West Nile virus neutralizing antibodies in wild birds from southern Spain

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Abstract

West Nile virus (WNV) is an emerging vector-borne arbovirus with a zoonotic life-cycle whose main reservoir hosts are birds. In humans and horses, WNV infections rarely result in clinical disease but on occasions – depending on factors such as climatic conditions, insect communities and background immunity levels in local populations – they can lead to outbreaks that threaten public and animal health. We tested for the presence of WNV antibodies in 149 birds belonging to 32 different species. Samples were first tested using a bird-specific ELISA kit and then both positive and doubtful results were confirmed by neutralization tests using WNV and Usutu virus. WNV antibodies were confirmed in a resident Sylvia melanocephala juvenile, supporting the idea of local transmission of WNV in southern Spain in 2013. In addition, the serum from an adult blackbird (Turdus merula) showed neutralization of both WNV and Usutu virus. We discuss our results in light of the occurrence of WNV on horse farms in southern Spain in 2013.

Keywords: Avian species; flavivirus; Usutu virus; vector-borne pathogens

Introduction

West Nile virus (WNV) is an emerging arbovirus with a zoonotic life-cycle [1]. Virus transmission between birds (the virus reservoirs) requires the bite of an infected mosquito, although other transmission routes including oral transmission have been demonstrated experimentally [2, 3]. WNV has a complex eco-epidemiology that involves a wide range of vectors and great host diversity and is considered to be the most geographically widespread of all mosquito-borne flaviviruses [4]. In humans and horses, both incidental hosts of the virus, WNV infections rarely result in clinical disease but can occasionally cause outbreaks that seriously affect animal and public health [5]. In humans, 80% of infections are asymptomatic, the remaining 20% being associated with influenza-like symptoms; despite this, in a few cases (<1%) the disease may appear as aseptic meningitis or encephalitis. It is important to note that these proportions vary according to the viral strain involved [6].

In the New World, the spread of WNV has had marked consequences and has resulted in the death of millions of birds since 1999 [7]. European birds infected with WNV rarely develop clinical symptoms and avian mortality is only reported infrequently in the wild [8]. Nevertheless, recent changes in the virus epidemiology suggest that an increase in its virulence has occurred [9]. Additionally, experimental infections in the laboratory have confirmed the pathogenic effect of many European WNV strains in birds from the Old World [3, 10], which highlights the importance of this virus in both public health and biological conservation [11].

In Spain, in addition to the arrival of trans-Saharan migrant birds that are potentially exposed to WNV during their stay in Africa [12], local transmission events are thought to have occurred since the 1960s [8]. Conclusive evidence of WNV circulation in Spain came in the early 2000s when many bird species were detected with

WNV antibodies [13] and the virus was identified in mosquitoes [14].

We analysed the presence of WNV antibodies in different migrant and resident species captured during 2013 as a part of an extensive study on WNV transmission in southern Spain. WNV and Usutu virus (USUV) belong to the same serogroup (Japanese encephalitis group; family: Flaviviridae) and a cross-reaction between these viruses may occur [15]. As is the case for WNV, USUV actively circulates in southern Spain [14, 16]. Therefore, we confirmed our results by comparative neutralization tests using WNV and USUV in parallel. USUV, an African vector-borne flavivirus, has been recorded in recent years in a number of European countries [17], with birds from the genus Turdus usually suffering the highest mortality rates [16, 18].

Methods

In July–October 2013, birds were trapped in the provinces of Huelva, Cádiz and Sevilla (Fig. 1). Birds were captured using mist-nets and subsequently ringed, with sex and age recorded [19]. Birds were released at the capture site after sampling without injury. A blood sample (volume <1% of body mass) was obtained from the jugular vein of each bird using sterile syringes. Blood samples were maintained at 4 °C for 24 h prior to centrifugation for 10 minutes at 1700 g to separate serum and cellular fractions. Serum samples were frozen at −80 °C until the subsequent virus neutralization test (VNT) was performed. Experimental procedures were approved by the CSIC Ethics Committee on 9 March 2012.

