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Detection of West Nile Virus and other common equine viruses in three locations from the Leeward Islands, West Indies

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ABSTRACT

Equines in the West Indies are used for recreational purposes, tourism industry, racing and agriculture or can be found in feral populations. Little is known in the Caribbean basin about the prevalence of some major equine infectious diseases, some with zoonotic potential, listed as reportable by the OIE.

Our objective was to study the prevalence of antibodies for West Nile Virus (WNV), Equine Herpes Virus-1 and 4 (EHV-1 and EHV-4), Equine Influenza (EI), Equine Viral Arteritis (EVA) and Equine Infectious Anemia Virus (EIAV) using a retrospective serological convenience study. We used 180 equine serum samples, 140 from horses and 40 from donkeys in St. Kitts, Nevis, and Sint Eustatius, collected between 2006 and 2015 that were tested with ELISA kits and virus neutralization (for WNV and EVA).

Combining ELISA with virus neutralization testing, 25 (13.8%) equine sera were WNV positive (a mixture of indigenous and imported equines) and 3 sera (1.6%) showed doubtful results. For EHV-1, 41 equines (23.7%), mean age 6.7 years, were seropositive. For EHV-4, 138 equines were found seropositive (82.8%), mean age 6.3 years. For EI, 49 equines (27.2%), mean age 7.5 years, were seropositive on ELISA, some previously vaccinated horses. No antibodies against EAV were found on virus neutralization testing, although one animal (0.6%), was EAV positive on ELISA. All samples were EIAV negative.

The seroprevalence for EHV-1 and EHV-4 is similar to other parts of the world. For the first time in the study location serologic evidence of antibodies against WNV and EI is reported. This was found in both indigenous and imported animals, highlighting the need for developing proper surveillance plans based on complementary methods of virus detection. Further studies will be needed to define the prevalence, rates of transmission, characterize local virus strains, and study their impact on these populations.

1. Introduction

The equine industry will all its components is having an increasing economic impact worldwide nowadays. With the rapidly changing nature of infectious diseases, knowledge on equine diseases (some with zoonotic potential) in a certain area plays a pivotal role in designing biosecurity measures.

Diseases caused by West Nile Virus (WNV), Equine Herpes Virus-1 and 4 (EHV-1 and EHV-4), Equine influenza virus (EIV), Equine arteritis virus (EAV) and Equine infectious anemia virus (EIAV) are among the OIE-listed diseases, infections and infestations in force in 2017 (OIE, 2016). WNV is known to be highly pathogenic for birds in the Americas, with over 250 species of birds detected to harbour the virus and it can be transmitted to different species by mosquito vectors (WHO, 2011). The presence of *Culex nigripalpus* and *C. quinquefasciatus*, competent vectors of WNV, has been confirmed on St. Kitts and Nevis (Mohammed et al., 2015). Several hundred species of birds migrate through the Caribbean islands to South America (Raffaele et al., 1998; Rappole et al., 2000; Reed et al., 2003) as part of the Caribbean Island/Western North Atlantic Route (Rappole et al., 2000) (Fig. 1). 159 migratory bird species were recently recorded for St Kitts and Nevis (Rusk, 2014). In recent years, the resurgence of WNV in North America and Europe (Gray and Webb, 2014) has underscored the importance of close surveillance of diseases. EHV-1 and EHV-4 are endemic in horse

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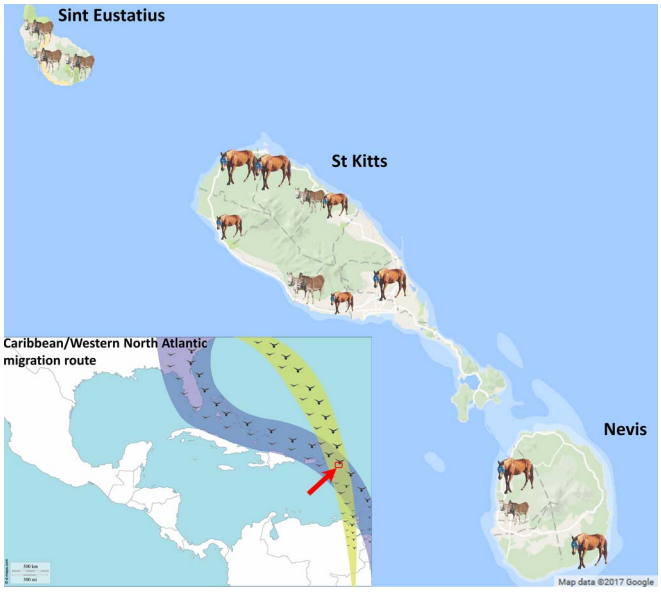

