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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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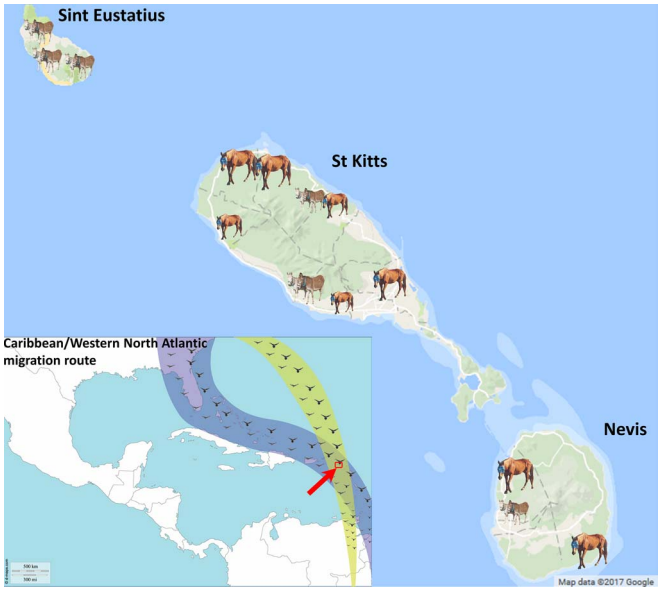

Fig. 1. Study location: equines originated from different parts of the three islands. Inset: origin of equine samples (red square indicated by the arrow) in the Leeward Islands on the Caribbean/Western North Atlantic pathway of migratory birds (green and blue shades). (For interpretation of the references 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).