ORIGINAL ARTICLE

Seroepidemiology of Selected Alphaviruses and Flaviviruses in Bats in Trinidad

N. N. Thompson[1], A. J. Auguste[2,3], A. P. A. Travassos da Rosa[3], C. V. F. Carrington[2], B. J. Blitvich[4], D. D. Chadee[5], R. B. Tesh[3], S. C. Weaver[3] and A. A. Adesiyun[1]

[1] School of Veterinary Medicine, The University of the West Indies, St. Augustine, Trinidad and Tobago
[2] Department of Pre-Clinical Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago
[3] Institute for Human Infections and Immunity and Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX, USA
[4] Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[5] Department of Life Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago

Impacts

- Bats in Trinidad were screened for selected viruses, including several of zoonotic and public health significance.
- Serological evidence of the past infection with VEEV, TABV, RBV and an undetermined flavivirus(es) was detected.
- Factors associated with bats being seropositive for TABV included bat species, location and feeding preference, and for VEEV, roost type and location were associated.

Summary

A serosurvey of antibodies against selected flaviviruses and alphaviruses in 384 bats (representing 10 genera and 14 species) was conducted in the Caribbean island of Trinidad. Sera were analysed using epitope-blocking enzyme-linked immunosorbent assays (ELISAs) specific for antibodies against West Nile virus (WNV), Venezuelan equine encephalitis virus (VEEV) and eastern equine encephalitis virus (EEEV), all of which are zoonotic viruses of public health significance in the region. Overall, the ELISAs resulted in the detection of VEEV-specific antibodies in 11 (2.9%) of 384 bats. Antibodies to WNV and EEEV were not detected in any sera. Of the 384 sera, 308 were also screened using hemagglutination inhibition assay (HIA) for antibodies to the aforementioned viruses as well as St. Louis encephalitis virus (SLEV; which also causes epidemic disease in humans), Rio Bravo virus (RBV), Tamana bat virus (TABV) and western equine encephalitis virus (WEEV). Using this approach, antibodies to TABV and RBV were detected in 47 (15.3%) and 3 (1.0%) bats, respectively. HIA results also suggest the presence of antibodies to an undetermined flavivirus(es) in 8 (2.6%) bats. Seropositivity for TABV was significantly ($P < 0.05$; χ^2) associated with bat species, location and feeding preference, and for VEEV with roost type and location. Differences in prevalence rates between urban and rural locations were statistically significant ($P < 0.05$; χ^2) for TABV only. None of the aforementioned factors was significantly associated with RBV seropositivity rates.

Introduction

The role of bats in the maintenance and transmission of rabies and other lyssaviruses has long been known, and they are now increasingly recognized as reservoirs for other viruses (e.g. members of the families *Paramyxoviridae*, *Togaviridae*, *Flaviviridae*, *Bunyaviridae*, *Reoviridae*, *Herpesviridae*, *Coronaviridae*) that 'spill over' or cross species barriers to infect humans, domestic animals and other wildlife (Calisher et al., 2006). Given their abundance, wide distribution, high mobility and apparent ability to harbour highly pathogenic viruses with little or no effect, bats may present a greater risk of zoonotic transmission than do other animals (Calisher et al., 2006). There is therefore considerable interest in

characterizing viral diversity in these animals. Recent efforts applying metagenomic sequencing and/or consensus-degenerate PCR primers and conventional sequencing techniques to bat faecal/oral swabs and other bat tissues have resulted in the detection of a wide range of viruses including bat influenza A viruses (Tong et al., 2012), several paramyxoviruses (Drexler et al., 2012), hepaciviruses and pegiviruses (Quan et al., 2013), and close relatives of the Middle East respiratory syndrome (MERS) coronavirus (Ithete et al., 2013; Memish et al., 2013).

Earlier serological studies suggest an even wider range including members of the *Alphavirus* and *Flavivirus* genera to which some of the most important human pathogens belong. Alphavirus antibodies previously detected in bats include eastern (EEEV), Venezuelan (VEEV) and western (WEEV) equine encephalitis viruses (Price, 1978a; McLean et al., 1979; Ubico and McLean, 1995). All but the latter are associated with deadly epidemics in humans and/or horses. Included among the flaviviruses for which antibodies have been detected are important pathogenic arboviruses such as WNV, SLEV, Japanese encephalitis virus (JEV), and dengue viruses (DENV) (Price, 1978a; Herbold et al., 1983; Ubico and McLean, 1995; Bunde et al., 2006; Aguilar-Setien et al., 2008; Cui et al., 2008; Machain-Williams et al., 2013). However, it is still unclear whether bats serve as important amplification/reservoir hosts for any of these viruses.

There are also several flaviviruses that are considered bat viruses, such as Rio Bravo (RBV) and Tamana bat virus (TABV), which belong to the 'no known vector' group. Although not arthropod-borne and phylogenetically distinct from SLEV (Grard et al., 2009), RBV is closely related antigenically to SLEV (Hendricks et al., 1983). TABV has only ever been isolated once, from *Pteronotus parnellii* in Trinidad (Price, 1978b; de Lamballerie et al., 2002). Trinidad is also the only country to have reported the presence of RBV antibodies in bats and in humans (Price, 1978b).