Fig. 1. Study location: equines originated from different parts of the three islands. Inset: origin of equine samples (red square indicated by the arrow) in the Leeward Islands on the Caribbean/Western North Atlantic pathway of migratory birds (green and blue shades). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

populations throughout the world, causing respiratory disease, abortions, neonatal death, and equine herpesvirus myeloencephalopathy (EHV-1) or febrile rhinopneumonitis (EHV-1 and EHV-4) (Ma et al., 2013; Patel and Heldens, 2005). Equine influenza (EI) can occur in equids in all continents as a highly contagious disease (Na et al., 2016), and can cross species barriers, resulting in outbreaks in dogs (Daly et al., 2011; Landolt, 2014). Equine viral arteritis (EVA) also has a worldwide distribution and can be responsible for respiratory and reproductive disease limited to the Equidae family (Balasuriya, 2014). Equine infectious anemia (EIA), a blood borne disease that can be transmitted mechanically by hematophagous insects, causes persistent infections in equines including recurring episodes of fever, thrombocytopenia, and wasting symptoms (Cook et al., 2013). Insect vectors for EIAV, e.g., tabanids, are documented in the Caribbean area linked with several diseases of veterinary importance (Callan, 1952; Section, 2002).

There is a relative dearth of sound data on the prevalence of major equine infectious diseases in the Caribbean Basin. Previous data in the tested islands exists only about equine piroplasmosis (Li et al., 2015; Loftis et al., in press) but not about the viral diseases selected for serological testing in our study. As part of a regional effort to improve and harmonize surveillance and expand the knowledge base of animal diseases including zoonoses (e.g. WNV), our data will be used as part of the efforts from RUSVM to join the Caribbean animal health network (CaribVET). The aim of the current research was to document the seroprevalence of some important equine viral infections using convenient equines serological samples from the islands of St Kitts, Nevis and St Eustatius. One of the main goals of the study was to establish the presence of equine antibodies against zoonotic agents like WNV.

2. Materials and methods

2.1. Study location, ecosystem information and sample collection

Serum samples were collected between 2006 and 2015 and stored at −80 °C in the Diagnostic Laboratory of RUSVM in St Kitts. Since the seroprevalence of all the diseases was unknown at the time of the study design, we calculated an overall sample size of 186 equids assuming 20% seroprevalence, a precision of ± 0.05 and a 95% confidence interval and assuming a test with imperfect sensitivity and/or specificity (Humphry et al., 2004).

We have included in our study approximately 25% (180 out of 730) of the total number of equines, from different areas of the three islands, to capture the heterogeneity of the population. Overall approximately 78% of the horse population and 7% of the donkey population on these three islands was tested. The 180 equine samples tested were from 31 donkeys (St Eustatius), 140 horses and 9 donkeys (St Kitts and Nevis − from previous serology studies (Loftis et al., in press) or from the equines belonging to the RUSVM Large Animal Teaching Facility). The estimated equine population in the area is around 730 animals for the 3 islands (for 2015), with around 180 horses in Saint Kitts and Nevis (120 in St Kitts and 60 in Nevis) and no horses present in St Eustatius. A total of around 550 donkeys should be present within the 3 islands: 140 donkeys are in Saint Kitts (out of which around 100 belong to RUSVM), 300 in Nevis (mainly feral) and around 110 in St Eustatius (mainly feral). This valuable data was collected with the kind support of the Chief Veterinary Officer from the Department of Agriculture in St Kitts and Nevis and the president of the Eastern Caribbean Public Health Foundation from Sint Eustatius. Our research was performed in accordance with an approved Institutional Animal Care and Use protocol.

The tested equines were a combination of feral donkeys, donkeys and horses used in tourism industry, for recreational purposes, in racing industry, from farmers in remote areas owning individual animals as well as donkeys and horses used for teaching, belonging to RUSVM. We tested 140 males and 40 females with a mean age of 6.3 years (ranging 1–28 years old). 43 serum samples originated from horses that were used in the equine racing industry, mostly imported at least 1 year before sampling. These race horses were previously vaccinated against EI and tested negative for EIAV at the time of importation. Also, they were not vaccinated against WNV. The donkeys belonging to RUSVM were immunized against tetanus, but not against any other disease.

