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SUPPLEMENT ARTICLE

# Serological Evidence for Henipa-like and Filo-like Viruses in Trinidad Bats

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Bat-borne zoonotic pathogens belonging to the family Paramxyoviridae, including Nipah and Hendra viruses, and the family Filoviridae, including Ebola and Marburg viruses, can cause severe disease and high mortality rates on spillover into human populations. Surveillance efforts for henipaviruses and filoviruses have been largely restricted to the Old World; however, recent studies suggest a potentially broader distribution for henipaviruses and filoviruses than previously recognized. In the current study, we screened for henipaviruses and filoviruses in New World bats collected across 4 locations in Trinidad near the coast of Venezuela. Bat tissue samples were screened using previously established reverse-transcription polymerase chain reaction assays. Serum samples were screened using a multiplex immunoassay to detect antibodies reactive with the envelope glycoprotein of viruses in the genus *Henipavirus* and the family Filoviridae. Serum samples were also screened by means of enzyme-linked immunosorbent assay for antibodies reactive with Nipah G and F glycoproteins. Of 84 serum samples, 28 were reactive with ≥1 henipavirus glycoprotein by ≥1 serological method, and 6 serum samples were reactive against ≥1 filovirus glycoproteins. These data provide evidence of potential circulation of viruses related to the henipaviruses and filoviruses in New World bats.

**Keywords.** Filovirus; Henipavirus; Trinidad; Bats; Screening; Serology; Luminex; RT-PCR

Since 1994, >350 human fatalities from Hendra (HeV) or Nipah virus (NiV) disease outbreaks have been reported [1–3]. Periodic outbreaks of Ebola and Marburg virus disease caused by members of the family Filoviridae have resulted in approximately 13 700 recorded human fatalities since 1976 [4, 5]. In addition to public health concerns, henipavirus and filovirus spillover events continue to have severe economic and ecological impacts [6–9]. Bats are natural reservoirs for some paramyxoviruses (NiV, Hendra virus, Cedar virus, Menangle virus, and Achimota virus 1 and 2 ) and some filoviruses (Marburg and Bombali viruses) and are the putative reservoirs for other paramyxovirus and filovirus species [10–21]. The geographic distribution of henipaviruses has yet to be determined outside South and Southeast Asia, Africa, and Australia [2, 22]. In the context of filoviruses, the broader ecology and circulation within their respective natural reservoirs and the extent of the geographic distribution of filoviruses are still largely unknown [23].

Henipaviruses have only been isolated from pteropid bats in Southeast Asia and Australia [13–15]. However, multiple studies have presented evidence for the presence of henipaviruses in Africa [16, 22, 24–30], with full genome sequences of the bat-borne Ghana henipavirus recovered in Ghana [18]. In addition, recent serological data suggest that African henipaviruses are capable of spilling over into human and husbandry animal populations, although these data have not been associated with any recorded morbidity and mortality events [24, 28, 29]. A serological study by de Araujo et al found henipavirus-like antibodies in Brazilian bats. Given the distribution of bat species in Latin America that were serologically positive for the Brazilian henipa-like virus, it is possible that these viruses are circulating in Trinidad and Tobago.

The discovery of filoviruses outside Africa, including Reston virus (RESTV) in the Philippines, Lloviu virus (LLOV) in Spain, and Měnglà, Xīlǎng, and Huángjiāo viruses in China, demonstrates the broad geographic range of filoviruses [31–34]. Serological and polymerase chain reaction (PCR) evidence for filoviruses in China, Singapore, Bangladesh, and Hungary also suggest the possibility that uncharacterized filoviruses may circulate in bat populations beyond the currently described geographic range [35–39]. Han et al [40] used published filovirus surveillance data to predict bat species that may be potential filovirus reservoirs based on behavior, life history, and ecological

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traits; their study predicted that several New World bats, including several bat species with populations in Trinidad and Tobago, may be potential hosts of uncharacterized filoviruses.

In 2012, bats of 6 species were captured from 4 locations in Trinidad. Malmlov et al [41] screened these bat samples and found evidence of the circulation of Tacaribe virus. We describe here the results of surveillance efforts for evidence of henipa-like and filo-like viral infection in the same sample set, because the breadth of the host range and geographic distribution are still largely unknown for these virus families.

## METHODS

### Ethics Statement

All field work was performed under the approval of the Ethics Committee, Faculty of Medical Sciences, The University of the West Indies (UWI), St. Augustine Campus, and under a special game license from the Wildlife Section, Forestry Division, Ministry of Agriculture, Land and Fisheries, Republic of Trinidad and Tobago. All work with infectious henipaviruses and filoviruses was performed under biosafety level 4 conditions at the Rocky Mountain Laboratories, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, according to standard operating protocols approved by the Institutional Biosafety Committee.

