Prevalence of Neutralizing Antibodies to West Nile Virus in Eleonora's Falcons in the Canary Islands

Laura Gangoso,[1,5,6] Juan Manuel Grande,[2] Francisco Llorente,[3] Miguel Ángel Jiménez-Clavero,[3] Jesús M. Pérez,[4] and Jordi Figuerola[5] [1] Department of Ecology and Evolution, University of Lausanne CH-1015, Lausanne, Switzerland; [2] Department of Biology, University of Saskatchewan, 112 Science Pl., Saskatoon, SK S7N 5E2, Canada; [3] Centro de Investigacion en Sanidad Animal, INIA, Ctra. Algete-El Casar s/n, Valdeolmos, E-28130, Madrid, Spain; [4] Department of Animal and Vegetal Biology and Ecology, University of Jaen, Campus Las Lagunillas s/n, E-23071, Jaen, Spain; [5] Department of Wetland Ecology, Estacion Biologica de Donana, CSIC, Avda. Americo Vespucio s/n, E-41092, Seville, Spain; [6] Corresponding author (email: laura.gangoso@unil.ch)

ABSTRACT: Birds are the major amplifying host for West Nile virus (WNV), a flavivirus that may affect humans and transmitted by bloodsucking vectors. Eleonora's Falcons (Falco eleonorae) migrate to the Canary Islands annually from WNV-endemic regions. To investigate the possible role of Eleonora's Falcons in the circulation of WNV, we measured WNV-specific antibodies in 81 falcons captured in 2006. None of the nestlings but 14.8% of the adults had WNV-neutralizing antibodies. RT-PCR did not detect flaviviruses in nonculicine ectoparasites (n5231) of the falcons. These findings suggest that WNV infection did not occur locally, but rather on the wintering grounds or during migration.

Key words: Canary Islands, Eleonora's Falcon, louse flies, neutralizing antibodies, West Nile Virus.

West Nile virus (WNV; family Flaviviridae, genus Flavivirus) is a vector-borne virus belonging to the Japanese encephalitis virus (JEV) serocomplex. Its natural transmission cycle involves birds as major amplifying hosts and arthropod vectors, mainly mosquitoes (Hubálek, 2008). Although originally identified in Africa, WNV is now distributed through all continents except Antarctica. Birds also seem to be involved in the dispersal of WNV (Hubálek, 2008).

The Canary Islands are in the northeast Atlantic Ocean, approximately 110 km off the northwest African mainland (Fig. 1). Many birds move yearly between the Canary Islands and Africa and Europe; some of them come from WNV-endemic regions, suggesting that the risk for the disease to reach the islands could be high. Eleonora's Falcon (Falco eleonorae) is a medium-sized raptor that breeds colonially over the entire Mediterranean basin (Walter, 1979). Eleonora's Falcons stay in the Canary Islands for breeding from July to October and then cross Africa to reach their wintering grounds in eastern Africa and the Indian Ocean, especially Madagascar and the surrounding islands (Gschweng et al., 2008, Fig. 1). In fall and spring migration, as well as during the wintering period, falcons cross through or stay at areas where WNV is endemic (Dauphin et al., 2004). Other Falconidae species have been found susceptible to WNV disease (Nemeth et al., 2006) and antibody prevalence studies have found that populations of European and African falcons have been exposed to WNV (Banet-Noach et al., 2004). To assess the potential role of Eleonora's Falcons in the possible introduction of WNV to the Canary Islands, we examined the prevalence of WNV-neutralizing antibody in a breeding population. In addition, as no culicine mosquitoes are present in the islets where these birds breed (Gangoso and Grande, pers. obs.), and WNV RNA has been detected in louse flies (Diptera, Hippoboscidae) collected from wild raptors (Farajollahi et al., 2005), we tested for flavivirus presence in these ectoparasites commonly feeding on falcon blood.

