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Surveillance for West Nile Virus in Wild Birds from Northern Europe

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Abstract

A total of 1935 migratory birds from 104 different species were captured in southeastern Sweden in 2005–2006 and tested for antibodies against West Nile virus (WNV). Overall, 46 birds (2.4%; binomial confidence limits, 1.8–3.2) were positive by blocking-ELISA, but only 2 (0.10%; binomial confidence limits, 0.0–0.4) had antibodies detectable by both blocking-ELISA and WNV neutralization test. ELISA-positive birds included long- and short-distance migrants likely exposed to WNV while wintering in or migrating through areas enzootic for WNV. Exposure to a cross-reactive *Flavivirus* was suspected for short-distance migrants of the Turdidae family, but no cross-neutralization with tick-borne encephalitis and Usutu viruses was observed.

Key Words: Antibodies—Migratory birds—Sweden—West Nile.

West Nile virus (WNV) is considered the most widespread arbovirus in the world. Naturally transmitted between birds and ornithophilic mosquitoes, it is occasionally responsible for disease in humans and equines (Dauphin et al. 2004). Formerly known as an Old World pathogen, it was introduced in 1999 in the New York City area, where it caused notable disease and death in humans, equines, and birds, before expanding within a few years over the whole American continent (Dauphin et al. 2004). Evidence of WNV has never been reported in Northern Europe although the virus is regularly circulating in Eastern Europe, Africa, and the Mediterranean basin (Dauphin et al. 2004). Because migratory birds are known to be potential dispersal vehicles for WNV (Owen et al. 2006), we carried out serologic and molecular investigations on birds migrating through southern Sweden to assess the risk of WNV introduction into Northern Europe.

Between March 2005 and May 2006, 1935 migratory birds from 104 species (Table 1), mostly Passeriformes (79%, 65 species) and Charadriiformes (16%, 20 species), were captured at Ottenby Bird Observatory, southeastern Sweden (56°12′N, 16°24′E). Each bird was aged, ringed, and bled from the jugular vein, and resulting serum was stored at −70°C. All samples were tested by a blocking-ELISA method (Jozan et al. 2003) using the *Flavivirus* group–specific monoclonal antibody 4G2 produced by the hybridoma cell line HB-112 (American-Type Culture Collection; LGC Standards AB, Boras, Sweden) and a WNV antigen prepared from both a lineage 1 (WN_0304, Israel) and a lineage 2 (MgAn 798, Madagascar) WNV strain. A threshold cut-off value of ≥30% was used. Positive samples were further tested for WNV-neutralizing antibodies (Table 2) using WN_0304 strain and a 96-well plate method adapted from Vorndam and Beltran (2002). They were also tested for neutralizing antibodies against tick-borne encephalitis virus (strain 93/783) to rule out cross-reaction with this *Flavivirus* known to occur in birds migrating through southeastern Sweden. Usutu virus (USUV) neutralization assay (using Austrian strain 939 as in Hubalek et al. 2008) was also done for some Song Thrushes (*Turdus philomelos*) and European Blackbirds (*Turdus merula*), two short-distance migrant species frequently positive for USUV in some parts of Central Europe (Meister et al. 2008).

Out of the 1935 birds sampled, 46 (2.4%; binomial confidence limits, 1.8–3.2) were positive by blocking-ELISA, but only 2 (0.10%; binomial confidence limits, 0.0–0.4) had

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Table 1. Results of Serological Investigations (by Blocking-ELISA) Performed on Migratory Birds Sampled at Ottenby Bird Observatory from Spring 2005 to Spring 2006

