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Monitoring of West Nile virus, Usutu virus and Meaban virus in waterfowl used as decoys and wild raptors in southern Spain

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ABSTRACT

In the last decade, the number of emerging flaviviruses described worldwide has increased considerably, with wild birds acting as the main reservoir hosts of these viruses. We carried out an epidemiological survey to determine the seroprevalence of antigenically related flaviviruses, particularly West Nile virus (WNV), Usutu virus (USUV) and Meaban virus (MBV), in waterfowl used as decoys and wild raptors in Andalusia (southern Spain), the region considered to have the highest risk of flaviviruses circulation in Spain. The overall flaviviruses seroprevalence according to bELISA was 13.0% in both in decoys (n = 1052) and wild raptors (n = 123). Specific antibodies against WNV, USUV and MBV were confirmed by micro virus neutralization tests in 12, 38 and 4 of the seropositive decoys, respectively. This is the first study on WNV and USUV infections in decoys and the first report of MBV infections in waterfowl and raptors. Moreover we report the first description of WNV infections in short-toed snake eagle (*Circaetus gallicus*) and Montagu's harrier (*Circus pygargus*). The seropositivity obtained indicates widespread but not homogeneous distribution of WNV and USUV in Andalusia. The results also confirm endemic circulation of WNV, USUV and MBV in both decoys and wild raptors in southern Spain. Our results highlight the need to implement surveillance and control programs not only for WNV but also for other related flaviviruses. Further research is needed to determine the eco-epidemiological role that waterfowl and wild raptors play in the transmission of emerging flaviviruses, especially in decoys, given their close interactions with humans.

1. Introduction

During the last decade, the spread of many flaviviruses (Genus *Flavivirus*) has been reported, representing an emerging threat for both animal and human health [1]. Most of these viruses are transmitted within an enzootic cycle involving ornithophilic mosquitoes or ticks as competent vectors, and wild birds that play a central role in flavivirus epidemiology as they represent the main amplifying hosts in the wild and may contribute to their dispersion through their migratory behavior [2]. Mammal species including humans are considered dead-end or incidental hosts, because they can get infected but are not thought to be able to transmit the viruses [3]. In humans, other routes of flavivirus transmission such as blood transfusion, organ transplantation, intrauterine transmission, handling of infected carcasses and breast feeding, have been also described [4]. Even though infections by flaviviruses are generally asymptomatic or result in mild illness, they can potentially cause fever, encephalitis and mortality both in birds and in other vertebrate species, including humans [5].

Encephalitis and mortality associated with West Nile virus (WNV), Usutu virus (USUV), Bagaza virus (BAGV), tick-borne encephalitis virus (TBEV) and Louping ill virus (LIV) have been reported in humans (WNV, TBEV), wild birds (WNV, USUV, BAGV), horses (WNV, TBEV) and small ruminants (LIV) in Europe in the last few years [6–12]. Additionally, Meaban virus (MBV) infections have been identified in wild birds and ticks in several European countries

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Table 1
Results of bELISA and VNTs for flaviviruses (West Nile virus, Usutu virus and Meaban virus) detection in decoys from Andalusia (southern Spain).

Species	Seropositive	VNT Results							
		WNV	USUV	MBV	WNV and/or USUV	USUV and MBV	WNV and MBV	WNV and/or USUV and MBV	Flaviviruses[a]
Greyland goose	116/842 (13.77%)	10	28	1	8	4	3	4	58
mallard ducks	21/210 (10.0%)	2	10	3	3	1	0	0	2
Total	137/1052 (13.0%)	12	38	4	11	5	3	4	60

[a] Flaviviruses: Positive sera by ELISA could not be analyzed by VNT (n = 45) due to low volume or serum citotoxicity and positive sera by ELISA showed negative results against the three flaviviruses tested using VNT (n = 15).

[13]. Most of these flaviviruses have shown a high propensity to spread or have been unexpectedly reported in European countries in the last few years [14].

Due to its strategic geographic location on the migratory flyway of wild birds, the high number of wetlands and the high density of competent vectors and susceptible host species, Spain is considered as an at risk country for flaviviruses introduction. In fact, five (WNV, USUV, MBV, BAGV and LIV) out of the six flaviviruses detected in Europe in the last decade, have circulated in Spain. Serological evidence of WNV circulation in Spain has been detected in wild birds [15], humans [16], horses [7], camels [17], wild boars, Iberian pigs and red foxes [18]. However, clinical disease and mortality associated to WNV infection has been reported only in wild birds, horses and humans from central and southern regions of Spain [19,20]. To date, a total of 105 WNV outbreaks in horses [21] and two clinical cases in humans [7] have been reported in Spain. Usutu virus has been detected in both mosquitoes [22] and in migratory birds [6]. Mortality associated to BAGV infection was confirmed in free-living game birds in south-western Spain in 2010 [8]. Meaban virus has been found in both yellow-legged gull (*Larus michaelis*) and ticks (*Ornithodoros maritimus*) in north-eastern Spain [13]. Finally, mortality associated to LIV infection was also detected in sheep and goats and suspected in chamois (*Rupicapra pyrenaica*) in northern Spain [11].

