

# Prevalence of West Nile Virus Neutralizing Antibodies in Spain Is Related to the Behavior of Migratory Birds

GUILLERMO LÓPEZ,<sup>1</sup> MIGUEL ÁNGEL JIMÉNEZ-CLAVERO,<sup>2</sup>  
CONCHA GÓMEZ TEJEDOR,<sup>3</sup> RAMÓN SORIGUER,<sup>1</sup> and JORDI FIGUEROLA<sup>1</sup>

## ABSTRACT

West Nile virus (WNV) is a bird flavivirus capable of infecting horses and humans that is transmitted by blood-sucking vectors. In Europe and Africa, sporadic infections and outbreaks causing human illness and deaths have occurred and have led to 2 mutually nonexclusive hypotheses regarding the circulation of WNV in Europe: (1) the occurrence of endemic sylvatic cycles that occasionally result in human or equine infection, or (2) sporadic seeding of WNV by migratory birds from areas where the virus is endemic in Africa or elsewhere that cause local epizootic foci and eventually lead to infection in humans. To investigate these 2 possibilities, we used a micro virus-neutralization test to examine the prevalence of WNV neutralizing antibodies in 574 individuals belonging to 25 species of birds captured in spring 2004 in Seville (southern Spain). Trans-Saharan migrant species had both higher prevalences and antibody titers than resident and short-distance migrants. This result suggests that trans-Saharan migrants spend part of their life cycles in areas with greater circulation of WNV, or a closely related flavivirus, before their arrival in Spain. On the other hand, seroprevalences assessed in resident birds suggest a low level of WNV circulation in the studied locality. Aside from the question of local circulation, it thus seems that the risk for introduction of strains of WNV from Africa by migratory birds merits further field and experimental studies in Spain. **Key Words:** WNV—Virus dispersal—Bird migration—Long-distance dispersal.

## INTRODUCTION

**W**EST NILE VIRUS (WNV) is an arbovirus (arthropod-borne virus) belonging to the Japanese encephalitis group (family *Flaviviridae*, genus *Flavivirus*). Virus transmission between hosts can occur both by means of a vector (usually mosquitoes or ticks, Chevalier et al. 2004, Lawrie et al. 2004) or, less commonly, by direct transmission (Banet-Noach et al. 2003). Although WNV causes illness and outbreaks of disease in humans and equines (Autorino et al. 2002, Durand et al. 2002, Del Giudice et al. 2004, Sanchez-Seco and Navarro 2005), the natural hosts of the virus are wild birds, which act as amplifying reservoirs (Zeller and Schuffenecker

2004). WNV normally causes an asymptomatic infection in Eurasian birds (Petersen and Roerigh 2001), and its presence in Eurasia has been known since 1958 (Bárdos et al. 1959). This situation differs greatly from the situation in the Americas, where the introduction of WNV in a single event in 1999 (Asnis et al. 2000) generated a fast and explosive spread over the whole continent which caused numerous outbreaks and mortality in humans, horses (Asnis et al. 2001, Blitvich et al. 2003), and wild native birds (Kramer and Bernard 2001). Despite WNV's origin in the Old World, it has been studied more intensively in North America since its introduction in 1999 than in Europe, as judged by the number of papers included in the ISI Web

<sup>1</sup>Estación Biológica de Doñana, CSIC, Seville, Spain.

<sup>2</sup>CISA-INIA, Valdeolmos (Madrid), Spain.

<sup>3</sup>Laboratorio Central de Veterinaria, Algete (Madrid), Spain.

of Knowledge (129 vs. 693 up to November 2007). The intensity of circulation and the rapid spread of WNV in America, as well as the sensibility of American birds to the disease, are thought to have converted migrant birds into important vectors for the transport of the virus throughout the continent. Peterson et al. (2003), for instance, found that migratory birds could explain the spread of WNV throughout North America. However, Rappole et al. (2003, 2006) did not find any basis to support this idea, as the dispersal patterns of WNV in North America are better explained by short-to-medium-distance dispersive movements of resident birds. The known ecology and epidemiology of WNV in Europe have provided 2 potential hypotheses to explain the disease's outbreak dynamics (Hubálek 2000). First, a sylvatic circulation cycle could exist locally, which would maintain the virus in enzootic foci throughout the year and, under proper conditions, cause outbreaks in humans due to enhanced virus cir-

ulation. Second, migrant birds may act as virus carriers, repeatedly seeding the infection from areas where the virus is endemic in habitats suitable for the infection to progress in Europe in different areas and years (Hubálek 2000, Malkinson et al. 2001, 2002, Malkinson and Banet 2002). To assess whether long-distance migrant birds are more exposed to WNV than resident birds, which would support the second of the 2 hypotheses, we carried out a seroprevalence study in small passerines. We hypothesized that long-distance migrant birds have a higher risk for exposure to WNV and thus may show higher seroprevalence than short-distance migrant or resident birds.