Taxonomic order (number of species sampled)	*Migratory status*	*Overall*		*Sampling period*			*Age class*		
		Tested	*Prevalence*	*Spring*	*Summer*	*Autumn*	*Juvenile*	*Adult*	*Unknown*
Passeriformes (65)	S or L or S/L	44/1521	2.9% [2.1–3.9]	16/698	2/124	26/699	24/676	20/839	0/6
Charadriiformes (20)	S or L or S/L	2/308	0.6% [0.1–2.3]	2/185	0/122	0/1	0/12	2/294	0/2
Anseriformes (7)	S	0/53	<6.7%	0/37	0/15	0/1	0/6	0/46	0/1
Falconiformes (5)	S	0/28	<12.3%	0/1	0/6	0/21	0/24	0/2	0/2
Other[a] (7)	S or L or S/L	0/25	<13.7%	0/12	0/7	0/6	0/6	0/19	0
Total (104)	S or L or S/L	46/1935	2.4% [1.8–3.2]	18/933	2/274	26/728	24/724	22/1200	0/11
Short-distance migrants (59)	S	40/1339	3.0% [2.2–4.0]	13/604	1/144	26/591	24/553	16/780	0/6
Passeriformes only (35)	S	38/993	3.8% [2.7–5.2]	11/402	1/28	26/563	24/512	14/480	0/1
Long-distance migrants (41)	L	6/539	1.1% [0.4–2.4]	5/300	1/126	0/113	0/162	6/372	0/5
Passeriformes only (29)	L	6/494	1.2% [0.4–2.6]	5/286	1/96	0/112	0/157	6/332	0/5
Short- or long-distance migrants (4)	S/L	0/57	<6.3%	0/29	0/4	0/24	0/9	0/48	0
Passeriformes only (1)	S/L	0/34	<10.3%	0/10	0	0/24	0/7	0/27	0

In the Prevalence column, within brackets are binomial confidence limits.
[a]Strigiformes ($n = 8$), Piciformes ($n = 8$), Gruiformes ($n = 6$), Columbiformes ($n = 2$), and Pelecaniformes ($n = 1$).
Sampling period: spring = March to May; summer = June to August; autumn = September to November. Migratory status: S = species strictly short-distance migrant; L = species strictly long-distance migrant; S/L = species in which some individuals are short-distance migrants whereas others are long-distance migrants.

antibodies detectable by both WNV neutralization test and blocking-ELISA (Table 1). Because the neutralization assay might be less sensitive, ELISA-positive birds may have had antibodies against WNV at levels undetectable by the neutralization assay. However, cross-reactions with other flaviviruses are also likely and nonspecific falsely positive reactions cannot be excluded.

Considering the ELISA results, seroprevalence was significantly higher in Passeriformes (2.9%) than in other taxonomic orders (0.5%) (Pearson's Chi-squared test with Yates' conti-

Table 2. Results of Seroneutralization and RT-PCR Assays Performed on ELISA-Positive Sera from Migratory Birds Sampled at Ottenby Bird Observatory from Spring 2005 to Spring 2006

Taxonomic order	*Taxonomic family*	*Species*	*Migratory status*[a]	*Blocking-ELISA*[b]		*Seroneutralization*			*PCR WNV*[f]
						WNV[c]	TBEV[d]	USUV[e]	
Passeriformes	Sylviidae	*Phylloscopus trochilus*	L	1/146	0.7% [0.0–3.8]	0/1	0/1	NT	0/10
	Laniidae	*Lanius collurio*	L	1/33	0.3% [0.1–15.8]	0/1	0/1	NT	0/10
	Sylviidae	*Sylvia communis*	L	1/24	4.2% [0.1–21.1]	0/1	0/1	NT	0/10
	Motacillidae	*Anthus trivialis*	L	1/18	5.6% [0.1–27.3]	NT	NT	NT	0/10
	Hirundinidae	*Delichon urbica*	L	1/15	6.7% [0.2–31.9]	1/1	0/1	NT	0/15
	Fringillidae	*Carpodacus erythrinus*	L	1/5	20.0% [0.5–71.6]	1/1	0/1	NT	0/5
Charadriiformes	Scolopacidae	*Calidris alpina*	S	2/237	0.8% [0.1–3.0]	0/2	0/2	NT	0/11
Passeriformes	Turdidae	*Erithacus rubecula*	S	4/266	1.5% [0.4–3.8]	0/4	0/4	NT	0/10
		Turdus philomelos	S	27/124	21.8% [14.9–30.1]	0/27	0/27	0/15	0/10
		Turdus merula	S	6/92	6.5% [2.4–13.7]	0/6	0/6	0/4	0/10
		Sturnus vulgaris	S	1/67	1.5% [0.0–8.0]	0/1	0/1	NT	0/10
Species positive by blocking-ELISA			S or L	46/1027	4.5% [3.4–5.9]	2/45	0/45	0/19	0/111