From 10 September to 17 October 2006, we monitored a population of Eleonora's Falcons in the Alegranza Islet, the northernmost islet of Lanzarote, Canary Islands (27u379–29u259N, 13u209–18u199W, Fig. 1). Adult Eleonora's Falcons were captured at nests with the use of net traps when nestlings were 10–

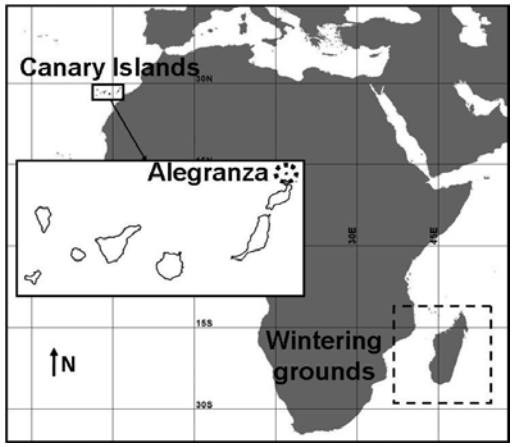

FIGURE 1. Study site for Eleonora's Falcons in the Canary Islands 10 September–17 October 2006 and wintering grounds in eastern Africa, Madagascar, and surrounding islands.

25 days old or with roost traps at a pond where they regularly bathe and drink. Nestlings were sampled at nests when they were 25–30 days old. Age in days was calculated following Ristow and Wink (2004). Blood samples (1 ml) from the brachial vein were collected into Eppendorf tubes, allowed to clot at ambient temperature, and placed into coolers until centrifugation later the same day. Sera were stored at 220 C. All birds were marked with numbered aluminum rings and released after handling. The research protocols were authorized by the Regional Government of the Canary Islands.

All sampled birds were inspected for ectoparasites (louse flies) during a 3-min period. Louse flies were removed and initially stored in Eppendorf tubes filled with RNA-Later® (QIAgen, Madrid, Spain) for up to 3 wk at 4 C. In the laboratory, samples were stored at 280 C until identification and subsequent analysis. Identification of louse flies was made on a subsample of 10 individuals on the basis of features such as scutellum, abdominal tergites, wing length, and venation as well as presence of hair in the wing membrane (Hutson, 1984).

West Nile Virus strain Eg101 (isolated in Egypt in 1951) and the E6 clone of Vero cells used for virus propagation were obtained from Hervé Zeller (Institut Pasteur, Lyon, France). Neutralizing antibody titers to WNV were determined by a micro-virus-neutralization test (micro-VNT) in 96-well plates, as described (Figuerola et al., 2007). Only samples yielding positive neutralization at 1:20 dilution were scored as positives and further titrated by analyzing serial serum dilutions from 1:20 to 1:640. Neutralizing serum titer was the highest value of the reciprocal serum dilution giving a complete absence of cytopathic effect (CPE). The specificity of the assay was assessed first, by analyzing a panel of sera from an external quality assessment, consisting of serum samples containing antibodies from four other flaviviruses (Niedrig et al., 2007), that proved negative in our micro-VNT assay, while duplicate testing of all WNV antibody-positive serum samples proved positive ($1:20) for neutralization titers. Second, by comparing the neutralizing antibody titers of 18 samples tested in parallel for WNV and Usutu virus (USUV, a closely related JEV group flavivirus) to determine the specificity of the antibody responses to WNV in comparison to the closely related USUV (see Figuerola et al., 2007 for details).