## MATERIALS AND METHODS

We trapped 574 birds belonging to 25 different species (mainly passerines; Table 1) during prenuptial migration (March, April, and May)

TABLE 1. SPECIES, NUMBER OF SAMPLED INDIVIDUALS, AND TITERS FOR INDIVIDUAL BIRDS WITH WNV NEUTRALIZING ANTIBODIES SAMPLED BETWEEN MARCH AND MAY 2004 IN SEVILLE, SPAIN

Species	Sampled (n)	Titers						Migratory status <sup>a</sup>
		20	40	80	160	320	640	
<i>Acrocephalus scirpaceus</i>	5							T
<i>Alectoris rufa</i>	1							R
<i>Carduelis carduelis</i>	10							R
<i>Carduelis chloris</i>	26							R
<i>Erithacus rubecula</i>	1							M
<i>Ficedula hypoleuca</i>	19							T
<i>Galerida cristata</i>	2							R
<i>Lanius senator</i>	17				2			T
<i>Luscinia megarhynchos</i>	3							T
<i>Merops apiaster</i>	1							T
<i>Muscicapa striata</i>	5							T
<i>Oriolus oriolus</i>	1							T
<i>Passer domesticus</i>	79							R
<i>Passer montanus</i>	1							R
<i>Phoenicurus ochruros</i>	1							M
<i>Phoenicurus phoenicurus</i>	2		1					T
<i>Serinus serinus</i>	54							R
<i>Streptopelia decaocto</i>	23							R
<i>Sturnus unicolor</i>	1							R
<i>Sylvia atricapilla</i>	59							M
<i>Sylvia borin</i>	183	7	2	2	1	2	1	T
<i>Sylvia communis</i>	3							T
<i>Sylvia hortensis</i>	2							T
<i>Turdus merula</i>	74	3	1					R
<i>Upupa epops</i>	2							M

<sup>a</sup>Each species was scored as resident (R), short-distance migrant (M), or trans-Saharan migrant (T).

in 2004 in a forestry nursery near Seville city (37° 23' N, 5° 57' W). We chose this place because of their proximity to human inhabited areas, where the potential for mosquito cofeeding in humans and birds is likely to be high. Additionally, passerines present high levels of virosis when infected with WNV and may be good candidates for virus amplification and dispersal (Komar et al. 2003, Owen et al. 2006). Birds were captured in 20 12-meter-long mist nets operating from sunrise to sunset. Individuals were marked with numbered aluminum rings and their body mass (to the nearest 0.1 g) was recorded. For each individual we drew a blood sample from the jugular vein using 29 G sterile insulin syringes (always less than 1% of the body mass). The blood was placed in a vial, kept for several hours at ambient temperature (15°C–25°C) to allow clotting, and then centrifuged for 10 minutes at 6000 rpm in an Eppendorf Minispin centrifuge to separate the serum from the blood clots. The sera were frozen at –20°C until subsequent analysis. The presence of WNV neutralizing antibodies was determined in each serum by a micro virus-neutralization test (micro-VNT) in 96-well plates as previously described (Niedrig et al. 2006, Figuerola et al. 2007a). Serum samples were inactivated at 56°C for 30 minutes prior to analysis. Dilutions of test sera (25  $\mu$ L) were incubated for 1 hour at 37°C with 100 tissue-culture infectious doses (TCID<sub>50</sub>) of WNV strain Eg101 in the same volume (25  $\mu$ L) in Eagle's medium (EMEM) supplemented with L-glutamine, nonessential amino acids, sodium pyruvate, penicillin (100 U/mL), and streptomycin (100  $\mu$ g/mL). Then, 50  $\mu$ L of a suspension ( $5 \times 10^5$  cells/mL) of Vero E6 cells was added to the same medium, along with fetal calf serum, to reach a final concentration of 5%. The mixture was further incubated for 6 days at 37°C until a cytopathic effect (cpe) was observed in control wells containing 10 TCID<sub>50</sub> of virus. The screening of samples was performed at 1/10 and 1/20 dilutions of the tested sera (dilutions considered before the addition of virus, i.e., in a volume of 25  $\mu$ L). Samples yielding positive neutralization (absence of cpe) at one or both of the dilutions tested were confirmed and further titrated by analyzing serial serum dilutions from 1/10 to 1/640. Controls