The last survey to detect arboviral antibodies in bats in Trinidad was performed almost 40 years ago (Price, 1978a, b), when sera from bats of 39 species were screened by either hemagglutination inhibition assays (HIA) or neutralization tests. Of almost 1000 bat sera tested by HIA, 0.6% of bats of four species were EEEV-seropositive and 15.3% (representing 11 species) were SLEV-seropositive (Price, 1978a). Additionally, antibodies against RBV and TABV were detected at rates of 14.1% (125 of 887) and 8.5% (72 of 850), respectively (Price, 1978b).

In the present study, 384 bats (14 species) sampled from 24 locations in Trinidad were screened for antibodies to VEEV, EEEV, WEEV, SLEV, WNV, RBV and TABV using HIA and also using epitope-blocking ELISA in the cases of VEEV, EEEV and WNV. Additionally, associations between seropositivity and bat species, gender, age class (juvenile or adult), location, roost type and feed type were determined.

Materials and Methods

Determination of sample size

An estimated sample size (n_o) of 296 was determined using $n_o = t^2 (p) (1-p)/d^2$ (Thrusfield, 2007), where distribution (t) = 1.96 (at 95% confidence level), prevalence (p) = 26% from a similar study performed in the region on different bat populations (Guatemala) where 87 of 332 samples tested were seropositive for one or more of 10 viruses assayed for (Ubico and McLean, 1995), and precision at a type 1 error (d) = 5%.

Sampling locations

Global positioning system (GPS) coordinates at roost locations were recorded with a Garmin C76CSX unit (Garmin, Olathe, KS, USA) along with the names of the towns and mapped with Arcmap 9.2, ArcGIS 9 (ESRI, Redlands, CA, USA). Locations were defined as urban (200 or more persons per sq km) or rural (fewer than 200 persons per sq km) (CSO, 2001).

Bat sampling strategy

Three hundred and eighty-four (384) bats of 14 species were captured between 13 November, 2006 and 2 May, 2008 from 27 locations in 24 towns by systematic random sampling such that after randomly selecting a starting location, every third location was selected from a list obtained from the Anti-Rabies Unit (ARU) of the Ministry of Agriculture, Land and Marine Resources (MALMR) in Trinidad. This was followed by non-random opportunistic sampling from known roosts of varying types (categorized as trees, tunnels, caves, bridges, houses and other buildings) at the selected location.

To limit ecological impact, fieldwork was performed under close direction and with the assistance of ARU staff that monitor bat populations and movement within Trinidad. Permission was given to trap up to 30 bats per roosts based on their prior knowledge of the population sizes at roosts/locations visited and adjustments were made in the field based on the observed population sizes.

Trapping was carried out by ARU staff using mist-nets (36 mm mesh) or hand-held nets (Kunz and Kurta, 1988) between 5 pm and 11 pm when the bats emerged from their roosts for feeding. Vampire bats were collected at periodic roost exterminations associated with the MALMR's rabies eradication exercises. Bats were transported live in covered metal cages to the School of Veterinary Medicine at the University of the West Indies where they were taxonomically classified based on morphology. Bat specimens were identified to species firstly by experienced ARU and MALMR personnel and then confirmed by

our laboratory by descriptions made in Goodwin, 1961. Age class and gender were also noted. Apart from mild dehydration, all bats studied were apparently healthy. A repository of voucher specimens collected in this study is housed at the Zoology museum, UWI, St Augustine campus, for future reference.

Serum collection

Bats were anesthetized with 2% Bomazine (Bomac Laboratory Limited, Manukaau, Auckland, New Zealand) and 10% ketamine (Dutch Farm Veterinary Pharmaceuticals, Loosdrecht, the Netherlands) administered intraperitoneally (Heard et al., 1996; Fowler and Cubas, 2001). Cardiac exsanguination was then performed with 1 ml syringes and 25–27 gauge needles. Sera were harvested and stored at −70°C until further processing.

Hemagglutination inhibition assay

Hemagglutination inhibition assay (HIA) was performed as previously described (Shope, 1963; Beaty et al., 1989). Sucrose-acetone antigens (hemagglutinins; HA) were prepared and stored at −70°C. They were Tr 126865 strain for RBV, Tr 127154 strain for TABV, BeAr 23379 strain for SLEV, NY-385-99 for WNV, the TC-83 (Subtype IAB) vaccine strain for VEEV, McMillan 1963 strain for WEEV and North American strain NJ/60 for EEEV. Testing began at serum dilutions of 1 : 20 with titrations through to the HI endpoint. A positive control serum titrated through to its endpoint was included for each antigen. Sera were deemed positive for antibodies to a given virus if there was a fourfold or greater difference in antibody titres over other virus antigens.

Epitope-blocking ELISA for detection of flavivirus antibodies

Epitope-blocking enzyme-linked immunosorbent assays (ELISAs) were performed following the procedure of Blitvich et al. (2003) with minor modifications. A serum dilution of 1 : 10 was used along with monoclonal antibody (MAb) 3.1112G (Chemicon, Temecula, CA, USA), which reacts specifically with WNV (Scherret et al., 2001) and 6B5A-2 MAb (Chemicon), which reacts more specifically with (SLEV) (Scherret et al., 2001) but also with viruses closely related to SLEV (SLE-like viruses). The antigens used were WNV NY-385-99 strain and SLEV strain TBH-28.

