Circulation of Zoonotic Arboviruses in Equine Populations of Mallorca Island (Spain)

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Abstract

The presence of major arbovirus vector species, climate change that promotes the expansion and increase of their populations, and potential animal reservoirs mean that vector-borne diseases represent a significant health risk for Mallorca’s inhabitants. Microbiological monitoring of circulating arboviruses, particularly flaviviruses causing encephalitis, was initiated using domestic horses from localities near wetlands as “sentinel” hosts. A total of 291 blood samples were taken from 172 horses between 2011 and 2012, using paired samples to highlight seroconversion events. A multiplex immunoassay and confirmatory reference serological assays were used to screen sera for immunoglobulin G antibodies against West Nile (WNV), Usutu (USUV), and tick-borne encephalitis (TBEV) viruses. The seroprevalence was 6.4% (confidence interval [95% CI] 3.2%–11.0%) for WNV, 1.2% (95% CI 0.1%–4.1%) for USUV, and 0.6% (95% CI 0.0%–3.2%) for TBEV. In addition, eight horses (4.6%; 95% CI 2.0%–8.9%) were found positive for unidentified flaviviruses. Seroconversion events were detected for WNV and USUV, reflecting recent arboviral infections. These results highlight the active transmission of zoonotic arboviruses in Mallorca wetlands.

Keywords: arbovirus, equine, seroprevalence, serosurvey, tick-borne encephalitis, Usutu, vectorborne, West Nile

Introduction

Arthropod-borne viruses (arboviruses) responsible for human diseases have become very important in the global health landscape, due to their epidemic potential and unprecedented spread (Weaver and Reisen 2010). Neurotropic viruses belonging to the Japanese encephalitis and tick-borne encephalitis serocomplexes in the Flaviviridae family are the prominent cause of arboviral encephalitis in vertebrate hosts, including humans. While viruses belonging to the Japanese encephalitis serocomplex, such as West Nile (WNV) and Usutu (USUV) viruses, are primarily spread through mosquitoes (Vázquez et al. 2011), members of the tick-borne encephalitis serocomplex, such as the tick-borne encephalitis virus (TBEV), are mainly transmitted by ticks of the genus Ixodes (Dobler 2010).

WNV has expanded its geographic range and is commonly found in Africa, Europe, the Middle East, North America, Australia, and West Asia (WHO 2015). Large outbreaks of raised clinical severity have been reported in Southern and Eastern Europe (Hubař et al. 1999, Gubler 2010). In recent years, WNV circulation was confirmed in Spain by the detection of specific neutralizing antibodies in humans, wild bird species, and horses (García-Bocanegra et al. 2011, 2012, López et al. 2011). In addition, WNV has been detected in mosquitoes from Spain and many other European countries (Sánchez-Seco et al. 2009, Dobler 2010, Vázquez et al. 2010, Engler et al. 2013). Evidence suggests that USUV was
introduced in Europe (Italy) in a pathogenic form in the 1990s (Weissenböck et al. 2013). Since then, it has emerged in central Europe, causing mainly disease and mortality in several species of resident passerines and raptors (Vázquez et al. 2011) and humans (Cavrini et al. 2009, Pecorari et al. 2009). Moreover, a USUV genetically different from strains circulating in central Europe has so far only been found in mosquitoes from Spain (Vázquez et al. 2011). TBEV causes a potentially fatal disease of the central nervous system, mainly in humans, but also in dogs and horses. WNV, USUV, and TBEV exploit complex biotic and trophic interactions between vectors and reservoirs to persist in a particular area. These interactions are influenced by ecological and environmental factors that may confer unique characteristics to the virus cycle in a particular ecosystem, including differential ways of transmission and persistence (Calzolari et al. 2013).