for cytotoxicity in the absence of virus were included for every sample at 1/10 dilution. Cytotoxic samples were excluded from the analyses. The neutralizing serum titer was considered to be the highest value of the reciprocal serum dilution giving a complete absence of cpe. Birds were scored positive when neutralization at 1/20 or higher dilutions occurred. The specificity of the VNT employed in our study has been analyzed previously using a panel of sera with specificity for different flaviviruses (WNV, yellow fever virus, dengue virus, and tick-borne encephalitis virus), and titers of 10 or higher were only detected in sera from WNV-infected individuals (Figuerola et al. 2007a). Additionally, previous studies have shown a higher specificity to WNV in neutralization tests run in parallel against Usutu virus (another bird *Flavivirus*), confirming that the flavivirus causing the immune reaction is more closely related to WNV than to other flaviviruses (Figuerola et al. 2007b). Each species was scored as resident, short-distance migrant (wintering in southern Europe or in Africa north of the Sahara), or trans-Saharan migrant (long-distance migrants wintering south of the Sahara) as per Cramp and Perrins (1994). WNV neutralizing antibody prevalence was analyzed with the GENMOD procedure (SAS Institute 2000a) in the SAS 9.1 statistical package. Deviances from the model were scaled with the square root of the ratio deviance/degrees of freedom to correct for overdispersion. Log-transformed values of antibody titers from individuals with antibodies were compared using a *t*-test for unequal variance groups with JMP 5.0 (SAS Institute 2000b).

## RESULTS

For species with more than 20 individuals sampled, the prevalences ranged from 0% (*Carduelis chloris*, 95% CI: 0%–13.2%; *Passer domesticus*, 0%–4.6%; *Serinus serinus*, 0%–6.6%; *Streptopelia decaocto*, 0%–14.8%; *Sylvia atricapilla*, 0%–6.1%) to 8.1% (*Sylvia borin*, 4.7%–13.2%). Intermediate prevalences were found in a resident species (5.4%, 1.5%–13.3%, *Turdus merula*), suggesting that local circulation of WNV exists.

Antibody prevalence was related to migra-

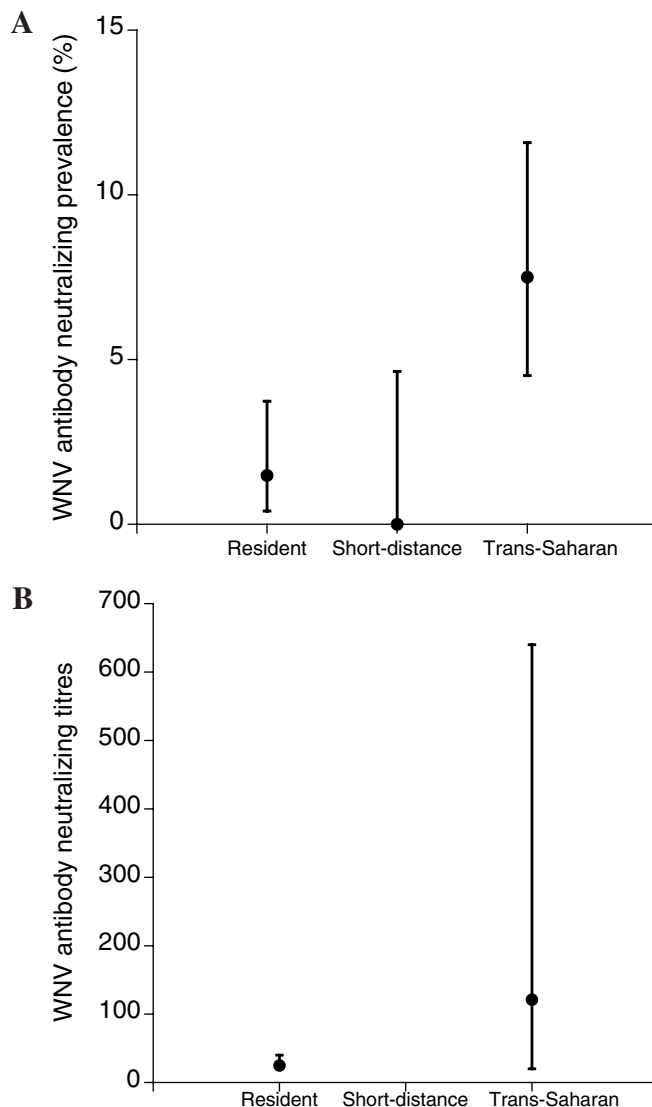
tion distance ( $\chi^2 = 19.28$ ,  $df = 2$ ,  $p < 0.0001$ ). None of 63 sampled short-distance migrants presented WNV neutralizing antibodies. Antibodies to WNV were more prevalent in trans-Saharan (18 of 240 individuals, 4.5%–11.6%) than in resident birds (4 of 271, 0.4%–3.7%, a posteriori least square means comparison,  $\chi^2 = 10.23$ ,  $df = 1$ ,  $p = 0.001$ ; Fig. 1A). Most of the sera from trans-Saharan birds correspond to *S. borin*, however, this is not likely to introduce a bias in the analyses because the GLM method used controls for differences in sample size and because a trend for higher prevalence in trans-

