Surveillance for West Nile virus in wild birds from Northern Europe

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West Nile virus (WNV) has never been reported in Northern Europe although it is regularly circulating in Southern, Central and Eastern Europe.

QUESTIONS:

- Do migratory birds that breed in Northern Europe carry antibodies against WNV?

- If yes, are they likely to introduce WNV into Northern Europe?

1. Blocking-ELISA

1 Only **2.4%** (binomial confidence limits: [1.8-3.2], n=46) of the 1935 birds tested were positive by blocking-ELISA.

2 Seroprevalence was significantly higher in Passeriformes than in other taxonomic orders.



**Pearson's Chi-squared test with Yates continuity correction: $\chi 2=7.14$, df=1, p=0.008

3 Short-distance migrant passerines were more frequently positive than longdistance migrant passerines.



4 Within short-distance migrant passerines, birds of the Turdidae family were more frequently positive than other birds.



5 Seroprevalence was not significantly different between juvenile and adult passerines. However, whereas none of the juvenile long-distance migrants were positive, 24 short-distance migrant juveniles were



CONCLUSIONS

• Antibodies against WNV were detectable by ELISA in a small proportion (2.4%) of birds.

· The lack of reaction by neutralization compared to ELISA suggests that the ELISA-positive birds had not recently been exposed to WNV and were therefore unlikely to introduce the virus.

· Seropositive long-distance migrants had likely been exposed to WNV or a cross-related Flavivirus in endemic or epidemic areas.

• The detection among short-distance migrants of ELISA-positive juvenile birds suggests that these birds were likely exposed in Northern Europe to a Flavivirus different from WNV, TBEV and USUV.

Overall, these results suggest that the probability of WNV introduction into Northern Europe by migratory birds is low. However, it seems that northern bird populations might be exposed to another WNV-related Flavivirus.

to search for viremic birds (~10 birds were tested for each species).



Between March 2005 and May 2006, 1935 migratory birds from

104 species were captured at Ottenby Bird Observatory (Southeastern

Resulting serum was tested by a blocking-ELISA method¹ and

positive samples were further tested for WNV (n=45), TBEV (tick-

borne encephalitis virus, n=45) and USUV (Usutu virus, n=19)

For species positive by ELISA (n=11), real time RT-PCR³ was used

Sweden, see map) and bled from the jugular vein.

neutralizing antibodies².

further tested by neutralization, only 2 had detectable WNV neutralizing antibodies (none was positive for antibodies against TBEV or USUV).

Other ELISA-positive birds:

· may have had antibodies against WNV at levels undetectable by the neutralization assay; • may have been exposed to a cross-reactive Flavivirus:

· non-specific falsely positive reactions cannot be excluded.

3. Real time RT-PCR

The 111 serum samples tested for the presence of WNV RNA were negative. This result is not surprising because viremia is believed to only lasts a few days and the probability of finding a viremic bird in nature is hence minute.

Technical notes

1 with a threshold cut-off value of 2 using WNV strain 0304, TBEV strain 93/783 and USUV Austriar 30% and using the Flavivirus groupspecific monoclonal antibody 4G2 and an antigen prepared from both Israel 0304 (lineage 1) and Madagasstrain 939 ³targeting the 5' non-coding region of all available WNV sequences car 798 (lineage 2) WNV strains including Rabensburg lineage

Bird species positive by ELISA



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Within the ELISA-positive birds

2. Seroneutralization