Mallorca is the largest island of the Balearic archipelago, located in the western Mediterranean region, near the east coast of the Iberian Peninsula and about 270 km from Algeria coast. The island has different wetlands where many bird species stop in their migratory route between Africa and Europe (Gargallo et al. 2011). The presence of abundant mosquito populations in these wetlands, including the principal vector species of arboviruses (*Culex* and *Aedes* species), represents a significant health risk for humans (Engler et al. 2013). Therefore, monitoring arboviral infections in these areas is crucial in terms of public health. Equines are excellent “sentinels” to detect the introduction or circulation of arboviruses, especially in the context of the surveillance of zoonotic flaviviruses, as they develop an intense and long-lasting immune response to flaviviral infections (Chevalier et al. 2011, Mattar et al. 2011). To identify zoonotic arboviruses circulating in Mallorca, horses from different localities of the island were subjected to a serosurvey.

We report the results of the first large-scale serosurvey of major arboviruses conducted in horses from Mallorca. The aim of this study was (1) to assess the presence of arbovirus seropositivity in horses in Mallorca, (2) to know whether there is active arbovirus circulation on the island, and (3) to identify possible risk factors associated with arbovirus seropositivity.

**Materials and Methods**

**Study setting and sample collection**

Mallorca is divided into three geomorphological units: the North Mountains, the central depression where most of the people live, and the East Mountains. The major wetlands are located in the northern and southern outermost parts of the central depression. The survey was conducted in 2011 and 2012 in these two important wetland areas. The horse population from Mallorca ranged between 8400 and 7700 horses in 2011 and 2012, respectively. We sampled 23 stables (16 located in the northern area and 7 in the southern area). These stables were distributed in seven townships (Alcúdia, Muro, Pollença, Sa Pobla, and Santa Margalida in the north and Campos and Ses Salines in the south) (Fig. 1). We sampled the greatest number of horses possible for each stable. In 2011, two sampling sessions were conducted, the first between May and June (104 horses sampled) and the second between September and October (82 horses sampled). In 2012, one sampling session was carried out in early autumn September and October (105 horses sampled). Resampling the same horses allowed us to highlight seroconversion events. For each individual, 5 mL of blood was obtained with a disposable syringe and stored at 4°C for a few hours. Samples were centrifuged for 15 min at 12,000 rpm, and the serum was extracted and stored at –80°C until testing.

For each horse, we obtained information about the following: age (1–5, 6–10, 11–15, and >15 years), sex, how long the equine had lived on the property (named years of permanency: 1–2, 3–4, 5–6, and >6 years), and the size of the

**FIG. 1.** Relative location of sentinel equine populations in Mallorca.
herd. Only one stable located in Campos with the largest herd size (52 horses) had complete information.

**Multiplex immunoassay**

All sera collected at the three different time points were screened simultaneously using an in-house microsphere-based multiplex immuno-assay (MIA) (Balasuriya et al. 2006, Wang et al. 2010). The sensitivity was found to be 97% for WNV and 98% for TBEV. The specificity was found to be 92% and 100% for WNV and TBEV, respectively (Beck et al. 2015). Purified recombinant envelope domain III proteins of WNV, USUV, and TBEV were used for the capture of specific IgG antibodies, whereas purified recombinant O6-methylguanine DNA methyltransferase protein was used as control antigen. Distinct MagPlex microsphere sets (Luminex Corp., Austin, TX) were, respectively, bound to viral and control antigens using the amine coupling kit (Bio-Rad Laboratories, Hercules, CA) according to manufacturers’ instructions. The MIA procedure was performed as described previously (Cao-Lormeau et al. 2016). Briefly, microsphere mixtures were sequentially incubated in the dark under constant shaking with a 1:200 dilution of equine serum samples, with 2 μg/mL anti-equine IgG biotin-conjugated antibody (Jackson Immunoresearch, West Grove, PA) and 2 μg/mL streptavidin-R-phycocerythrin (Life technologies). After the final incubation, the median fluorescence intensity (MFI) of each microsphere set was quantified using a Bio-Plex 200 instrument (Bio-Rad Laboratories). Samples were considered seropositive if the ratio of MFI values obtained for the viral antigen to the control antigen was superior to 8.0.