Epitope-blocking ELISA for detection of alphavirus antibodies

An identification algorithm of murine monoclonal antibodies (MAbs) for subtyping VEEV was employed as described by Roehrig and Bolin (1997) except that MAb 5B4D-6 was used for detecting VEEV (TC-83 vaccine strain) and MAb 1B1C-4 for detecting EEEV, (Roehrig et al., 1990). MAb 1B1C is cross-reactive for both North American (NJ/60) and South American (BeAn 5122) EEEV. All MAbs were from Chemicon. ELISAs were performed according to Wang et al. (2005) with minor modifications to detect alphaviral antibodies. Optimal concentrations of viral antigens were first estimated by titration with each MAb. The antigens used were VEE-TC83 (subtype 1) strain for VEEV and North American Strain (NJ/60) for EEEV.

Seropositivity (% inhibition) calculation

The seropositivity for both flaviviruses and alphaviruses was calculated using the formula: % inhibition = 100 − $\{(TS − B)/(CS − B)\} \times 100$, where TS = optical density (OD) of test sera, CS = OD of control sera and B = background OD (Hall et al., 1995). For TS, CS and B, the mean optical densities from duplicate tests were used. An inhibition value of 30% or greater was considered to indicate the presence of viral antibodies (Blitvich et al., 2003). Due to the small volumes available, some sera were tested only by ELISAs.

Statistical analyses

The seropositivity results of the HIA and epitope-blocking ELISA were compared and the factors such as species, gender, age class (juvenile or adult), location (urban or rural town), roost type and types of food eaten were subjected to the chi-squared test (χ^2) with the level of significance determined at an alpha level of 0.05. Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc., Chicago, Il, USA) was used for analysis of the data.

Ethical approval and permits

The study was approved by the Ethics Committee of the Faculty of Medical Sciences, and the permit for capture of the bats was received from the Wildlife Section of the Ministry of Agriculture, Land and Marine Resources.

Results

Distribution and characteristics of sampled bats
Fig. 1 shows the 24 sampled locations and the number of bats captured at each. In total, 384 microchiropteran bats (174 males, 210 females) representing 10 genera and 14 species were sampled from six roost types. Additional details (i.e. species, roost type, gender and feeding preference) are summarized in Table 1.

Seropositive rates by epitope-blocking ELISA and HIA

The details of the HIA and ELISA seropositive bat species are shown in Table 1. Of the 384 sera tested by epitope-blocking ELISA for antibodies against WNV, VEEV and EEEV, 11 (2.9%) representing five species were positive for VEEV-specific antibodies and none had antibodies to EEEV
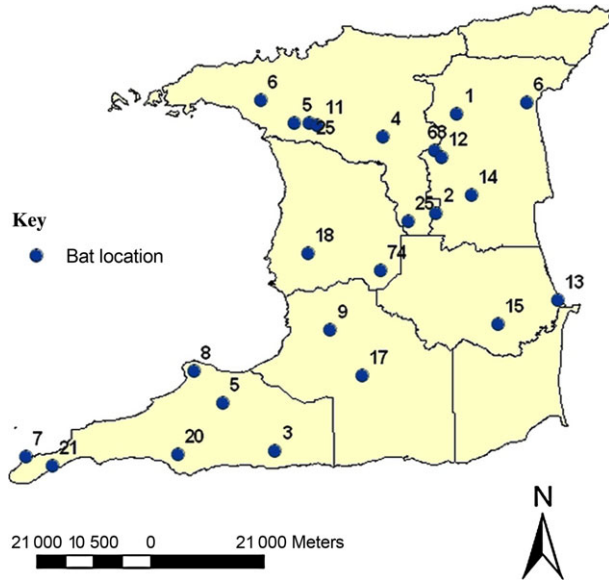

Fig. 1. Locations in Trinidad where bats were collected with number of bats collected from each site indicated.

or WNV (See Table 1). Seven sera (from four bat species) contained antibodies that specifically block the binding of 6B5A-2 MAb to SLEV antigen (Table 1). As this antibody also binds to other related viruses, the conclusion was that these bats had antibodies to SLEV and/or a SLEV-like viruses. Of the 308 sera that were also tested by HIA for antibodies against SLEV, WNV, VEEV, EEEV, WEEV, TBV and RBV, 47 (15.3%) representing eight species were TABV–seropositive, and three (1.0%) representing two species were seropositive for RBV. For the eight TABV-seropositive species (of the 13 species tested), rates ranged between 2.6% and 45.7%. The latter was for the hematophagous vampire bat species *Desmodus rotundus*, which was also the most common species (35.0%) among the TABV-positive bats (Table 1).

In addition, there were six sera for which HIA detected antibodies that were cross-reactive against SLEV and RBV (i.e. less than fourfold difference in titres), and two that were cross-reactive against SLEV, RBV and TABV, suggesting the presence of antibodies against an undetermined flavivirus(es) at a rate of 2.6%. No antibodies to EEEV, WEEV and WNV were detected among the sera sampled by either HIA or ELISA.

There is no evidence of flavivirus–alphavirus co-infections (i.e. no VEEV-positive bat was seropositive for any other virus), but there was evidence of flaviviral co-infection where two *Molossus major* bats were SLEV-seropositive by epitope-blocking ELISA and TABV-seropositive by HIA.