Anseriformes and Charadriiformes are susceptible to WNV infection [3]. Clinical disease and mortality associated to WNV infections have been described in captive waterfowl [23]. Experimental infections demonstrated that oral and cloacal shedding of WNV from infected waterfowl may increase the risk of infection of other birds [24]. Decoys are domestic waterfowl species, including Anseriformes and Charadriiformes, which act as lures for hunting purposes (Decree 182/2005, 26 July). In Spain, the use of decoys is considered an important economic activity with great relevance in regions with presence of wetlands. Waterfowl hunting is carried out in extensive waterlands which, due to the variety of vertebrate host species and the suitable presence of aquatic environments that support the development of competent arthropod vectors, are considered important risk areas for flaviviruses circulation [25]. Because of the frequent contact of decoys with wild birds during the hunting season as well as their convenience to be sampled, domestic waterfowl are potentially good sentinel species for the monitoring of WNV [26].

As wild raptors are located at the top of the food chain, they are considered useful for the monitoring of pathogens [27]. Even though several raptor species have been found to be particularly susceptible to WNV infection [28], surveillance studies on birds of prey are still scarce. In Spain, antibodies against antigenically related flaviviruses have been detected in non-migratory wild raptors [29], which suggest local circulation of these viruses. In addition, mortality due to WNV infection in wild birds in Spain has been detected in raptor species, including the critically endangered Spanish imperial eagle (*Aquila adalberti*) and the golden eagle (*Aquila chrysaetos*) [6,19].

The aim of this study was to determine the seroprevalence of antigenically related flaviviruses, particularly WNV, USUV and MBV, in decoys and wild raptors in Andalusia (southern Spain), the region considered to have the highest risk of flaviviruses circulation in Spain.

2. Material and methods

2.1. Ethical statement

This study was carried out in strict accordance with the current national and regional laws regarding ethics and animal use for scientific purposes in Andalusia where samples were collected (R.D.1021/2005; BOJA-55/2012; N/Ref: SGYB/FOA/AFR/CFS). Samples from decoys were collected by official veterinarians from the Regional Government of Andalusia (Consejería de Agricultura, Pesca y Desarrollo Rural) as part of the Regional Avian influenza surveillance program (BOJA-246/2010). Samples from wild raptors were collected from birds admitted at the Wildlife Rehabilitation Centres by official veterinarians from the Regional Government of Andalusia (Consejería de Medio Ambiente y Ordenación del Territorio). Samples were collected in compliance with the Ethical Principles in animal research guidelines in Wildlife Rehabilitation Centres. Blood samples from decoys and wild raptors were collected from the basilic or medial metatarsial vein. Handling procedures were designed to reduce stress and minimize suffering for subjects, according to European (86/609) and Spanish laws (R.D. 223/1988, R.D.1021/2005).

2.2. Sampling

Between November and December 2011, all decoys' flocks (n = 143) present in Andalusia (southern Spain) were sampled. The sampling was designed with the aim of detecting a prevalence in flavivirus infection of 0.2% with a 95% confidence level. Based on the total census of decoys in Andalusia, a total of 1052 decoys (45.4% of the total census in Andalusia), including 842 Greyland goose (*Anser anser*) and 210 mallard ducks (*Anas platyrhynchos*), had to be tested (Table 1). The number of birds analyzed within flocks ranged from 1 to 22 (mean = 8.0). All birds were individually identified by metal rings. In addition, 123 sera samples from wild raptors from 16 species (Table 2) were obtained in Wildlife Rehabilitation Centers from Andalusia between 2011 and 2014. Samples were collected into sterile tubes without anticoagulant and centrifuged at 400*g* for 15 min. Then, serum was separated and stored at −20 °C until analyzed. Moreover, data of the individual identification, species, date of sampling, location and census of the flock, were recorded. Clinical signs compatible with flaviviruses infection were not observed in any of the birds analyzed.

2.3. Serological assays

Serum samples were tested for antibodies against one epitope of the preMembrane-Envelope (prM-E) protein common with other viruses of the Japanese encephalitis serocomplex. A commercial blocking enzyme-linked immunosorbent assay (bELISA 10.WNV.K3 INGEZIM West Nile COMPAC®, Ingenasa, Madrid, Spain) was used