**Confirmatory reference serological methods**

All positive sera by MIA were sent to the National and European Reference Laboratory for viral equine diseases (ANSES, Maisons-Alfort) for confirmatory testing by plaque reduction neutralization test (PRNT90) or microvirus neutralization test (micro-VNT). PRNT90 was performed to confirm the presence of WNV-, USUV-, and TBEV-specific antibodies and was derived from the OIE PRNT90 method, with threefold serially diluted sera and WNV lineage 1 98-81 strain (Ahmadnejad et al. 2011), USUV It 2012 strain, or TBEV Hypr strain. Titer values were expressed as the reciprocal of the highest serum dilution yielding >90% reduction in the number of viral plaques. Micro-VNT allowed a larger number of samples to be screened using cell microplates and was performed as previously described (Beck et al. 2015) to help confirm the presence of WNV-, USUV-, or TBEV-specific antibodies. In cases of cross-reactive sera for several viruses, the serum was classified as positive for a specific flavivirus (e.g., WNV) if the corresponding neutralizing titer was at least fourfold higher than the titers observed for the other two flaviviruses.

A commercial competition ELISA (ID Screen West Nile competition ELISA kit; ID Vet) was used to screen highly cytotoxic sera for antibodies against WNV and related flaviviruses (Beck et al. 2015). Horses found seropositive in ELISA, but inconclusive VNTs were considered infected by an unidentified flavivirus. The ELISA test was found to have a sensitivity of 98% and a specificity of 100% for WNV IgG detection (Van Maanen et al. 2010).

**Data analysis**

As a consequence of the low number of seropositive horses found in the sampled population, statistical analyses were performed for the overall flavivirus seroprevalence (WNV, USUV, TBEV, or other unidentified flaviviruses). We used a generalized linear mixed model (GLMM) and assumed a binomial distribution and number of horses in each stable. Resampling and clustering of horses in stables were taken into account by introducing the horses and stables as random effects in this logistic model. We performed a second analysis on the largest sampled stable (situated in Campos) as information on years of permanency and age was available for 52 horses. For this analysis, we only included all horses sampled in this stable during the second year. We used a generalized linear model and assumed a binomial distribution to investigate the relationships among flavivirus seroprevalence and two categorical variables (years of permanency and age). Years of permanency and age variables were not included in the same model since these two variables had a high correlation. The strength of the association between flavivirus seroprevalence and the variables considered was reported as odds ratio with corresponding 95% confidence intervals (CIs) and p-values. Association was considered statistically significant when p < 0.05. All analyses were performed using R software version 3.0.2.

**Results**

**Sentinel horse population of Mallorca**

A total of 291 blood samples were taken from 172 horses: 104 samples between May and June 2011, 82 between September and October 2011, and 105 samples in 2012. Eighty-five horses (49.4%) were sampled once, 55 horses (32.0%) twice, and 32 horses (18.4%) thrice. The size of the sampled herds ranged from 1 to 53 horses (mean = 7) (Table 1 and Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/vb2). The overall females/males ratio of the analyzed horses was 1 (86/86). However, the sex ratio was highly variable between stables with nine stables having only males and three having only females.

**Flavivirus seroprevalence in the sentinel horse population**

Out of the 291 blood samples, 37 samples (collected from 21 horses) were found seropositive against at least one flavivirus according to the MIA screening method and sent to the OIE Reference Laboratory for confirmatory testing. All 37 samples found seropositive by MIA were tested by micro-VNT. A total of 27 samples (collected from 14 horses), out of these 37 samples, were found to be seropositive against WNV, USUV, or TBEV in the micro-VNT assay (titer range: 30–2430). All 27 sera found seropositive by micro-VNT were retested by the gold-standard PRNT90 method and confirmed to be seropositive against one of the three flaviviruses (titer range: 10–810). The 10 samples found seropositive by MIA, but seronegative by micro-VNT were retested by WNV competition ELISA, as the performance of this assay is not affected by the cytotoxic nature of the sera. The 10 sera (8 horses) were confirmed to be seropositive against WNV according to the ELISA. The specific serological results for these 10 sera were, however, considered to be inconclusive due to the discrepancy
between the positive MIA/ELISA results and the negative micro-VNT results. As a consequence, these sera were classified as seropositive against an “unknown Flavivirus.”