2.2. Serological analyses

Commercially available ELISA testing kits were used to test for the presence of antibodies: WNV-IDVet multispecies test for detection of anti-pr-E antibodies (WNC ver. 1014); EHV-1 and EHV-4-SVANOVIR EHV1/EHV4-Ab Discriminating Test targeting anti-EHV-1 or EHV–4 G antibodies, SVANOVA (art. 10-3100-02), solid phase indirect ELISA; EI-IDvet (FLUACA ver. 0914), blocking test, multi-species test detecting antibodies against the internal nucleocapsid of the Influenza A virus; EAV-VMRD cELISA (Cat # 272-5) that would detect anti-GP5 antibodies; and EIA-VMRD (Cat # 290-5), targeting antibodies against EIAV p26 antigen. These kits offer high analytical sensitivity and repeatability. For the IDVet WNV competition kit, analytical sensitivity has been evaluated as superior to 99.9% and intra-plaque and inter-plaque variations were estimated at 4–7% and 10–12% respectively; however, this kit suffers from poor specificity, detecting antibodies against West Nile and other varied flaviviruses [French Agency for Food, Environmental and Occupational Health & Safety − ANSES data]. For EAV the test has 98.9% sensitivity and 98.3% (Pfahl et al., 2016). Both EAV and EIA kits used are USDA approved as screening test, with positives needing to be confirmed by seroneutralization (EVA) or AGID (EIA). Each of the ELISA tests were performed in duplicate at RUSVM, in compliance with each of the test kit's instructions and all readings were performed on the same microplate photometer.

All of the WNV-ELISA positive sera (n = 31) with sufficient sample size were retested, together with 11 randomly selected WNV negative samples, for immunoreactivity against different flaviviruses as previously described (Beck et al., 2015). The flavivirus Luminex technology was first used and these results were confirmed by WNV and Usutu virus (USUV-control flavivirus not present in the tested area) virus neutralization tests (Beck et al., 2015). Flavivirus luminex and WNV VNT offers comparable performances and high analytical and diagnostic specificity, the Flavivirus Luminex being slightly more sensitive than WNV VNT (Beck et al., 2015).

Table 1
Breed, sex and origin of animals as risk factors for WNV, EHV-1, EHV-4, EI, EAV and EIAV based on the initial ELISA testing. The disease seroprevalence with corresponding 95% confidence intervals and chi-square or Fisher's exact test P values are also shown.

Disease	[a]Overall sero-prevalence	[b]Breed		[b]Sex		[b]Origin of sample		
		Horse (n = 140)	Donkey (n = 40)	Female (n = 40)	Male (n = 140)	St. Kitts (n = 120)	Nevis (n = 29)	S. Eustatius (n = 31)
WNV	17.8% (32/180)	18.6% [12.9%–25.9%]	15.0% [6.84%–29.8%]	17.5% [8.5%–32.6%]	17.8% [12.3%–25.2%]	10.7% [6.3%–17.7%]	50.0% [32.1%–67.9%]	16.1% [6.8%–33.6%]
EHV-1	23.7% (41/173)	27.6% [20.6%–35.9%]	10.3% [3.9%–24.5%]	10.2% [3.86%–24.5%]	27.6% [20.6%–35.8%]	28.4% [20.9%–37.4%]	18.5% [7.8%–37.7%]	10.0% [3.2%–27.0%]
EHV-4	82.8% (144/174)	90.0% [83.7%–94.0%]	52.9% [36.3%–68.9%]	59.0% [43.0%–73.3%]	91.0% [84.7%–94.8%]	95.0% [89.3%–97.8%]	71.4% [52.2%–85.1%]	36.0% [19.8%–56.2%]
EI	27.2% (49/180)	34.3% [26.8%–42.6%]	2.5% [0.3%–16.0%]	20.0% [10.3%–35.3%]	29.3% [22.3%–37.4%]	25.0% [17.9%–33.4%]	67.9% [48.7%–82.5%]	0%
EAV	0.6% (1/180)	0%	2.5% [0.3%–16.0%]	2.5% [0.3%–16.0%]	0%	0%	0%	3.2% [0.44%–20%]
EIA	0% (0/180)	0%	0%	0%	0%	0%	0%	0%

[a] Overall seroprevalence − percentage of seropositive animals (seropositives/total tested).
[b] For each parameter (breed, sex and origin) the results illustrate the percentage of seropositive animals and the 95% confidence intervals within brackets.