Table 1. Seropositivity for flavivirus and alphavirus antibodies in bats by species, gender and feeding preference

Species	Gender			Epitope-blocking ELISA			HIA		
	No. (%) male	No. (%) female	Feed preference	No. tested	No. SLEV positive (%)	No. VEEV positive (%)	No. tested	No. TABV positive (%)	No. RBV positive (%)
Artebius sp.	9 (20.0)	36 (80.0)	Fruits	45	3 (6.7)	2 (4.4)	39	1 (2.6)	0 (0.0)
Carollia perspicillata	27 (46.6)	31 (53.4)	Fruits	58	0 (0.0)	4 (6.9)	47	11 (23.4)	0 (0.0)
Desmodus rotundus	12 (29.3)	29 (70.7)	Mammalian blood	41	0 (0.0)	2 (4.9)	35	16 (45.7)	0 (0.0)
Diaemus youngi	4 (57.1)	3 (42.9)	Mammalian & avian blood	7	0 (0.0)	0 (0.0)	6	0 (0.0)	0 (0.0)
Glossophaga soricina	16 (48.5)	17 (51.5)	Nectar	33	1 (3.0)	2 (6.1)	21	2 (9.5)	0 (0.0)
Molossus ater	3 (27.3)	8 (72.7)	Insects	11	0 (0.0)	0 (0.0)	8	0 (0.0)	0 (0.0)
Molossus major	4 (14.3)	24 (85.7)	Insects	28	2 (7.1)	0 (0.0)	23	7 (30.4)	0 (0.0)
Mormoops megalophylla	9 (50.0)	9 (50.0)	Insects	18	0 (0.0)	0 (0.0)	5	0 (0.0)	0 (0.0)
Mormoops sp.	0 (0.0)	2 (100.0)	Insects	2	0 (0.0)	0 (0.0)	2	0 (0.0)	0 (0.0)
Noctilio leporinus	8 (57.1)	6 (42.9)	Fish	14	0 (0.0)	1 (7.1)	14	0 (0.0)	1 (7.1)
Phyllostomus discolor	10 (62.5)	6 (37.5)	Omnivorous	16	0 (0.0)	0 (0.0)	16	2 (12.5)	0 (0.0)
Phyllostomus hastatus	27 (96.4)	1 (3.6)	Omnivorous	28	1 (3.6)	0 (0.0)	27	6 (22.2)	0 (0.0)
Pteronotus parnellii	45 (54.9)	37 (45.1)	Insects	82	0 (0.0)	0 (0.0)	65	2 (3.1)	2 (3.1)
Vampyrodes sp.	0 (0.0)	1 (100.0)	Fruits	1	0 (0.0)	0 (0.0)	0	0 (0.0)	0 (0.0)
Total	174 (45.3)	210 (54.7)		384	7 (1.8)	11 (2.9)	308	47 (15.3)	3 (1.0)

RBV, Rio Bravo virus; SLEV, St. Louis encephalitis virus; TABV, Tamana bat virus; VEEV, Venezuelan equine encephalitis virus. All samples tested by ELISA were negative for antibodies to WNV and EEEV; all samples tested by HIA were negative for WNV, SLEV, ILHV, EEEV, WEEV and VEEV antibodies.

Factors associated with seropositivity

Chi-square analysis was used to test for associations between seropositivity and age (adult/juvenile; data not shown), species (Table 1), gender (Table 1), feed preference (Table 1), location (data not shown), location type (i.e. urban/rural; data not shown) and roost type (data not shown).

No statistically significant associations were found with age or gender. For VEEV, seropositive rates were associated with roost types ($P = 0.001$; $\chi^2 = 20.70$) and location ($P < 0.001$; $\chi^2 = 85.32$) (Table 2). For TABV, rates were significantly associated ($P < 0.05$; χ^2) with bat species ($P < 0.001$; $\chi^2 = 64.17$), feeding preference ($P < 0.001$; $\chi^2 = 40.81$), location ($P < 0.001$; $\chi^2 = 106.78$) and location type ($P < 0.001$; $\chi^2 = 20.54$) (Table 2). In the case of RBV, none of the factors tested was significantly associated with seropositivity rates.

Discussion

Using epitope-blocking ELISAs, VEEV-specific antibodies were detected in 2.9% of the 384 sera tested. No EEEV or

Table 2. TABV- and VEEV-seropositive bats by location and roost type

	No. TABV-positive (%)	No. VEEV-positive (%)
Location		
Urban		
Arima	1 (2.1)	2 (18.2)
Couva 1	0 (0.0)	1 (9.1)
Couva 2	0 (0.0)	1 (9.1)
Mt. Hope	0 (0.0)	2 (18.2)
Princes Town	2 (4.3)	0 (0.0)
Tabaquite	3 (6.4)	0 (0.0)
Rural		
Aripo savannah	7 (14.9)	1 (9.1)
Cedros	4 (8.5)	0 (0.0)
Erin	10 (21.3)	0 (0.0)
Fyzabad	2 (4.3)	2 (18.2)
Manzanilla	1 (2.1)	0 (0.0)
Morne Diablo	0 (0.0)	1 (9.1)
Moruga	2 (4.3)	0 (0.0)
Rio Claro	2 (4.3)	0 (0.0)
Talparo	7 (14.9)	0 (0.0)
Tamana	0 (0.0)	1 (9.1)
Wallerfield	6 (12.8)	0 (0.0)
Total	47 (100)	11 (100)
Roost type		
Bridge	0/2 (0.0)	1/3 (33.3)
Cave	1/13 (7.7)	0/35 (0.0)
House	1/18 (5.6)	3/29 (10.3)
Other building	24/136 (17.6)	3/158 (1.9)
Tree	18/73 (24.7)	4/84 (4.8)
Tunnel	3/66 (4.5)	0/75 (0.0)
Total	47/308 (15.3)	11/384 (2.9)

TABV, Tamana bat virus; VEEV, Venezuelan equine encephalitis virus.