Ectoparasite samples were transferred to polypropylene tubes with screw caps (pooled to a maximum of three individuals/tube) containing 1 ml phosphate-buffered saline (PBS), and homogenized in a Tissue Lyser (QIAgen) homogenizer. Nucleic acid extraction from aliquots of 0.1 ml of each homogenate (or, in hippoboscides, due to their larger size, using 0.1 ml of the homogenate diluted 1:10) was performed with the use of Biosprint 15-DNA Blood kit (optimized for viral RNA; QIAgen) and an automated nucleic acid extractor (Biosprint 15; QIAgen), following manufacturer's instructions. Pan-flaviviral heminested reverse-transcription polymerase chain reaction (RT-PCR) was performed as described (Scaramozzino et al., 2001), with the use of 4 ml of each RNA extract and

the generic primers CFD2 and MAMD for the first RT-PCR reaction, carried out with One step RT-PCR kit (QIAgen), and 0.5 ml of the RT-PCR product for the heminested reaction, performed with the generic primers CFD2 and FS788 and Taq Gold DNA polymerase.

Of the 81 Eleonora's Falcons tested (27 adults and 54 nestlings), four individuals (4.9%) had WNV neutralizing antibodies. All of the antibody-positive falcons were adults (14.8% of the adults subsample), and the virus-neutralization titers were 1:40. None of the 18 individuals tested (including all the positive individuals for WNV) had USUV-neutralizing antibodies.

The louse flies collected were identified as Ornitophila gestroi. This genus has only two described species, of which O. gestroi had been reported on Eleonora's Falcon (Wink et al., 1979), but never in the Canary Islands. None of the 231 louse flies (collected from 108 individuals) were positive for flaviviruses by RT-PCR.

Only a small proportion of Eleonora's Falcons breeding on the Canary Islands in 2006 were antibody positive for WNV. Because no bird showed antibodies against USUV, we demonstrated that the antibodies corresponded to WNV or a very closely related flavivirus. The prevalence of antibodies in adult falcons is comparable to that of other long-distance migrant birds captured in southern Spain that winter or move through African areas where WNV is endemic (Figuerola et al., 2007), and to those of resident bird species captured in Morocco (Figuerola et al., 2009).

The presence of WNV-neutralizing antibodies in sera of adult falcons indicates previous contact with the virus, survival of initial infection, and subsequent development of immunity (Nemeth et al., 2008). Detection of antibodies, however, does not explain when and where the infection occurred. Although it has been demonstrated that WNV antibodies remain detectable for several years (Nemeth et al., 2008), other studies indicate a rapid reduction in antibody titer in other host species after exposure to the virus (Figuerola et al., 2007) suggesting that our data could underestimate the proportion of birds that had been previously infected with WNV.

Our results suggest that WNV is not circulating locally in the Eleonora's Falcon population studied, because none of the nestlings presented antibodies and none of the louse flies were flavivirus positive (WNV or any other member of the family). Consequently, exposure to WNV in adult birds is more likely to have occurred during the migratory journey or in the wintering grounds, where the virus actively circulates (Dauphin et al., 2004; Figuerola et al., 2009). The probability that falcons could introduce WNV into the Canary Islands far from distant wintering grounds seems to be low, given the duration of viremia in bird blood ($>$7 days; Komar et al., 2003) although they could probably carry the virus from much closer areas such as Morocco (150 km). Although our study demonstrates WNV exposure in the Eleonora's Falcon, further studies of this and other wild bird species migrating to the Canary Islands from WNV-endemic areas are needed to assess the risk of WNV circulation.

The Obra Social de la Caja de Canarias funded this research with Eleonora's Falcon. Our WNV research is funded by a European Union project (GOCE-2003-010284EDEN) and FAU/2008-00002-00-00 from INIA. This publication is catalogued by the EDEN Steering Committee as EDEN (http://www.eden-fp6project.net). J.M.G. and L.G. received postdoctoral fellowships from the Spanish Ministry of Science and Innovation and from the Isabel María López Memorial Scholarship (J. M.G.).We thank J. J. Moreno, J. L. Barroso, E. Luque, P. Vicent, G. Tejera, D. Lagares, I. Luque, and M. de la Riva, for their help during the fieldwork.