The ELISA positive EVA result was retested using virus neutralization test recommended by the World Organization of Animal Health (OIE), as previously described (Laabassi et al., 2014).

3. Results

3.1. Serology results

Results of ELISA testing are shown in Table 1. For WNV, EHV-1, EHV-4 and EI there was at least one seropositive animal per disease after duplicate testing. There were 7 equivocal results in case of EHV-1 and 6 equivocal results for EHV-4 which were discarded for further statistical analysis. All sera tested negative for EIAV, hence no further analysis on risk factors could be determined for this disease.

On ELISA testing: 17.8% equines were seropositive for WNV; antibodies against EI were detected in 27.2% samples with 5.5% from the total tested serum samples for originating from indigenous equines, with no vaccination history, whereas the rest of seropositives were vaccinated at some prior point in time.; 23.7% of the tested equines were EHV-1 seropositive; EHV-4 was the most prevalent of the six diseases tested with 82.8% overall seroprevalence; only 1 sample was EAV positive (0.6%) − a female donkey from Sint Eustatius.

Samples with positive results for WNV and EAV were tested with more specific tests (Table 2). Of the 31 ELISA-positive sera, 28 were positive in the flavivirus Luminex test and 25 sera were confirmed to be truly WNV positive in WNV neutralization testing (representing 13.8% of the total sera tested in the study). 3.3% of the total tested equines were indigenous positive equines that had WNV exposure at one point, and the rest of the positives represented imported equines, that might have been exposed before arriving in the Eastern Caribbean. The sera did not, or poorly neutralized Usutu virus (USUV), the negative control flavivirus. Using virus neutralization test, the one EAV ELISA-positive sample was found to be negative.

Table 2
Results of serum neutralization test on equine serum samples initially ELISA positive.

		Initial ELISA testing (number of samples/% from total tested)	Virus neutralization (number of samples/% from total tested)
EVA	Positive	1 (0.6%	0 (0%)
WNV	Positive	32 (17.8%)[a]	25 (13.8%) positive 3 (1.6%) doubtful 3 (1.6%) negative
	Negative	11	11

[a] 1 ELISA WNV positive sample was not retested due to insufficient sample size.

3.2. Factors associated with disease seropositivity

Information was available for the horses in our study that were part of the racing industry regarding the number of years they had been present in the Caribbean, as a risk factor for exposure. In case of the WNV positive racing horses (26 out of the total 32 seropositives), they have been in the area between 3–9 years (average 5.3 years), time in which they were not vaccinated against WNV. This is significant in the fact that there was more than 12 months since their last movement. Thus, the likelihood that they carry WNV vaccination antibodies (assuming they were previously vaccinated) is extremely low, proving a real exposure, similar to the indigenous equines (6 out of the total 32 seropositives, representing 4 donkeys and 2 horses), which were born and raised in one of the three islands.

Location seems to play a role regarding seropositivity to WNV and EI. It appears as the more than half of the equines tested for WNV originating from the island of Nevis (> 50%) were seropositives, compared to only around 10% for St. Kitts and around 16% for Sint Eustatius. Equines located on the island of Nevis also had a higher percentage of EI antibodies (67.9%) compared to equines being housed in St. Kitts (only 25%) and Sint Eustatius (0%). For both EHV-1 and EHV-4 seroprevalence was higher in male horses compared to females or donkeys. However, only for EHV-4, location seems to play an important role: equines located in St. Kitts had the highest seroprevalence (95%), followed by Nevis (71.4%) and Sint Eustatius (36%). Since there were no seropositive samples for EAV or EIAV, no assumptions could be drawn about breed, sex or geographical origin representing risk factors for these diseases.

4. Discussion

One exciting observation from our study is that both for WNV and EI, there were positive samples from indigenous equines (born and raised in the 3 islands, with no prior vaccination) besides imported equines (possibly previously exposed to WNV and vaccinated against EI). Collectively, our data confirms the possibility of WNV spread on Sint Eustatius, St. Kitts and Nevis as well as possibly other locations within the Lesser Antilles. The composition of the equine population and the vector biology is similar in different parts of the Eastern Caribbean. Taking into consideration the epidemiological situation regarding the tested diseases in the area, most of the results were

expected and comparable with neighbouring islands which have active surveillance and reporting programmes in place. An example would be Guadeloupe, which with arising awareness has implemented a surveillance system to monitor the spread of WNV in 2002 (Lefrancois et al., 2006). It is recommended that other countries in the area implement similar surveillance systems to monitor WNV and take leadership in the Caribbean to contribute to the lacking database of such ubiquitous, zoonotic disease. One way to do this is by joining the Caribbean animal health network (CaribVET) initiative, for which RUSVM has applied for membership.