Initial screening for the detection of antibodies against WNV and other related flaviviruses was performed using the epitope blocking ELISA kit Ingezim West Nile Compac (Ingenasa Spain), which, according to the manufacturer's instructions, requires 10 µl bird serum to measure antibodies [20]. Samples giving ELISA positive or doubtful results were subsequently analysed by VNT. For this test we used the micro-assay format (96-well plates) described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [21] and elsewhere [13] with the following modifications: (1) we used Vero instead Vero E6 cells, and (2) the incubation of sample dilutions with viral antigens was performed in the presence of 0·1% bovine serum albumin. The VNTs were performed in the BSL-3 laboratory at CISA in accordance with all current biosafety guidelines. Neutralizing antibody titres were determined in parallel for each serum sample against WNV (strain Eg-101) and USUV (strain SAAR1776) by using serial (twofold) dilutions (1:10–1:1280) of each serum sample in a VNT. Specific responses to viruses were based on the comparison of VNT titres obtained in parallel against the two flaviviruses: the neutralizing immune response observed was considered specific when VNT titres for a given virus were >fourfold higher than the titre obtained for the other virus [13].

Results

In all, blood samples from 149 wild birds belonging to 32 different species were analysed in this study (Table 1). With the ELISA kit, positive and doubtful reactions were observed in six and seven individuals, respectively. Only one female juvenile (born in the same calendar year) Sardinian warbler (Sylvia melanocephala) had specific WNV-neutralizing antibodies, with a titre of 1:80. Serum from an adult male blackbird (Turdus merula) neutralized WNV at a titre of 1:40 and USUV at 1:80. These two birds were captured at the beginning of September in the province of Huelva, the former at an equestrian centre and the latter in wetland area.

Discussion

We found WNV antibodies in the resident species S. melanocephala. This result supports the idea of local transmission of WNV in southern Spain in 2013, thereby providing more information on WNV transmission dynamics in the area. In 2013, there were WNV outbreaks on horse farms in 34 locations in southern Spain, 28 and six in the provinces of Sevilla and Huelva, respectively (Fig. 1). The closest location with a declared WNV case (S. melanocephala) in horses was 27 km from the capture site, a location with many horses. This indicates that the virus was in fact circulating in a larger area than that suggested by the known cases of disease in horses, and highlights the importance of wild bird surveillance when attempting to detect the circulation of WNV in the absence of the disease [22].

Unlike in other bird groups such as rallids [23], raptors [11] and crows [24] (see [3] and references therein), only a small proportion of songbirds – the most extensively sampled avian group – were found to have WNV-neutralizing antibodies. Although migration is likely to be an important factor affecting the exposure of avian species to WNV, i.e. trans-Saharan migratory species usually show higher values than migrant species travelling short distances or resident species [12, 25], we did not detect WNV antibodies in any migratory species. Possible explanations of these results include inter-annual variations in the proportion of seropositive birds, differences between the species sampled in studies or, simply, the fact that in autumn juvenile birds had not yet migrated to Africa; in fact, in total we only sampled 10 adults of trans-Saharan migratory species (20% of the individuals captured).

Finally, our results strongly support the need to use VNTs to confirm WNV in all positive and doubtful samples detected by ELISA kits in order to increase the accuracy of estimates of pathogen seroprevalence in wild birds. We found that only one of the six ELISA-positive samples reacted in the VNT. The other five birds may have had antibodies that were specific to another flavivirus not studied here such as Marisma Mosquito virus (see [26]). Obviously, these results suggest the need for a conservative approach, which will reduce the number of positive individuals. The use of VNT will be especially important in areas where related flaviviruses co-circulate in order to prevent overestimates of the presence of WNV antibodies [5].

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Figure 1. Place of origin of the avian serum samples analysed in this study (◦) and those with at least one positive sample by ELISA (•). Place of origin of birds with each WNV neutralizing antibody (◆) and flavivirus neutralizing antibody (◇) are shown. The locations with positive cases of WNV infections in horses during 2013 are indicated by ▲.