Saharan species remains even when the analyses are repeated without *S. borin* ( $\chi^2 = 5.56$ ,  $df = 2$ ,  $p = 0.06$ ). Antibody titers were also higher in trans-Saharan migrants than in resident birds ( $t_{17.54} = 2.90$ ,  $p = 0.01$ ; Fig. 1B).

## DISCUSSION

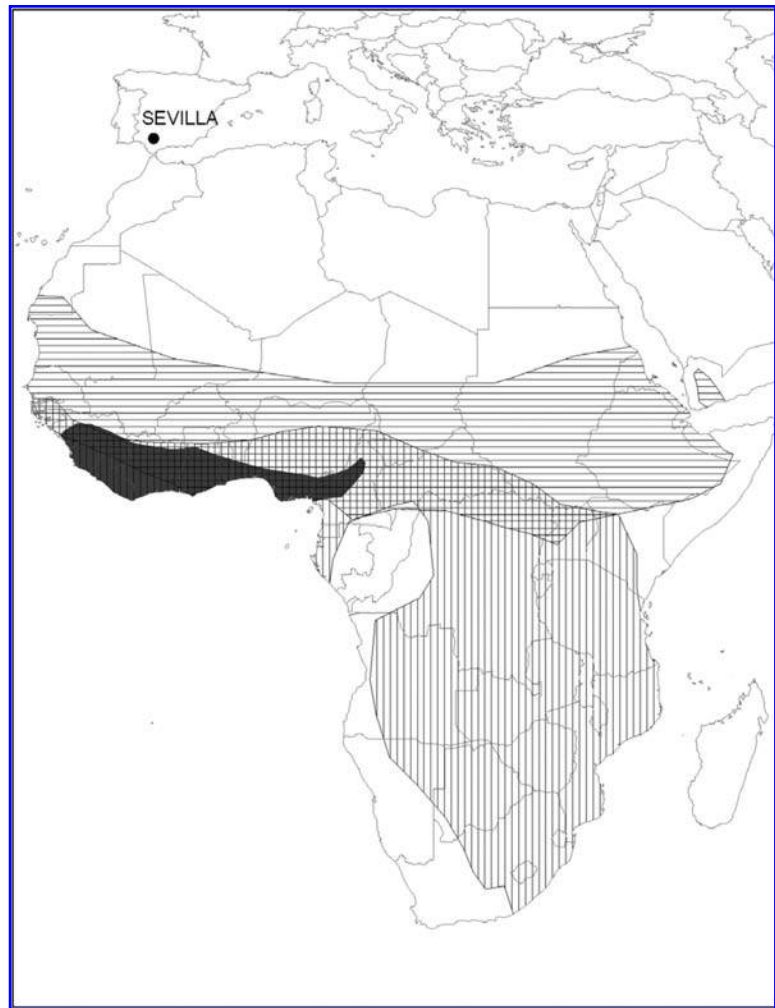
Our results confirm that long-distance migrants are exposed during their migratory journeys and/or their winter stay in Africa to higher levels of WNV circulation, or a closely antigenically related flavivirus, than the levels found in their breeding grounds in Europe. In particular, species wintering south of the Sahara (Figs. 1 and 2) presented higher seroprevalences than species wintering in northern Africa and Spain. In addition, higher WNV antibody titers were found among trans-Saharan migrants, probably reflecting a recent (or repeated) exposure to the virus in individuals with higher titers. Although some studies done in captivity suggest that WNV antibodies remain detectable for more than a year (Gibbs et al. 2005), detailed analyses of animal serology both in the laboratory (Komar et al. 2003) and in the field (Figuerola et al. 2007b, Cabre et al. 2006) suggest a rapid reduction in antibody titers after exposure to the virus. In addition, both trans-Saharan and short-distance migrants captured in this study were on their journeys toward their breeding grounds (some in Spain, but also in northern Europe). There is no a priori reasons to expect differences in the geographical distribution of both groups during the breeding season, and both groups of species breed in sympatry and winter in allopatry. For this reason, we favor the hypothesis of a higher exposure to the virus in Africa rather than during the previous breeding season in Europe.