WNV specific antibodies were detected by ELISA, and of the seven viruses screened for by HIA, only RBV (1.0%)- and TABV (15.3%)-specific antibodies were detected in the 308 serum samples tested. As HIA generally has a lower sensitivity than ELISA (Beaty et al., 1989), the detection of VEEV by ELISA in samples that were VEEV negative by HIA is unsurprising.

The absence of antibodies to WEEV is consistent with the results of a 1978 serosurvey of almost 1000 Trinidad bats (Price, 1978a). However, in contrast to our study, the latter detected antibodies to EEEV, albeit at a low rate (0.6%) (Price, 1978a). In the case of WNV, a recent study demonstrated the presence of WNV reactive antibodies among horses in Trinidad, but all livestock and wildlife (including birds) tested were seronegative (Thompson et al., 2012).

The VEEV seropositivity rate of 2.9% ($n = 11$) obtained by ELISA was higher than the rate of 0.4% determined by Price (1978b) using the HIA. This difference may be a consequence of the higher sensitivity of ELISA, or it may reflect the fact that Price (1978b) screened for antibodies specific for subtype IIIA VEEV (i.e. Mucambo virus) while the VEEV serotype or strain responsible for the seropositivity in our study was not determined. During the period when our serosurvey was conducted, Mucambo virus was repeatedly isolated from mosquitoes collected from forested regions of Trinidad (Auguste et al., 2009, 2010) including one of the seropositive locations. It is therefore possible that this strain is the one circulating in bats in Trinidad.

The five VEEV-seropositive species were all first reports for Trinidad, but similar findings have been previously reported in Brazil, Guatemala and Mexico (Scherer et al., 1971; Correa-Giron et al., 1972; Calisher et al., 1982; Ubico and McLean, 1995). A significantly higher percentage of VEEV-seropositive bats roosted in close association with people (e.g. houses, bridges and other buildings), compared to in caves and tunnels further from human habitations (Table 2), raising the issue of risk of infection for humans and their livestock.

No SLEV-specific antibodies were detected by HIA; however, antibodies to SLEV or a related 'SLEV-like' virus were detected in 1.8% of the bats tested by epitope-blocking ELISA. In contrast, Price (1978a) detected SLEV-reactive antibodies at a much higher rate of 15.3% using HIA, but these positive sera were negative by neutralization tests, suggesting that the SLEV-reactive antibodies were specific to another virus (e.g. Rio Bravo virus). In fact the lower seropositivity rate (1.8%) of SLEV/SLE-like virus in our study is closer to the 4.5% SLEV seropositivity reported from Guatemala by Ubico and McLean (1995).

The flaviviruses for which antibodies were detected (i.e. RBV and TABV) have been previously reported in bats in Trinidad (Price, 1978a,b). The overall RBV seropositivity

rate was 1.0% compared to 14.1% previously reported in Trinidad (Price, 1978b) and 19.0% in Guatemala (Ubico and McLean, 1995). The two RBV-seropositive species in our study were *Pteronotus parnellii* (an insectivore) and *Noctilio leporinus* (a fish-eating bat). Both were also included among the species screened in the earlier Trinidad study (Price, 1978b) and only the *Pteronotus parnellii* bats were RBV-seropositive. However, the number of *Noctilio leporinus* tested ($n = 4$) was small.

Our results indicate a seropositivity rate of 15.9% for TABV, which is higher than the 8.5% previously recorded in Trinidad, but there was no difference in the species of bats found to be seropositive compared to the earlier study (Price, 1978b). There are no other published serological reports of TABV for comparison, and with only one record of isolation (Price, 1978b), there is a dearth of information on the biology of this virus. This single isolation was from the saliva of a bat. Transmission via oral secretions could explain the observed associations with bat species ($P < 0.001$; $\chi^2 = 64.17$) and feeding preference ($P < 0.001$; $\chi^2 = 40.81$) for this 'no-known vector' virus. For example, in the case of frugivorous and nectar-feeding bats, transmission could be facilitated when a large number of bats are feeding on the same fruits and flowers.

In the case of *Desmodus rotundus*, it is conceivable that the successful spread of TABV is facilitated by its aggressive nature and habit of regurgitating its blood meal to roost mates (Wilkinson, 1990), large roosts sizes and the social habits of mutual grooming and biting during courtship and mating (Wong et al., 2007). Given the hematophagous feeding nature of this bat species, there is also presumably a risk of infection of humans and livestock, particularly in rural areas where seroprevalence rates were significantly higher ($P < 0.05$; χ^2) and where bat bites in livestock are not uncommon.