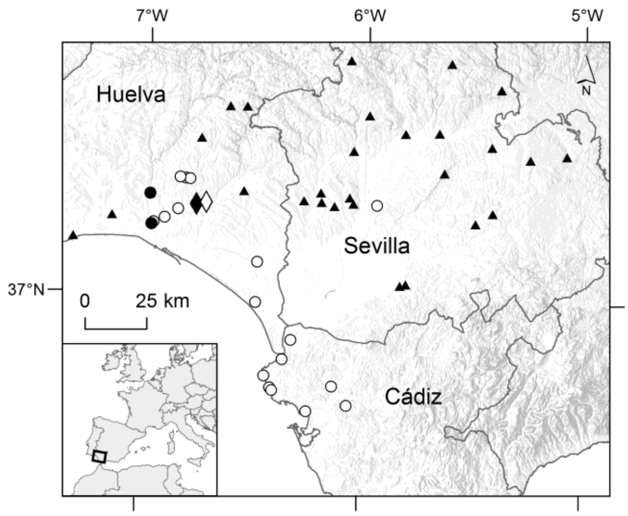

Table 1. Bird species sampled and analysed for WNV antibodies using ELISA. Positive and doubtful samples using ELISA were subsequently tested using VNT.

Orden	Family	Species name	Common name	Sampled individuals	Elisa positive	Elisa doubtful	VNT positive,
Columbiformes	Columbidae	*Streptopelia decaocto*	Eurasian collared dove	1			
Coraciiformes	Upupidae	*Upupa epops*	Hoopoe	2	2		2 (WNV <1:10, USUV <1:10)
Cuculiformes	Cuculidae	*Cuculus canorus*	Common cuckoo	1			
Passeriformes	Acrocephalidae	*Hippolais polyglotta*	Melodious warbler	1			
	Alaudidae	*Galerida cristata*	Crested lark	1			
	Certhiidae	*Certhia brachydactyla*	Short-toed treecreeper	1			
	Cisticolidae	*Cisticola juncidis*	Streaked fantail warbler	1			
	Corvidae	*Cyanopica cyanus*	Azure-winged magpie	12	2	4	6 (WNV <1:10, USUV <1:10)
		Pica pica	Common magpie	1			
	Fingillidae	*Carduelis carduelis*	European goldfinch	2			
		Carduelis chloris	European greenfinch	1			
	Hirundinidae	*Delichon urbicum*	House martin	3			
	Laniidae	*Lanius senator*	Woodchat shrike	1			
	Motacillidae	*Motacilla flava*	Western yellow wagtail	6		1	1 (WNV <1:10, USUV <1:10)
	Muscicapidae	*Erithacus rubecula*	European robin	1			
		Ficedula hypoleuca	Pied flycatcher	12			
		Luscinia megarhynchos	Common nightingale	4			
		Muscicapa striata	Spotted flycatcher	1			
		Oenanthe oenanthe	Wheatear	1			
		Phoenicurus phoenicurus	Common redstart	3		1	1 (WNV <1:10, USUV <1:10)
	Paridae	*Parus major*	Great tit	4			
	Passeridae	*Passer hispaniolensis*	Willow sparrow	37			

		Passer montanus	Eurasian tree sparrow	1			
	Phylloscopidae	*Phylloscopus trochilus*	Willow warbler	3			
	Sylviidae	*Acrocephalus scirpaceus*	Eurasian reed warbler	4		1	1 (WNV <1:10, USUV <1:10)
		Cettia cetti	Cetti's warbler	1			
		Sylvia atricapilla	Eurasian blackcap	3			
		Sylvia borin	Garden warbler	5			
		Sylvia communis	Common whitethroat	5			
		Sylvia melanocephala	Sardinian warbler	19	1		1 (WNV 1:80, USUV <1:10)
	Turdidae	*Turdus merula*	Blackbird	9	1		1 (WNV 1:40, USUV 1:80)
Pelicaniformes	Ardeidae	*Bubulcus ibis*	Cattle egret	2			