Among resident species, the case of *Passer domesticus* merits further discussion. Given the anthropogenic behavior of this species, it has often been proposed as a key species in the understanding of transmission to humans and as a focal species for WNV monitoring (Komar et al. 2001, Jourdain et al. 2007). However, none of the individuals sampled in our study presented antibodies, unlike the case of another



**FIG. 1.** Mean prevalence  $\pm$  95% confidence interval (**A**) and mean and range of titers (**B**) of antibodies against WNV in resident, short-distance migrant, and trans-Saharan migrant bird species trapped during spring migration in Spain.

FIG. 2. Wintering areas of trans-Saharan migrant species captured with WNV neutralizing antibodies between March and May 2004 in Seville, Spain: *Ficedula hypoleuca* (gray area), *Sylvia borin* (vertical lines), and *Lanius senator* (horizontal lines), based on Cramp and Perrins (1994).



resident species of larger size, *Turdus merula*. Interestingly, recent detailed analyses of the dynamics of WNV in relation to bird ecology identified the conspecific species *Turdus migratorius* as a likely key species in explaining the transmission of WNV to humans in North America (Kilpatrick et al. 2006). Probably, we still lack the basic knowledge of the nature of the WNV-vector-host interactions needed to understand the regulatory factors of these interactions; thus, interspecific comparative studies are still necessary to understand the ecological factors regulating WNV-vector-host interactions.

Malkinson and Banet (2002) compared WNV strains isolated from birds in eastern Europe that had arrived from Africa with others isolated in Africa and suggested that migratory routes can explain the occurrence of West Nile foci in Europe. The winter distributions of the

3 species of trans-Saharan migrant passerines that show seropositivity for WNV neutralizing antibodies overlap in a region of Africa that includes parts of Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, and Cameroon. The highest seroprevalence (8.2%) was found for *Sylvia borin*, a species with a geographically wide winter distribution in Africa extending from 13°N to 35°S. A recent serosurvey of horses in sub-Saharan Africa reporting a high seroprevalence of WNV neutralizing antibodies (up to 97% in some countries, Cabre et al. 2006) further supports these conclusions. While it is true that some birds may be infected while on migration, the low seroprevalence of antibodies in short-distance and resident birds suggests that exposure to WNV occurs mainly south of the Sahara.

Do our results imply that there is a high risk for infection by WNV caused by the arrival of



migratory birds? While this is a possibility, we consider that it is highly unlikely. First, we have previously shown that local circulation of WNV occurs in Spain without necessarily leading to illness among humans (Figuerola et al. 2007b), thus supporting the hypothesis that WNV—or a closely related cross-reacting flavivirus—remains essentially undetected and sylvatic in Europe. Second, for successful long-distance dispersal of WNV by migratory birds, a successive series of highly unlikely events must occur over a short period of time: (1) a WNV-infected mosquito must infect an immunologically naïve bird; (2) the bird then must survive the infection, accumulate fat reserves, and cross the Sahara in a continuous or intermittent flight of 40 hours over 2000 km of inhospitable terrain (Schmaljohann et al. 2007); (3) the bird must then be bitten after arrival by a susceptible vector while still viremic (viremia lasts less than 7 days in birds; see Komar et al. 2003); and, finally, (4) the infected vector must then feed and infect other susceptible birds in the new locality. Most of the parameters needed to calculate the likelihood of this process are still unknown. Recently, Owen et al. (2006) have demonstrated that individuals of 2 passerine species show migratory restlessness while still viremic, although no information is available yet on the impact of WNV on the capacity of birds to accumulate energetic reserves and perform serious exercise while viremic, or on the effect of the stress produced by migration on viremia. The parameterization of the different factors that affect transmission rates of WNV at local and long-distance scales is essential if we are to understand the real role of migratory and resident bird species in the dispersal dynamics of the virus.

