this family in Mexico) and experiences high mortality rates from WNV infection; 13 species of American corvids are of global conservation concern (Stattersfield *et al*. 1999). In the United States, Florida Scrub-jay *Aphelocoma coerulescens*, a corvid already considered endangered owing to concerns about habitat preservation, may also be vulnerable to WNV-mediated mortality. Given apparently strong WNV-mediated mortality in the wild in grouse (Bernard *et al*. 2001), as well as some as-yet unpublished inoculation experiments demonstrating dramatic mortality in grouse (D. Naugle pers. comm.), the recently described Gunnison Sage-grouse *Centrocercus minimus* would be another species of considerable concern.

Figure 5. Example distributions — presently recognized species of Corvidae endemic to Mexico north of the Isthmus of Tehuantepec. Triangles, *Cyanolyca mirabilis*; plus signs, *Aphelocoma guerrerensis*; crosses in east, *C. nana*; vertical squiggle, *Cyanocorax dickeyi*; undotted squares, *C. sanblasianus*; dotted squares, *C. beecheii*; dotted circles, *Corvus imparatus*; crosses in the west, *C. sinaloae*.

Across the Western Hemisphere, large numbers of species are considered endangered or threatened, and/or may have small geographic distributions (Bibby *et al.* 1992, Stattersfield *et al*. 1999). Such species are potentially vulnerable to WNV effects, as modest elevations of mortality rates could interact with existing pressures (e.g. habitat destruction, trapping for the pet trade) to increase extinction risk. For example, California Condor *Gymnogyps californianus* was until recently extinct in the wild. Now, captive-bred individuals have been reintroduced with mixed success, and a new source of mortality could increase extinction risk substantially.

Some of the globally threatened species that seem to be most vulnerable to potential negative effects from a new avian flavivirus include Hawaiian Crow *Corvus hawaiiensis* (now apparently extinct in the wild regardless) on Hawaii, Ridgway's Hawk *Buteo ridgwayi* and White-necked Crow *Corvus leucognaphalus* on Hispaniola, Cuban Kite *Chondrohierax wilsoni* and Gundlach's Hawk *Accipiter gundlachi* on Cuba, and Cozumel Thrasher *Toxostoma guttatum* on Cozumel Island, as well as many others of the approximately 80 critically endangered bird species in the Americas. As a previous review (Male 2003) pointed out, however, the exact dimensions of the effects on endangered species from WNV are not yet known, and given that WNV may have unexpected interactions with antibodies for other flaviviruses, it may behave distinctly in tropical avifaunas.

Future evolution

Why is WNV causing epidemics in humans and widespread mortality in birds in the Western Hemisphere, and what is the future of WNV in this new distributional area? The answer to both questions lies in the theory of host–parasite coevolution. WNV is highly coevolved with birds in Africa, the Middle East and southern Europe. This long, shared history would be expected, based on some theoretical arguments (e.g. Clayton and Moore 1997), to lead to the evolution of avirulence in the virus towards particular hosts, such that these hosts infected with the virus can survive without ill-effects, or such that large-scale epidemics do not occur. The answer to the first question may be that the widespread mortality and the occurrence of large-scale epidemics are likely consequences, at least in part, of the novelty of the virus among the avian hosts present in the Western Hemisphere. However, some as yet unpublished data are emerging that indicate that the North American strain of WNV is particularly virulent for birds, when compared with other related strains. The recent increase in epidemic WNV activity in the Old World (e.g. Romania, Israel and South Africa) illustrates that the virus may continue to evolve new, potentially harmful strains (Le Guenno *et al*. 1996).

So, what will WNV be like in the future? The most likely answer is: like WNV in the Eastern Hemisphere. With time, in the Western Hemisphere, the great majority of populations will have had experience with WNV, and will have developed genetic resistance to severe disease, probably leading to lesser viraemias resulting in lesser virus amplification, lower prevalence and infrequent mortality. An alternative analogy in the Western Hemisphere is St Louis encephalitis virus (SLEV), an arbovirus held in an avian reservoir and transmitted by ornithophilous mosquito vectors. SLEV is also quite rare, with

low prevalence in bird populations. Most adult birds do not have antibodies to SLEV, and SLEV does not produce large mortality events in birds. In sum, SLEV represents a possible example of a future stage in the coevolution of WNV with Western Hemisphere birds. The effects of WNV may therefore prove to be relatively brief, in the order of decades, with effects diminishing over that time period, until the virus becomes yet another phantom, "native" arbovirus.

Wild birds and WNV surveillance

Many options exist for monitoring WNV in the Western Hemisphere (Komar 2001). For tracking WNV's spread, at least in populated and well-communicated areas in North America, dead bird surveillance is by far the most effective approach. Here, the fact that epizootics affect bird populations generally (particularly crows *Corvus* spp.) and produce dramatic avian mortality is key. Several studies have now demonstrated that the peak of avian mortality precedes the onset of human cases by 30–45 days in some areas (Eidson *et al*. 2001b, c), and that counties with early reports of WNV-infected birds were over six times more likely than other counties to report a human disease case (Guptill *et al*. 2003). However, in some places intensive mosquito surveillance has proven more effective than dead bird monitoring for predicting risk of human disease (Brownstein *et al*. 2004).