Of the sera tested by HIA, 6% were cross-reactive, suggesting the presence of antibodies to unknown or undetermined flaviviruses. However, cross-reactivity among flaviviruses (such as RBV, TABV, SLEV) in different complexes is frequently observed with HIA test especially in sequential flaviviral infections (Casals, 1957). To positively identify the causative virus(es), a wider panel of HIA antigens including other flavi- and alphaviruses previously detected in this region (i.e. Dengue, Rocio, Cacipacore, Iguape, VEEV IIIA (Mucambo), Una and Mayaro viruses) or plaque reduction neutralization tests would be necessary. Unfortunately, the small volumes of sera available did not allow for this. PCR might also be used to detect viral RNA but as arboviral infections are generally transient, the chance of the sera collected being viremic is extremely low. Enzyme-linked immunoassays and hemagglutination inhibition (HIA) test, among others, are classical tests that lack specificity and have inherent problems in interpretation, in areas that are hyperendemic for flaviviruses. Testing by these methods may result in broad cross-reactions and therefore difficulty in determining the etiologic virus (Beaty et al., 1989). Hemagglutintion inhibition requires a wide panel of viral antigens usually along with acute and convalescent sera to adequately determine specific etiologic agents by identifying fourfold rise or fall in titre values. ELISA inhibition methods increase specificity and sensitivity and this has been demonstrated when compared with HIA for dengue diagnosis and surveillance (Beaty et al., 1989; Fernández, 1990). Epitope-blocking ELISAs have been developed with diagnostic efficacy similar to PRNT for detection of arboviral antibodies including WNV and VEEV (Blitvich et al., 2003; Wang et al., 2005).

In conclusion, our study suggests that TABV, RBV and VEEV are currently in circulation in bats within Trinidad, including species not previously reported. Of the nine species (67 bats) found to be seropositive for at least one of these viruses, five are new records for VEEV antibodies and one for RBV. We also found statistically significant associations among VEEV-seropositive bats and roosts in close proximity to humans and their livestock, as well as for TABV-seropositive bats (especially vampires) and their feeding and roosting habits in rural communities. Our data suggest that bat flaviviruses may be more easily transmitted among bats than the alphaviruses or that there is a difference in the viruses in circulation at the time of this study. Bat species tested and other factors may also influence the different rates observed. However, there is not enough data here to suggest that bats are less infected by one of these genera. Further work would be required to determine the specific role of bats in the enzootic maintenance and spread of these viruses, how the risk factors identified relate to their biology and whether they pose a risk to human and livestock populations.

Conflict of Interest

Authors declare that there are no conflicts of interest.

Acknowledgements

antigens and positive controls. We thank Alva Stewart-Johnson of UWI and Maria Diaz Morales of UTMB for their laboratory assistance.

References

Aguilar-Setien, A., M. L. Romero-Almaraz, C. Sanchez-Hernandez, R. Figueroa, L. P. Juarez-Palma, M. M. Garcia-Flores, C. Vazquez-Salinas, M. Salas-Rojas, A. C. Hidalgo-Martinez, S. A. Pierle, C. García-Estrada, and C. Ramos, 2008: Dengue virus in Mexican bats. *Epidemiol. Infect.* 136, 1678–1783.

Auguste, A. J., S. M. Volk, N. C. Arrigo, R. Martinez, V. Ramkissoon, A. P. Adams, N. N. Thompson, A. A. Adesiyun, D. D. Chadee, J. E. Foster, A. P. Travassos Da Rosa, R. B. Tesh, S. C. Weaver, and C. V. Carrington, 2009: Isolation and phylogenetic analysis of Mucambo virus (Venezuelan equine encephalitis complex subtype IIIA) in Trinidad. *Virology* 392, 123–130.

Auguste, A. J., A. P. Adams, N. C. Arrigo, R. Martinez, A. P. Travassos da Rosa, A. A. Adesiyun, D. D. Chadee, R. B. Tesh, C. V. Carrington, and S. C. Weaver, 2010: Isolation and characterization of sylvatic mosquito-borne viruses in Trinidad: Enzootic transmission and a new potential vector of Mucambo virus. *Am. J. Trop. Med. Hyg.* 83, 1262–1265.

Beaty, B. J., C. H. Calisher, and S. Shope. 1989: Arboviruses, In Schmidt, N. J., E. , and R. W. (eds), Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 6th edn. pp. 797–855. American Public Health Association, Washington DC.

Blitvich, B. J., R. A. Bowen, N. L. Marlenee, R. A. Hall, M. L. Bunning, and B. J. Beaty, 2003: Epitope- blocking enzyme-linked immunosorbent assays for detection of west nile virus antibodies in domestic mammals. *J. Clin. Microbiol.* 41, 2676–2679.

Bunde, J. M., E. J. Heske, N. E. Mateus-Pinilla, J. E. Hofmann, and R. J. Novak, 2006: A survey for West Nile virus in bats from Illinois. *J. Wildl. Dis.* 42, 455–458.

Calisher, C. H., R. M. Kinney, O. de Souza Lopes, D. W. Trent, T. P. Monath, and R. B. Francy, 1982: Identification of a new Venezuelan equine encephalitis virus from Brazil. *Am. J. Trop. Med. Hyg.* 31, 1260–1272.