Among the 21 horses (12.2%) found positive for flaviviruses, 11 (6.4%; 95% CI 3.2%–11.0%) were found positive for WNV, 2 (1.2%; 95% CI 0.1%–4.1%) were found positive for USUV, and 1 (0.6%; 95% CI 0.0%–3.2%) was found positive for TBEV. In addition, eight horses (4.6%; 95% CI 2.0%–8.9%) were found positive for unidentified flaviviruses. The blood sample of one horse was positive for WNV and unidentified flavivirus. Out of 23 stables, 5 (21.7%) had horses seropositive for WNV, USUV, or TBEV. However, 76.2% of the seropositive horses for flaviviruses belonged to only one stable situated in Campos.

All WNV-seropositive horses had not been vaccinated for this virus. WNV-seropositive horses were found in only one township and 91% of total of seropositive horses (10/11) were found in one stable (Table 1). The presence of neutralizing antibodies against USUV was observed in two horses from two different stables (Pollença and Sta. Margalida municipalities). Another horse with neutralizing antibodies against TBEV was located in a stable of Alcúdia township. Of the eight horses found positive for unidentified flaviviruses, seven horses belonged to two stables (six and one horse, respectively) situated in Campos, and one horse in Pollença.

Out of 21 seropositive horses, 8 (38.1%) had not travelled out of Mallorca during the study period. These eight horses were positive for WNV (1), USU (2), TBEV (1), and unidentified flaviviruses (5).

### Predictors of Flavivirus seropositivity in sentinel horse populations

The results obtained from the GLMM to assess the association between several factors and flavivirus seropositivity in the sentinel equine population indicated that flavivirus seroprevalence was not positively associated with the number of horses by stable ($\beta = 0.094$, $z = 1.79$, $p = 0.07$). Also, the GLM indicated no significant differences in flavivirus seroprevalence by age class, and years of permanency, suggesting that risk of exposure to flaviviruses did not increase with age or length of residency in stables (Table 2 and Fig. 2).

### Time distribution

To highlight recent transmission of arboviral infections in the equine population of Mallorca, several horses were tested successively between May 2011 and October 2012 to identify potential seroconversion events. A seroconversion event was defined as the detection of IgG antibodies in a horse, which did not present specific detectable antibodies during the precedent sampling session. On 14 horses found positive for WNV, USUV, or TBEV antibodies, three seroconversion events were detected between 2011 and 2012: two horses from Campos showed seroconversion for WNV and one horse from Pollença showed seroconversion for USUV. Two of these horses had not travelled out of Mallorca. Three other horses from Campos (2) and Pollença (1) also showed seroconversion for unspecified flaviviruses and two of them had not travelled out of Mallorca.

### Discussion

Epidemiological surveys have demonstrated the circulation of flaviviruses in humans, horses, and various wild birds (resident and migratory species) in Spain since 2006 (Kaptoul et al. 2007, Garcia-Bocanegra et al. 2011, 2012, López et al. 2011). However, so far no information about the possible circulation of flaviviruses in the Balearic Islands was available.