Our ground work study brings a significant contribution to the current and future regional surveillance programmes in the Eastern Caribbean. The main finding in our study, was the detection of antibodies against WNV in indigenous equines (born and raised in the area) proving a real exposure. Equine WNV infections have only been sparsely and irregularly reported in the Caribbean region (OIE (Health, 2016b)): Cuba (disease limited to one or more zones; reports of infection/infestation); Haiti (disease suspected but not confirmed and limited to one or more zones; reports of infection/infestation); Guadeloupe (France) more than 100 asymptomatic infections reported so far in horses in 2003 (N = 54), 2007/2008 (N = 13, Promed data) and 2012/2013 (N > 30, unpublished data) (Health, 2016b). Furthermore, WNV was detected in the blood of migratory birds captured in Jamaica, Puerto Rico, and Mexico (Dupuis et al., 2003) and bird migration has been associated to WNV introduction in Guadeloupe (Lefrancois et al., 2006), another island in the Lesser Antilles, south of St Kitts. WNV antibodies were detected also in chickens, residing in Guadeloupe (Quirin et al., 2004), which is different to the situation in St Kitts, where no antibodies were detected in free roaming chickens so far [Bolfa, unpublished data]. No deaths were associated with the viral infection, partly due to the absence in the area of bird species known to be extremely susceptible to the infection (Corvidae), vector competence, ecological factors or the virus strain (Quirin et al., 2004). Two mosquito species that are competent WNV vectors and were confirmed on St. Kitts and Nevis (Culex nigripalpus and C. quinquefasciatus) (Mohammed et al., 2015) were also previously identified on Guadeloupe (Lefrancois et al., 2006), another island in the Lesser Antilles, south of St Kitts. Within this context and with flying carriers, spreading of disease is inevitable across these areas and many countries at highest WNV zoonotic risk, including the ones in the current study, are currently lacking surveillance.

Equine herpesvirus related disease data are scarce within the Caribbean islands, with only a few locations reporting outbreaks: Cayman Islands, Cuba, Dominican Republic and Jamaica. The relatively overall high prevalence demonstrated for EHV-4 antibodies in serum samples in our study (82.8%) together with the lower overall prevalence of EHV-1 (23.7%) is in accordance to those reported in other parts of the world (reviewed by (Patel and Heldens, 2005)). Previous reports show the seroprevalence of EHV-4 and EHV-1 to be 83.3% and 14.5%, respectively, in a population of over 260 horses, donkeys and mules in Turkey (Ataseven et al., 2009) and 96.7% and 5.0%, respectively, in a population of 181 horses from Costa Rica (Jimenez et al., 2014). Both EHV-1 and EHV-4 showed a significantly higher prevalence in male horses in our study, indicating sex as a risk factor for these two herpesviral infections, which to our knowledge was not reported before.

The second important aspect revealed by our study was that besides the horses that were previously vaccinated, and we expected to have some degree of seropositive response to EI, an additional 10 indigenous equines (9 horses and only 1 donkey) had serum antibodies against EI. Therefore, we assume that there must be serotypes of EI circulating within our tested area, mainly St Kitts and Nevis, since none of the equines from Sint Eustatius tested as positive. Interestingly enough, out of the 43 horses known to be used for the horseracing industry, 4 were seronegative following serological testing. A partial explanation for this fact might be the lack of revaccination of these horses. The lack of complete travel history of the horses makes it difficult to distinguish whether these horses have acquired the antibodies before or after moving into the region. The potential of antigenic shift and genetic reassortment and the highly variant nature of Influenza A warrants global surveillance (Alexander and Brown, 2000; Xie et al., 2016). Scarce reports of EI outbreaks exist in the Caribbean basin, according to OIE, mainly before 2004 in: Cayman Islands, Curacao, Dominican Republic, Haiti, Jamaica, Martinique and Trinidad and Tobago (Health, 2016b). Some of these reports, similar to our situation, are just serological evidence of disease and/or isolation of the causal agent, without clinical signs.