Calisher, C. H., J. E. Childs, H. E. Field, K. V. Holmes, and T. Schountz, 2006: Bats: important reservoir hosts of emerging viruses. *Clin. Microbiol. Rev.* 19, 531–545.

Casals, J., 1957: The arthropod-borne group of animal viruses. *Trans. N. Y. Acad. Sci. Ser.* 2, 219–235.

Correa-Giron, P., C. H. Calisher, and G. M. Baer, 1972: Epidemic strain of Venezuelan equine encephalomyelitis virus from a vampire bat captured in Oaxaca, Mexico, 1970. *Science* 175, 546–547.

CSO. 2001: CSO's household budget survey 1997-1998: Geographic Information Section, Central Statistical Office, Ministry of Finance, Trinidad and Tobago. Contract No.: Document Number 1998.

Cui, J., D. Counor, D. Shen, G. Sun, H. He, V. Deubel, and S. Zhang, 2008: Detection of Japanese encephalitis virus antibodies in bats in Southern China. *Am. J. Trop. Med. Hyg.* 78, 1007–1011.

Drexler, J.F. V.M. Corman, M.A. Müller, G.D. Maganga, P. Vallo, T. Binger, F. Gloza-Rausch, A. Rasche, S. Yordanov, A. Seebens, A. Oppong, Y. Adu Sarkodie, C. Pongombo, A.N. Lukashev, J. Schmidt-Chanasit, A. Stöcker, A.J. Carneiro, S. Erbar, A. Maisner, F. Fronhoffs, R. Buettner, E.K. Kalko, T. Kruppa, C.R. Franke, R. Kallies, E.R. Yandoko, G. Herrler, C. Reusken, A. Hassanin, D.H. Krüger, S. Matthee, R.G. Ulrich, E.M. Leroy, and C. Drosten, 2012: Bats host major mammalian paramyxoviruses. *Nat. Commun.* 3, 796. doi: 10.1038/ncomms1796

Fernández, R.J., and S. Vázquez, 1990: Serological diagnosis of dengue by an ELISA inhibition method (EIM). *Mem. Inst. Oswaldo Cruz* 85, 347–351.

Fowler, M. E., and C. Cubas 2001: Biology, medicine, and surgery of South American wild animals. Wiley Online Library.

Goodwin, G. G., 1961: A review of the bats of Trinidad and Tobago: descriptions, rabies infection, and ecology. *Bull. Am. Mus. Nat. Hist* 122, 210–283.

Grard, G., G. Moureau, R. N. Charrel, E. C. Holmes, E. A. Gould, and X. de Lamballerie, 2009: Genomics and evolution of Aedes-borne flaviviruses. *J. Gen. Virol.* 91, 87–94.

Hall, R. A., A. K. Broom, A. C. Hartnett, M. J. Howard, and J. S. Mackenzie, 1995: Immunodominant epitopes on the NS1 protein of MVE and KUN viruses serve as targets for a blocking ELISA to detect virus-specific antibodies in sentinel animal serum. *J. Virol. Methods*, 51, 201–210.

Heard, D. J., C. Beale, and J. Owens, 1996: Ketamine and Ketamine: xylazine for short-term immobilization of the Island flying fox (*Pteropus hypomelanus*). *J. Zoo Wildl. Med.* 27, 44–48.

Hendricks, D. A., J. L. Hardy, and W. C. Reeves, 1983: Comparison of biological properties of St. Louis encephalitis and Rio bravo viruses. *Am. J. Trop. Med. Hyg.* 32, 602–609.

Herbold, J. R., W. P. Heuschele, R. L. Berry, and M. A. Parsons, 1983: Reservoir of St. Louis encephalitis virus in Ohio bats. *Am. J. Vet. Res.* 44, 1889–1893.

Ithete, N. L., S. Stoffberg, V. M. Corman, V. M. Cottontail, L. R. Richards, M. C. Schoeman, C. Drosten, J. F. Drexler, and W. Preiser, 2013: Close relative of human middle east respiratory syndrome coronavirus in bat, South Africa. *Emerg. Infect. Dis.* 19, 1697–1699.

Kunz, T. H., and A. Kurta, 1988: Capture methods and holding devices. In Kunz, T. H. Ecological and Behavioral Methods for the Study of Bats. pp. 1–29, Smithsonian Institution Press, Washington, DC.

de Lamballerie, X., S. Crochu, F. Billoir, J. Neyts, P. de Micco, E. C. Holmes, and E. A. Gould, 2002: Genome sequence analysis of Tamana bat virus and its relationship with the genus Flavivirus. *J. Gen. Virol.* 83, 2443–2454.

Machain-Williams, C., M. López-Uribe, L. Talavera-Aguilar, J. Carrillo-Navarrete, L. Vera- Escalante, F. Puerto-Manzano, A.

Ulloa, J. A. Farfán-Ale, J. Garcia-Rejon, B. J. Blitvich, and M. A. Loroño-Pino, 2013: Serologic evidence of flavivirus infection in bats in the Yucatan Peninsula of Mexico. *J. Wildl. Dis.* 49, 684–689.

McLean, R. G., H. A. Trevino, and G. E. Sather, 1979: Prevalence of selected zoonotic diseases in vertebrates from Haiti, 1972. *J. Wildl. Dis.* 15, 327–330.