### Table 1. Sentinel Horse Population Characteristics and Observed Seroprevalence Estimates for Flaviviruses

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of horses</th>
<th>WNV, n (%)</th>
<th>USUV, n (%)</th>
<th>TBEV, n (%)</th>
<th>Unidentified flavivirus, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>172</td>
<td>11 (6.4)</td>
<td>2 (1.2)</td>
<td>1 (0.6)</td>
<td>8 (4.6)</td>
</tr>
<tr>
<td>Geographic area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area 1</td>
<td>92</td>
<td>11 (12.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>7 (7.6)</td>
</tr>
<tr>
<td>Campos</td>
<td>89</td>
<td>11 (12.4)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>7 (7.9)</td>
</tr>
<tr>
<td>Ses Salines</td>
<td>3</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Area 2</td>
<td>80</td>
<td>0 (0.0)</td>
<td>2 (2.5)</td>
<td>1 (1.2)</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Alcúdia</td>
<td>37</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (2.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Muro</td>
<td>12</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Pollença</td>
<td>9</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Sa Pobla</td>
<td>14</td>
<td>0 (0.0)</td>
<td>1 (7.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>St. Margalida</td>
<td>8</td>
<td>0 (0.0)</td>
<td>1 (12.5)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Herd size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–10</td>
<td>61</td>
<td>1 (1.6)</td>
<td>2 (3.3)</td>
<td>0 (0.0)</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td>11–20</td>
<td>38</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>&gt;20</td>
<td>75</td>
<td>10 (13.3)</td>
<td>0 (0.0)</td>
<td>1 (1.3)</td>
<td>6 (8.0)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>86</td>
<td>10 (11.6)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>4 (4.6)</td>
</tr>
<tr>
<td>Male</td>
<td>86</td>
<td>1 (1.2)</td>
<td>2 (2.3)</td>
<td>1 (1.2)</td>
<td>4 (4.6)</td>
</tr>
</tbody>
</table>

WNV, tick-borne encephalitis virus; USUV, Usutu virus; WNV, West Nile virus.
available. This archipelago is interesting from an epidemiological point of view as (1) it is placed between European and African coasts and could therefore serve as a sentinel region for diseases coming from these two continents; (2) different bird species stop in the region during their seasonal intercontinental migrations; and (3) it has extensive wetland areas.

Our results indicate that flavivirus seropositivity among horses is not associated with an increased number of horses within the holding. However, 76.2% of the horses found seropositive belonged to the stable with the highest number of horses. This finding is not in agreement with previous studies (Durand et al. 2005, García-Bocanegra et al. 2012). These differences could come from the association of the herd size with other ecoepidemiological factors, not addressed in this study. Analysis of additional factors on a larger number of horses will be necessary to identify independent risk factors of flavivirus seropositivity in equines.

The overall seroprevalence observed for WNV was slightly lower than from horse populations of Southern Spain (García-Bocanegra et al. 2012). However, these differences between other endemic areas may be due from different sampling and testing methodologies. Moreover, the high cytotoxicity found for several samples in this study could have prevented detection of low target antibody titers by PRNT and micro-VNT, thereby leading to an underestimation of the overall seroprevalence.

All individuals were seropositive for WNV live in the southern region of Mallorca, 10 of which came from the same stable. In this stable, the horses graze freely a few meters from the wetland area where there are a lot of mosquitoes, which may favor the horse infections.

USUV has previously been detected in Spain in mosquitoes (Engler et al. 2013); however, there is very little information about USUV infections in horses. The prevalence of USUV in Mallorcan horses was found to be much lower than WNV and can therefore be considered less of a health risk for host populations.

To date, TBEV has not been detected in Spain, although its geographic distribution has expanded during the last 30 years and thousands of human cases have been reported in European and Asian countries (Orlinger et al. 2011). Only one horse was found seropositive for TBEV, although its sera tested at the highest threshold serum dilution (1/20) yielded only 70% reduction in the number of viral plaques instead of