In the Blocking-ELISA column, within brackets are binomial confidence limits.
Primer sequence: 5′ GYC TGY GTG AGC TGA CAA ACT T 3′.
Reverse primer sequence: 5′ WCC GCG TTT TWG CAT ATT GAC A 3′.
Probe: 6-FAM-AAC CAG GAG GGC CCG G-MGB.
[a]S, species strictly short-distance migrant; L, species strictly long-distance migrant.
[b]Using Mab 4G2 (HB 112); using WNV lineage 1 strain WN_0304 and WNV lineage 2 strain MgAn 798; inhibition value ≥30%.
[c]Using WNV lineage 1 strain WN_0304; neutralization titer ≥20.
[d]Using TBEV strain 93/783; neutralization titer ≥20.
[e]Using USUV Austrian strain 939; neutralization titer ≥20.
[f]Real-time PCR method targeting the 5′ noncoding region of all available WNV sequences, including Rabensburg strain.
WNV, West Nile virus; TBEV, tick-borne encephalitis virus; USUV, Usutu virus; NT, not tested.

nuity correction: $\chi^2 = 7.14$, df = 1, $p = 0.008$). Short-distance migrant passerines (3.8%) were more frequently positive than long-distance migrant passerines (1.2%) ($\chi^2 = 6.96$, df = 1, $p = 0.008$), which contrasts with results previously observed in southern Europe (Lopez et al. 2008). Within short-distance migrant passerines, birds of the *Turdidae* family were more frequently positive (7.5%) than others (0.2%) ($\chi^2 = 28.66$, df = 1, $p < 0.001$); if they were excluded, then seroprevalence tended to be higher for long-distance migrant passerines (1.4%) than for other passerines (0.2%) (Fisher exact test, $p = 0.06$). No significant difference was observed between juveniles and adults both for short-distance ($\chi^2 = 1.66$, df = 1, $p < 0.198$) and long-distance migrant passerines (Fisher exact test, $p = 0.18$).

Overall, three categories of ELISA-positive birds could be distinguished (Table 2): (1) adult long-distance migrants of the order Passeriformes captured in May or early June ($n = 6$), that is, birds likely exposed to WNV or cross-reactive flaviviruses while wintering in sub-Saharan Africa (or Asia for the Common Rosefinch *Carpodacus erythinus*) or at stopover sites in the Mediterranean basin (Fransson and Hall-Karlsson 2008, Lopez et al. 2008); (2) adult short-distance migrants of the order Charadriiformes captured in May ($n = 2$), that is, shorebirds potentially exposed to WNV if they arrive early on their Mediterranean wintering grounds where WNV is regularly transmitted in late summer and autumn (Dauphin et al. 2004, Fransson and Hall-Karlsson 2008); (3) short-distance migrants of the order Passeriformes captured throughout the year ($n = 38$) and belonging essentially to the Turdidae family ($n = 37$).

We tried to find direct evidence of WNV in samples from all species with $\geq$1 ELISA-positive birds (Table 2) using a real-time RT-PCR method targeting the 5′ noncoding region of all available WNV sequences including Rabensburg lineage. All samples ($n = 111$) were negative, which is not surprising because viremia in birds experimentally infected with WNV only lasts a few days (Owen et al. 2006), and the probability of finding a viremic bird in nature is hence minute.

This study confirmed that antibodies against WNV are detectable in a small proportion (<3.2%) of the birds that migrate through southeastern Sweden and use Northern Europe as a breeding ground. The lack of reaction by seroneutralization compared to blocking-ELISA suggests that the ELISA-positive birds had not recently been exposed to WNV and were therefore unlikely to be in a viremic stage. Overall, these results suggest that WNV introduction into Northern Europe by migratory birds is unlikely. Additionally, although potential vectors such as the bird-feeding mosquitoes *Culex pipiens* and *Cx. torrentium* occur in Northern Europe (Becker et al. 2003), their abundance is probably too low and their emergence too late to reach the density required for bringing the WNV reproductive number above unity and allow the establishment of an enzootic bird–mosquito–bird transmission cycle.

Conversely, the capture in autumn of 24 ELISA-positive juvenile birds of the Turdidae family suggests that these birds had perhaps been exposed, on their northern breeding grounds, to a *Flavivirus* different from WNV, tick-borne encephalitis virus, and USUV, but antigenically related. Further investigations are needed to assess whether these turdid birds, which are ground-foraging and ideal medium-sized preys for both preimaginal ixodid ticks and ornithophilic mosquitoes (Lundström et al. 2001), were indeed exposed to a cross-reactive *Flavivirus* or if their serum is responsible for falsely positive reactions by blocking-ELISA.

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Disclosure Statement

No competing financial interests exist.

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