Memish, Z. A., N. Mishra, K. J. Olival, S. F. Fagbo, V. Kapoor, J. H. Epstein, R. Alhakeem, A. Durosinloun, M. Al Asmari, A. Islam, A. Kapoor, T. Briese, P. Daszak, A. A. Al Rabeeah, and W. I. Lipkin, 2013: Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. *Emerg. Infect. Dis.* 19, 1819–1823.

Price, J. L., 1978a: Serological evidence of infection of Tacaribe virus and arboviruses in Trinidadian bats. *Am. J. Trop. Med. Hyg.* 27, 162–167.

Price, J. L., 1978b: Isolation of Rio Bravo and a hitherto undescribed agent, Tamana bat virus, from insectivorous bats in Trinidad, with serological evidence of infection in bats and man. *Am. J. Trop. Med. Hyg.* 27, 153–161.

Quan, P.L. C. Firth, J.M. Conte, S.H. Williams, C.M. Zambrana-Torrelio, S.J. Anthony, J. A, . Ellison, A.T. Gilbert, I.V. Kuzmin, M. Niezgoda, M.O. Osinubi, S. Recuenco, W. Markotter, R.F. Breiman, L. Kalemba, J. Malekani, K.A. Lindblade, M.K. Rostal, R. Ojeda-Flores, G. Suzan, L.B. Davis, D.M. Blau, A.B. Ogunkoya, Alvarez Castillo, D. Moran, S. Ngam, D. Akaibe, B. Agwanda, T. Briese, J.H. Epstein, P. Daszak, C.E. Rupprecht, E.C. Holmes, and W.I. Lipkin, 2013: Bats are a major natural reservoir for hepaciviruses and pegiviruses. *Proc. Natl Acad. Sci. USA* 110, 8194–8199.

Roehrig, J. T., and R. A. Bolin, 1997: Monoclonal antibodies capable of distinguishing epizootic from enzootic varieties of subtype 1 Venezuelan equine encephalitis viruses in a rapid indirect immunofluorescence assay. *J. Clin. Microbiol.* 35, 1887–1890.

Roehrig, J. T., A. R. Hunt, G. J. Chang, B. Sheik, R. A. Bolin, T. F. Tsai, and D. W. Trent, 1990: Identification of monoclonal antibodies capable of differentiating antigenic varieties of eastern equine encephalitis viruses. *Am. J. Trop. Med. Hyg.* 42, 394–398.

Scherer, W. F., R. W. Dickerman, C. Campillo-Sainz, M. L. Zarate, and E. Gonzales, 1971: Ecologic studies of Venezuelan encephalitis virus in southeastern Mexico. V. Infection of domestic animals other than equines. *Am. J. Trop. Med. Hyg.* 20, 989–993.

Scherret, J. H., M. Poidinger, J. S. Mackenzie, A. K. Broom, V. Deubel, W. I. Lipkin, T. Briese, E. A. Gould, and A. Hall, 2001: The relationships between West Nile and Kunjin viruses. *Emerg. Infect. Dis.* 7, 697–705.

Shope, R. E., 1963: The use of a microhemagglutination-inhibition test to follow antibody response after arthropod-borne virus infection in a community of forest animals. *An. Microbiol.* 11, 167–171.

Thompson, N. N., A. J. Auguste, D. Coombs, B. J. Blitvich, C. V. Carrington, A. P. da Rosa, E. Wang, D. D. Chadee, M. A. Drebot, R. B. Tesh, S. C. Weaver, and A. A. Adesiyun, 2012: Serological evidence of Flaviviruses and Alphaviruses in livestock and wildlife in Trinidad. *Vector Borne Zoonotic Dis.* 12, 969–978.

Thrusfield, M., 2007: Presenting numerical data. *Vet. Epidemiol.* 3, 233.

Tong, S., D.A. Li, L.M. Rivailler, S. Conrardy, J.A. Castillo, C.T. Chen, I.A. Recuenco, A.S. Ellison, D. Davis, S. York, M. Turmelle, Y. Moran, M.R. Rogers, K. Shi, L.A. Tao, S. Weil, X. Tang, M. Rowe, K.A. Sammons, N.J. Xu, L.J. Frace, C.E. Lindblade, and R.O. Donis, 2012: A distinct lineage of influenza A virus from bats. *Proc. Natl Acad. Sci. USA* 109, 4269–4274.

Ubico, S. R., and R. G. McLean, 1995: Serologic survey of neotropical bats in Guatemala for virus antibodies. *J. Wildl. Dis.* 31, 1–9.

Wang, E., S. Paessler, P. Aguilar, D. R. Smith, L. L. Coffey, W. Kang, M. Pfeffer, J. Olson, P. J. Blair, C. Guevara, J. Estrada-Franco, and S. C. Weaver, 2005: 'A novel, rapid assay for detection and differentiation of serotype-specific antibodies to Venezuelan equine encephalitis complex alphaviruses. *Am. J. Trop. Med. Hyg.* 72, 805–810.

Wilkinson, G. S., 1990: Food sharing in vampire bats. *Sci. Am.* 262, 64–71.

Wong, S., S. Lau, P. Woo, and K. Y. Yuen, 2007: Bats as a continuing source of emerging infections in humans. *Rev. Med. Virol.* 17, 67–91.