### Table 2. Parameter Estimates (Logit Scale) from the Models on the Seroprevalence of Flaviviruses

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>No. of positives</th>
<th>No. of horses</th>
<th>β</th>
<th>Error</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age category</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>5</td>
<td>-0.41</td>
<td>0.91</td>
<td>-0.44 0.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–5 years</td>
<td>2</td>
<td>5</td>
<td>-0.55</td>
<td>1.05</td>
<td>0.58 0.79</td>
<td></td>
<td>-0.52</td>
<td>0.60</td>
</tr>
<tr>
<td>6–10 years</td>
<td>5</td>
<td>18</td>
<td>-0.21</td>
<td>1.03</td>
<td>0.81 0.99</td>
<td></td>
<td>-0.21</td>
<td>0.83</td>
</tr>
<tr>
<td>11–15 years</td>
<td>7</td>
<td>20</td>
<td>-1.67</td>
<td>1.40</td>
<td>0.19 0.89</td>
<td></td>
<td>-1.20</td>
<td>0.23</td>
</tr>
<tr>
<td>&gt;15 years</td>
<td>1</td>
<td>9</td>
<td>1.27</td>
<td>1.57</td>
<td>0.29 1.55</td>
<td></td>
<td>1.20</td>
<td>0.23</td>
</tr>
<tr>
<td>Years of permanency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>13</td>
<td>-0.47</td>
<td>0.57</td>
<td>-0.82 0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2 years</td>
<td>5</td>
<td>13</td>
<td>-1.20</td>
<td>0.85</td>
<td>0.30 0.60</td>
<td></td>
<td>-1.42</td>
<td>0.16</td>
</tr>
<tr>
<td>3–4 years</td>
<td>3</td>
<td>19</td>
<td>-0.24</td>
<td>0.88 0.99</td>
<td>0.23 0.71</td>
<td></td>
<td>0.28</td>
<td>0.78</td>
</tr>
<tr>
<td>5–6 years</td>
<td>4</td>
<td>9</td>
<td>-0.51</td>
<td>0.88 0.99</td>
<td>0.11 0.34</td>
<td></td>
<td>-0.58</td>
<td>0.56</td>
</tr>
<tr>
<td>&gt;6 years</td>
<td>3</td>
<td>11</td>
<td>1.27</td>
<td>1.57</td>
<td>0.29 1.55</td>
<td></td>
<td>1.20</td>
<td>0.23</td>
</tr>
</tbody>
</table>

*aReference.

FIG. 2. Number of seropositive horses by age (A) and years of permanency classes (B).
>90% in the confirmatory PRNT_{90} assay. The detection of antibodies against TBEV in horses from Mallorca could be due to cross-reactivity with IgG directed against closely related viruses. Indeed, the looping ill virus, a member of the TBEV serocomplex, has already been detected in ticks and livestock in Spain (Balseiro et al. 2012). Alternatively, the presence of TBEV could be explained by the introduction of infected ticks by several migratory birds (Waldenström et al. 2007) when they use the Balearic Islands as stopover or breeding site in their migratory routes (Gargallo et al. 2011). Furthermore, some horses on the island periodically spend some weeks in Germany for competitions and could carry infected ticks when they return. However, the horse seropositive for TBEV in Mallorca had not travelled outside the island, suggesting that it was infected in Mallorca.

Several Mallorcan horses also presented serum samples with difficult VNT interpretations (i.e., similar WNV and USUV titers or low antibody amounts), probably resulting from cross-reactivity with an unidentified flavivirus. Horses presenting antibodies against flaviviruses other than WNV, USUV, and TBEV are, however, not uncommon in sentinel serosurveys (Durand et al. 2005). The results obtained from horses that had not left the island indicate for the first time that these infections were acquired locally and there exists an active flavivirus circulation in Mallorca.

These findings will be complemented with a large-scale serosurvey of the human population and a molecular screening of collected mosquitoes to identify circulating arboviral strains and local vector species. These studies are currently in progress and to date no human case has been confirmed in the hospitals of Mallorca.

Conclusions

To improve early detection of arboviruses pathogenic to humans in Mallorca, we have implemented the first serological survey of equine sentinel populations in the Balearic Islands. Detection of IgG antibodies against WNV, USUV, and TBEV in the surveyed horse populations, as well as seroconversion events observed during the study, demonstrates that these human-pathogenic viruses are currently circulating within host populations in Mallorca.

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Author Disclosure Statement

No competing financial interests exist.

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