## ACKNOWLEDGMENTS

The Spanish Ministry of Health via the Thematic Research Net 'EVITAR' funded our research. EDEN (Emerging Diseases in a changing European Environment), FP6, RNM 118, and RNM 157 (PAI, Junta de Andalucía) also partially funded this study. Help from Oscar González, Manuel Vázquez, Beatriz Sánchez, and Ernesto García was essential in the captur-

ing and manipulation of birds. Alberto Álvarez, Alicia Cortés, Ángel Mejía, Ara Villegas, Beatriz Fernández, Carmen Gutiérrez, Chari Terceño, Cristina Sánchez, Elena Fierro, Enrique Sánchez, Esteban Serrano, Francisco Miranda, Grego Toral, Inma Cancio, Francisco Jamardo, Miguel Carrero, Manuel Sánchez, Joaquín Díaz, José Antonio Sánchez, Mari Carmen Roque, Olga Jiménez, Pedro Sáez, Rafael Reina, and Samuel del Río also assisted during the fieldwork. This is contribution number 79 of EDEN project.

## REFERENCES

- Asnis, D, Conetta, R, Teixeira, A, Waldman, G, et al. The West Nile virus outbreak of 1999 in New York: the Flushing Hospital experience. *Clin Infect Dis* 2000; 30:413–418.
- Asnis, DS, Conetta, R, Waldman, G, Teixeira, AA. The West Nile virus encephalitis outbreak in the United States (1999–2000). From Flushing, New York, to beyond its borders. *Ann NY Acad Sci* 2001; 951:161–171.
- Autorino, GL, Battisti, A, Deubel, V, Ferrari, G, et al. West Nile virus epidemic in horses, Tuscany region, Italy. *Emerg Infect Dis* 2002; 8:1372–1378.
- Banet-Noach, C, Simanov, L, Malkinson, M. Direct (non-vector) transmission of West Nile virus in geese. *Avian Pathol* 2003; 32:489–494.
- Bárdos, V, Adamcová, J, Dedei, S, Gjini, N, et al. Neutralizing antibodies against some neurotropic viruses determined in human sera in Albania. *J Hyg Epidemiol Microbiol Immunol* 1959; 3:277–282.
- Blitvich, BJ, Fernandez-Salas, I, Contreras-Cordero, JF, Marlenee, NL, et al. Serologic evidence of West Nile virus infection in horses, Coahuila State, Mexico. *Emerg Infect Dis* 2003; 9:853–856.
- Cabre, O, Grandadam, M, Marié, J-L, Gravier, P, et al. West Nile virus in horses, sub-Saharan Africa. *Emerg Infect Dis* 2006; 12:1958–1960.
- Chevalier, V, de la Rocque, S, Baldet, T, Vial, L, et al. Epidemiological processes involved in the emergence of vector-borne diseases: West Nile fever, Rift Valley fever, Japanese encephalitis and Crimean-Congo haemorrhagic fever. *Rev Sci Tech* 2004; 23:535–555.
- Cramp, S, Perrins, CM. *Handbook of the Birds of Europe, the Middle East and North Africa. The Birds of the Western Palearctic*. Oxford: Oxford University Press; 1994.
- Del Giudice, P, Schuffenecker, I, Vandenbos, F, Counillon, E, et al. Human West Nile virus, France. *Emerg Infect Dis* 2004; 10:1885–1886.
- Durand, B, Chevalier, V, Pouillot, R, Labie, J, et al. West Nile virus outbreak in horses, southern France, 2000: results of a serosurvey. *Emerg Infect Dis* 2002; 8:777–782.
- Figuerola, J, Jimenez-Clavero, M, Rojo, G, Gómez-Tejedor, C, et al. Prevalence of West Nile virus neutraliz-