LITERATURE CITED

BANET-NOACH, C., A. Y. GANTZ, A. LUBLIN, AND M. MALKINSON. 2004. A twelve-month study of West

Nile virus antibodies in a resident and a migrant species of kestrels in Israel. Vector-Borne and Zoonotic Diseases 4: 15–22.

DAUPHIN, G., S. ZIENTARA, H. ZELLER, AND B. MURGUE. 2004. West Nile: Worldwide current situation in animals and humans. Comparative Immunology, Microbiology and Infectious Diseases 27: 343–355.

FARAJOLLAHI, A., W. J. CRANS, D. NICKERSON, P. BRYANT, B. WOLE, A. GLASER, AND T. G. ANDREADIS. 2005. Detection of West Nile virus RNA from the louse fly Icosta Americana (Diptera: Hippoboscidae). Journal of the American Mosquito Control Association 21: 474–476.

FIGUEROLA, J., R. E. BAOUAB, R. SORIGUER, O. FASSI-FIHRI, F. LLORENTE, AND M. A. JIMENEZ-CLAVERO. 2009. West Nile virus antibodies in wild birds, Morocco, 2008. Emerging Infectious Diseases 15: 1651–1653.

———, R. SORIGUER, G. ROJO, C. GÓMEZ-TEJEDOR, AND M. A. JIMÉNEZ-CLAVERO. 2007. Seroconversion in wild birds and local circulation of West Nile virus. Spain. Emerging Infectious Diseases 13: 1915–1917.

GSCHWENG, M., E. K. V. KALKO, U. QUERNER, W. FIEDLER, AND P. BERTHOLD. 2008. All across Africa: Highly individual migration routes of Eleonora's Falcon. Proceedings of the Royal Society B 275: 2887–2896.

HUBÁLEK, Z. 2008. Mosquito-borne viruses in Europe. Parasitological Research 103(Suppl. 1): S29–S43.

HUTSON, A. M. 1984. Keds, flat-flies and bat-flies. Diptera, Hippoboscidae and Nycteribiidae. Handbooks for the identification of British insects, P. C. Barnard and R. R. Askew (eds.). Vol. 10, no. 7, Royal Entomological Society of London, London, UK, 40 pp.

KOMAR, N., S. LANGEVIN, S. HINTEN, N. NEMETH, E. EDWARDS, D. HETTLER, B. DAVIS, R. BOWEN, AND M. BUNNING. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerging Infectious Diseases 9: 311–322.

NEMETH, N. M., D. GOULD, R. BOWEN, AND N. KOMAR. 2006. Natural and experimental West Nile virus infection in five raptor species. Journal of Wildlife Diseases 42: 1–13.

———, G. E. KRATZ, R. BATES, J. A. SCHERPELZ, R. A. BOWEN, AND N. KOMAR. 2008. Naturally-induced humoral immunity to West Nile virus infection in raptors. EcoHealth 5: 298–304.

NIEDRIG, M., O. DONOSO MANTKE, D. ALTMANN, AND H. ZELLER. 2007. First international diagnostic accuracy study for the serological detection of West Nile virus infection. BMC Infectious Diseases 7: 72. doi:10.1186/1471-2334-7-72.

RISTOW, D., AND M. WINK. 2004. Seasonal variation in sex ratio of nestling Eleonora's Falcons. Journal of Raptor Research 38: 320–325.

SCARAMOZZINO, N., J. M. CRANCE, A. JOUAN, D. A. DEBRIEL, AND D. GARIN. 2001. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. Journal of Clinical Microbiology 39: 1922–1927.

WALTER, H. 1979. Eleonora's Falcon: Adaptations to prey and habitat in a social raptor. University of Chicago Press, Chicago, Illinois, 410 pp.

WINK, M., D. RISTOW, AND C. WINK. 1979. Biology of Eleonora's Falcon Falco-eleonorae, Part 3, Parasitemia of adult and juvenile falcons in relation to breeding season and growth. Journal für Ornithologie 120: 94–97.

.