Lack of antibodies against EVA and EIAV represented a third important aspect of our study. Interestingly, according to OIE Handistatus, serological evidence and/or isolation of the causal agent, but no clinical signs of EVA disease was reported for St Kitts and Nevis in the period between 1996–1998 (Health, 2016b). That represents the only reported presence of the disease within the Caribbean basin after 1996, according to the WAHID database. Our study revealed only one animal − a female donkey located on the island of Sint Eustatius − which was seropositive on ELISA (performed twice) but negative on virus neutralization. Because disease occurrence is uncommon, as exemplified in our study, the need for vaccination is generally obviated (Gilkerson et al., 2015) − which further underlines the importance of keeping incidence of disease low in a disease naïve population. None of the horses in the study tested positive for EIAV, which is similar to the situation reported in the nearby islands of Grenada and Carriacou (Chikweto et al., 2009). Within the Antilles, EIA was only reported in Haiti and the Dominican Republic (Health, 2016b), which would represent the closest geographical location to our study area. 43 horses from our study were part of the horseracing industry and were imported at some point to the Lesser Antilles area. Although current international trade regulation (Health, 2016a) suggests monitoring (testing or vaccinating, depending on the disease) for Glanders, African horse sickness, EI, EIA, equine piroplasmosis and Venezuelan equine encephalomyelitis, most likely these regulations were not in place at the time of sample collection. Nevertheless, previous trade regulations included EIAV screening, and that would at least partly account for the absence of this disease in the tested islands, combined with the fact that all imported horses tested negative upon importation on EIAV. Another explanation might be the reduced equine population density in the study area, together with the reduced movement of the equines. The majority of the equines within the 3 tested islands are confined within a certain area, with limited movement outside it. There are a few horses and donkeys that are used in the tourism industry on the 3 islands, together with some feral donkeys, but also in their case, the movement and especially interaction with other equines is limited.

As any serological study based on convenient sampling, our study has a few limitations that we tried to address. Due to the nature of our samples (serum) we used a combination of indirect methods for the detection of viral infections (ELISAs and virus neutralization assays) (Balasuriya et al., 2015). The serological methods used in our study are not very useful diagnostic tools for active infections, as pre-existing antibodies to any of the viruses studied here may be the result of prior vaccination (in the case of Equine influenza) or infection (for all the viruses tested) (Balasuriya et al., 2015). With the ELISA test employed in our study, EI seropositivity cannot distinguish between the vaccinated and naturally infected individuals (Kittelberger et al., 2011). We expected that the horses that were imported from other parts of the world to our study area, would show some degree of seropositivity for EI on the ELISA test used. Even though within the three tested islands, similar to most of the Caribbean area, donkeys are more abundant than horses, more samples from horses were tested in our study (77%). The main reasons for this were that the vast majority of horse samples were available from a previous study (Loftis et al., in press), with most of the donkeys outside RUSVM included in the study originating from feral animals located either in Nevis or Sint Eustatius, and with some limitations in capturing those animals. Since there was a functional race

track in St. Kitts and one in Nevis (currently both inactive), and some of the sera (n = 43) included in our study originated from race horses, these horses have been immunized against EI as well as pre-screened for EIAV (all with negative results). Before being moved into the islands, these horses had previously been living in Europe, North America, Central America or South America. For the WNV ELISA test employed, the monoclonal antibody used in the kit cross-reacts with other flavivirus infections (due to flaviviruses in the Japanese Encephalitis serocomplex to which WNV belongs, as well as to distant flaviviruses such as the tick-borne encephalitis virus). To counteract this, virus neutralization testing (VNT) was used for increasing the specificity of WNV as well as EAV testing.

5. Conclusions

The main findings of our study was the confirmation of the presence of WNV and EI antibodies within the Leeward Islands in indigenous and imported equines, which together with the presence of carrier migratory birds, mosquito vectors and competent resident birds could constitute a perfect scenario for a possible human outbreak of West Nile fever in the area, similar to other regions in the world. The prevalence of EHV-1 and EHV-4 in our serum samples was similar to other parts of the world. Another important observation was that EVA and EIAV antibodies were absent from the study area. Our data supports the need for future local surveillance programmes that could be designed and implemented, enhancing biosecurity measures for public health and equine owners. A future study using a similar sample size, but employing direct methods of virus detection in each of the equine species, sexes, and from different islands, with more comprehensive vaccination history could further validate our data.

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