- ing antibodies in colonial aquatic birds in southern Spain. *Avian Pathol* 2007a; 36:209–212.
- Figuerola, J, Soriguer, R, Rojo, G, Gómez-Tejedor, C, et al. Seroconversion in wild birds and local circulation of West Nile virus, Spain. *Emerg Infect Dis* 2007b; 13:1915–1917.
- Gibbs, SEJ, Hoffman, DM, Stark, LM, Marlenee, NL, et al. Persistence of antibodies to West Nile virus in naturally infected rock pigeons (*Columba livia*). *Clin Diagn Lab Immunol* 2005; 12:665–667.
- Hubálek, Z. European experience with the West Nile virus ecology and epidemiology: could it be relevant for the New World? *Viral Immunol* 2000; 13:415–426.
- Jourdain, E, Toussaint, Y, Leblond, A, Bicout, DJ, et al. Bird species potentially involved in introduction, amplification, and spread of West Nile virus in a Mediterranean wetland, the Camargue (southern France). *Vector-Borne Zoonotic Dis* 2007; 7:15–33.
- Kilpatrick, AM, Daszak, P, Jones, MJ, Marra, PP, et al. Host heterogeneity dominates West Nile virus transmission. *Proc Biol Sci* 2006; 273:2327–2333.
- Komar, N, Panella, NA, Burns, JE, Duszka, SW, et al. Sero-logic evidence for West Nile virus infection in birds in the New York City vicinity during an outbreak in 1999. *Emerg Infect Dis* 2001; 7:621–625.
- Komar, N, Langevin, S, Hinten, S, Nemeth, N, et al. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis* 2003; 9:311–322.
- Kramer, LD, Bernard, KA. West Nile virus infection in birds and mammals. *Ann NY Acad Sci* 2001; 951:84–93.
- Lawrie, CH, Uzcategui, NY, Gould, EA, Nuttall, PA. Ixodid and argasid tick species and West Nile virus. *Emerg Infect Dis* 2004; 10:653–657.
- Malkinson, M, Banet, C. The role of birds in the ecology of West Nile virus in Europe and Africa. *Curr Top Microbiol Immunol* 2002; 267:309–322.
- Malkinson, M, Weisman, Y, Pokamunski, S, King, R, et al. Intercontinental transmission of West Nile virus by migrating white storks. *Emerg Infect Dis* 2001; 7:540.
- Malkinson, M, Banet, C, Weisman, Y, Pokamunski, S, et al. Introduction of West Nile virus in the Middle East by migrating white storks. *Emerg Infect Dis* 2002; 8:392–397.
- Niedrig, M, Linke, S, Zeller, H, Drosten, C. First international proficiency study on West Nile virus molecular detection. *Clin Chem* 2006; 52:1851–1854.
- Owen, J, Moore, F, Panella, N, Edwards, E, et al. Migrating birds as dispersal vehicles for West Nile virus. *Eco-Health* 2006; 3:79–85.
- Petersen, LR, Roerigh, JT. West Nile virus: a reemerging global pathogen. *Rev Biomed* 2001; 12:208–216.
- Peterson, AT, Viegla, DA, Andreasen, JK. Migratory birds modeled as critical transport agents for West Nile virus in North America. *Vector-Borne Zoonotic Dis* 2003; 3:27–37.
- Rappole, JH, Hubalek, Z. Migratory birds and West Nile virus. *J Appl Microbiol* 2003; 94:47S–58S.
- Rappole, JH, Compton, BW, Leimgruber, P, Robertson, J, et al. Modelling movement of West Nile virus in the Western hemisphere. *Vector-Borne Zoonotic Dis* 2006; 6:128–139.
- Sanchez-Seco, MP, Navarro, JM. Infections due to Toscana virus, West Nile virus, and other arboviruses of interest in Europe. *Enferm Infecc Microbiol Clin* 2005; 23:560–568.
- SAS Institute, Inc. *SAS/STAT Software: User's Guide*. Cary, NC: SAS Institute, Inc.; 2000a.
- SAS Institute, Inc. *JMP User's Guide*. Cary, NC: SAS Institute, Inc.; 2000b.
- Schmaljohann, H, Liechti, F, Bruderer, B. Songbird migration across the Sahara: the non-stop hypothesis rejected! *Proc Biol Sci* 2007; 274:735–739.
- Zeller, HG, Schuffenecker, I. West Nile virus: an overview of its spread in Europe and the Mediterranean Basin in contrast to its spread in the Americas. *Eur J Clin Microbiol Infect Dis* 2004; 23:147–156.

Address reprint requests to:

Dr. Jordi Figuerola  
Department of Wetland Ecology  
Estación Biológica de Doñana, CSIC  
Avda. Maria Luisa, s/n  
41013 Seville  
Spain