The design of a dead bird surveillance system is key in its success or failure. An electronic (web-based) reporting facility is certainly an enormous help, as telephone-reporting creates a considerable time burden. Effective and regular publicity is also important; reports of dead birds are concentrated within a week or so of press releases, radio interviews, etc. Finally, careful choice of indicators and priority taxa is also important. Perhaps the best summary of a strategy (Eidson 2001) is that surveillance should initially include testing all species found dead, disregarding the presence of trauma (individuals not showing signs of trauma are generally 2–3 times more likely to prove WNV-positive) or pathological findings that may suggest non-viral causes of death. However, once viral activity is confirmed, for efficiency and cost-effectiveness, a more restricted pool of birds could be tested as indicators, e.g. crows that do not show signs of trauma. In general, testing of birds should be restricted to individuals not more than 24–48 hrs post-mortem, and should involve extraction of a variety of internal organs, including brain, heart, lung, liver, kidney and spleen, for detection of viral antigen or viral RNA, or for virus isolation through culture techniques (Panella *et al*. 2001). High concentrations of virus in pulp from growing flight feathers (Docherty *et al*. 2004) may permit considerable improvement in efficiency of collecting tissues for testing. Several new methods for testing bird specimens have become available recently and are useful tools in the ornithologist's arsenal for detecting WNV infections. The most useful method is detection of WNV RNA from any biological specimen (Lanciotti 2003). Also, serum antibodies can now be detected with very high specificity utilizing an ELISA platform that can be applied to any species of bird (Blitvich *et al*. 2003b). Finally, viral antigen can be detected in oral swabs of corvid carcasses using a rapid field test that detects WNV antigen with high specificity (Lindsay *et al*. 2003). Unfortunately this last method has not yet been tested with non-corvids.

A second monitoring option is that of sampling live resident birds for WNV-neutralizing antibodies. For example, Rock Pigeons have been indicated as excellent "sentinels" for WNV presence, as they are widely and easily available, have a brief and low-level viraemia, but also show a strong immune response to WNV infection (Komar *et al*. 2003a). This general approach has also proven useful in regions in which dead bird surveillance is not practical (low human densities, poor communication, no freezers, etc.). Sampling resident bird species has proven effective in detecting WNV presence in local transmission cycles in the Caribbean (Komar *et al*. 2003b), and is likely to continue to constitute an important strategy in tropical regions.

Other strategies for monitoring WNV presence are, in general, less effective. Possibilities include: (1) sampling and testing mosquitoes for WNV, although large sample sizes over large geographic areas are generally required (Nasci *et al*. 2001) and the lead time prior to human cases is generally only about two-thirds that of dead bird surveillance (Marfin *et al*. 2001); and (2) maintenance of sentinel chicken flocks, although this technique is generally not very sensitive. When resources for WNV ecological surveillance are unavailable, passive monitoring for human cases is a last resort. Such an approach has at least two disadvantages: (1) by the time a human case is detected, an epidemic is probably well under way; and (2) in tropical areas, diagnosis of WNV in humans is complicated by the similarity of symptoms to other flavivirus infections such as dengue fever.

Ornithologists involved in WNV surveillance and research should consider using appropriate biosafety measures, especially given that infected birds have been shown to shed large quantities of infectious virus particles (Komar *et al*. 2003a) and that WNV has been contracted by several surveillance workers and bird-handlers (CDC 2002b, 2003). Handlers should always consider the use of "universal precautions" which will guard against transmission of most infectious pathogens. These precautions involve protection of skin and mucous membranes, and respiratory protection, when in close contact with potentially infectious materials. Given that outdoor work is generally well ventilated, the most important precautionary measure is the use of disposable gloves when handling wild animals or their carcasses or parts.

WNV and bird conservation

Consideration of the implications of WNV for bird conservation in the Americas is not easy, given the uncertainties as to its future. If the ideas discussed above regarding evolution of reduced pathogenicity hold, then the period of threat to the avifauna may be relatively brief, in the order of a decade or so. If not, of course, WNV could represent a long-term tax on bird populations across the region (although high corvid mortality could also reduce nest predation pressure for some species). In North America alone, several species come immediately to mind as of special concern; such species are listed in the section on rare and endangered species above.

For conservation of bird species potentially threatened by this new disease, options are not abundant. First, the dimensions of the problem should be evaluated for certain key populations, e.g. restricted-range or endangered bird species, such as the ones discussed above. Baseline studies of populations of marked

birds, such as the crow studies described above, will be most useful. Risks posed by WNV may be serious for some populations, possibly leading to extirpations or even extinctions. Such risks could conceivably be mitigated by reduction of vector mosquitoes. Although several mosquito-control methods exist (including habitat management, biological control, application of biochemical larvicide, and spraying insecticides for control of adult mosquitoes), the expense of applying mosquito control specifically to benefit an endangered species rather than the human population makes such practices extremely unlikely.

Vaccination of birds may eventually be possible, although no vaccine is currently licensed for avian use. One experimental DNA vaccine (Davis *et al.* 2001) has been tested in Fish Crows *Corvus ossifragus* with promising results (Turell *et al.* 2003). Vaccination of fish crows with a single dose reduced mortality from 50% to 0 in challenged birds. Oral vaccination was ineffective, but may eventually prove feasible. Experimental vaccination of crows using a licensed equine vaccine was ineffective (Turell *et al*. 2003, CDC unpublished data). Vaccination and other conservation efforts face many challenges, chiefly due to the lack of applied research to orient such efforts.

Ornithologists studying WNV may be able to greatly increase collective knowledge of this virus. Much remains to be learned about the natural history of the virus, host relationships and species-specific disease impacts. The environmental impact of WNV in North America has not been studied, yet seemingly will be significant given the decimation (and even extirpation) of some bird populations.

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