E-mail: jordi@ebd.csic.es





**This article has been cited by:**

1. V. RODRÍGUEZ-PRIETO, B. MARTÍNEZ-LÓPEZ, M. MARTÍNEZ, M. J. MUÑOZ, J. M. SÁNCHEZ-VIZCAÍNO. 2012. Identification of suitable areas for West Nile virus outbreaks in equid populations for application in surveillance plans: the example of the Castile and Leon region of Spain. *Epidemiology and Infection* **140**:09, 1617-1631. [[CrossRef](#)]
2. Ana-Valeria Gutiérrez-Guzmán, Joaquín Vicente, Raquel Sobrino, Elisa Perez-Ramírez, Francisco Llorente, Ursula Höfle. 2012. Antibodies to West Nile virus and related flaviviruses in wild boar, red foxes and other mesomammals from Spain. *Veterinary Microbiology* . [[CrossRef](#)]
3. R. Lelli, P. Calistri, R. Bruno, F. Monaco, G. Savini, D. Di Sabatino, I. Corsi, I. Pascucci. 2011. West Nile Transmission in Resident Birds in Italy. *Transboundary and Emerging Diseases* no-no. [[CrossRef](#)]
4. Elena Sotelo, Jovita Fernández-Pinero, Miguel Ángel Jiménez-Clavero. 2011. La fiebre/encefalitis por virus West Nile: reemergencia en Europa y situación en España. *Enfermedades Infecciosas y Microbiología Clínica* . [[CrossRef](#)]
5. Veronique Chevalier , Sylvie Lecollinet , Benoit Durand . 2011. West Nile Virus in Europe: A Comparison of Surveillance System Designs in a Changing Epidemiological Context. *Vector-Borne and Zoonotic Diseases* **11**:8, 1085-1091. [[Abstract](#)] [[Full Text HTML](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]
6. Ignacio García-Bocanegra , Núria Busquets , Sebastián Napp , Ana Alba , Irene Zorrilla , Rubén Villalba , Antonio Arenas . 2011. Serosurvey of West Nile Virus and Other Flaviviruses of the Japanese Encephalitis Antigenic Complex in Birds from Andalusia, Southern Spain. *Vector-Borne and Zoonotic Diseases* **11**:8, 1107-1113. [[Abstract](#)] [[Full Text HTML](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]
7. Charles R. Brown, Valerie A. O'Brien. 2011. Are Wild Birds Important in the Transport of Arthropod-borne Viruses?. *Ornithological Monographs* **71**:1, 1-64. [[CrossRef](#)]
8. Guillermo López , Miguel Ángel Jiménez-Clavero , Ana Vázquez , Ramón Soriguer , Concha Gómez-Tejedor , Antonio Tenorio , Jordi Figuerola . 2011. Incidence of West Nile Virus in Birds Arriving in Wildlife Rehabilitation Centers in Southern Spain. *Vector-Borne and Zoonotic Diseases* **11**:3, 285-290. [[Abstract](#)] [[Full Text HTML](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]
9. Anna Papa , Parthena Perperidou , Anisa Tzouli , Concetta Castilletti . 2010. West Nile Virus–Neutralizing Antibodies in Humans in Greece. *Vector-Borne and Zoonotic Diseases* **10**:7, 655-658. [[Abstract](#)] [[Full Text HTML](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]
10. Diana Seidowski , Ute Ziegler , Jan A.C. von Rönn , Kerstin Müller , Kathrin Hüppop , Thomas Müller , Conrad Freuling , Ralf-Udo Mühle , Norbert Nowotny , Rainer G. Ulrich , Matthias Niedrig , Martin H. Groschup . 2010. West Nile Virus Monitoring of Migratory and Resident Birds in Germany. *Vector-Borne and Zoonotic Diseases* **10**:7, 639-647. [[Abstract](#)] [[Full Text HTML](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]
11. Benoit Durand, Gilles Balança, Thierry Baldet, Véronique Chevalier. 2010. A metapopulation model to simulate West Nile virus circulation in Western Africa, Southern Europe and the Mediterranean basin. *Veterinary Research* **41**:3, 32. [[CrossRef](#)]
12. Gilles Balança , Nicolas Gaidet , Giovanni Savini , Benjamin Vollot , Antoine Foucart , Paul Reiter , Alain Boutonnier , Rossella Lelli , François Monicat . 2009. Low West Nile Virus Circulation in Wild Birds in an Area of Recurring Outbreaks in Southern France. *Vector-Borne and Zoonotic Diseases* **9**:6, 737-741. [[Abstract](#)] [[Full Text HTML](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]
13. Elena Sotelo, Jovita Fernandez-Pinero, Francisco Llorente, Montserrat Agüero, Ursula Hoefle, Juan M. Blanco, Miguel A. Jiménez-Clavero. 2009. Characterization of West Nile virus isolates from Spain: New insights into the distinct West Nile virus eco-epidemiology in the Western Mediterranean. *Virology* **395**:2, 289-297. [[CrossRef](#)]