### Bat Capture

In February 2012, bats were captured with mist nets in Trinidad at 4 locations; Mount Hope (N 10.67120, W 061.28677), Lopinot (N 10.69792, W 061.32243), Santa Cruz (N 10.69596, W 061.44629), and Maracas Valley (N 10.70945, W 061.40177) (Figure 1). Cloth bags were used to individually confine and transport bats to laboratory facilities at the University of the West Indies, St. Augustine, for processing. Six bat species were obtained: 36 flat-faced fruit bats (*Artibeus planirostris trinitatis*), 31great fruit-eating bats (*Artibeus lituratus*), 3 Pallas's long-tongued bat (*Glossophaga soricina*), 7 greater sac-winged bats (*Sacropteryx bilineata*), 3 little yellow-shouldered bats (*Sturnira lilium*), and 4 Seba's short-tailed bats (*Carollia perspicillata*). Bats were euthanized through inhalation of isoflurane and exsanguination before necropsy. Tissue (lung, liver, kidney, spleen, brain, and blood) and serum samples were stored at −80° C before shipment on dry ice to Rocky Mountain Laboratories for further processing.

### Luminex Serology

The presence of immunoglobulins against henipavirus- and filovirus-soluble native-like oligomeric virus envelope glycoproteins was measured using a Luminex xMAP-based multiplex microsphere immunoassay (MIA) [37, 42]. Briefly, soluble tetrameric henipavirus receptor binding proteins ($sG_{tet}$) (Yan et al in review) and soluble trimeric ectodomains of filovirus envelope glycoproteins were produced, as described elsewhere [37]. Purified $sG_{tet}$and envelope glycoprotein antigens were coupled to Bio-Plex Pro magnetic COOH beads (Bio-Rad). Blood was collected into serum separating tubes by means of cardiac puncture with bats under deep anesthesia, and it was centrifuged at 1000*g* for 10 minutes before serum was collected and frozen at −80°C. We performed the Luminex assay on serial dilutions of negative control serum samples from 14 captive-bred *Rousettus aegyptiacus* bats to determine an appropriate dilution for screening bat serum samples with the Luminex assay. All negative control serum samples were negative at a final dilution of 1:500. Field-collected bat serum samples were heat inactivated at 56°C for 30 minutes and diluted 1:500 before screening, and each sample was run in duplicate.

### Enzyme-Linked Immunosorbent Assay

Nunc Maxisorp 96-well flat-bottom Immuno Plates (ThermoFisher) were coated with purified NiV F and G glycoproteins (50 ng in 100 µL per well, diluted in phosphate-buffered saline [PBS]) overnight at 4°C. Plates were washed 3 times with PBS with 0.1% Tween 20 (PBS-T) and then blocked with 5% nonfat milk in PBS-T (100 µL per well) for 1 hour at room temperature. After being washed 3 times with PBS-T, diluted bat serum samples (1:100, 1:250, or 1:500 in 5% nonfat milk) were added to the wells in duplicate (100 µL) and incubated for 1 hour at room temperature. Plates were washed 5 times with PBS-T. Secondary antibody (goat anti-bat immunoglobulin G [IgG; heavy and light] horseradish peroxidase conjugate; Bethyl; 1:2500) was added to wells (100 µL) and incubated for 1 hour at room temperature. After 5 washes with PBS-T, 100 µL of a 1:1 ratio of 3,3',5,5'-tetramethylbenzidine (TMB) solution and peroxide solution (Pierce TMB Substrate Kit; ThermoFisher) was added to wells. Plates were allowed to develop in the dark. After stopping the reaction with 100 µL of 2 mol/L sulfuric acid, plates were read at 450 nm.

### In Vitro Transcription

Bombali virus and LLOV have never been isolated. Therefore, in vitro transcripts were generated as positive controls. RNA-dependent RNA polymerase coding sequence segments of Bombali virus and LLOV were synthesized into pUC57 cloning vectors (Biobasic). Plasmids were transformed into Stellar Competent Cells, following protocol PT5055-2 (Clontech). Plasmids were isolated using a PureLink HiPure Plasmid Midiprep kit (Invitrogen). Linear templates were generated by a single digestion with restriction enzyme EcoR1, according to the manufacturer's protocol (New England Biolabs). Negative-sense RNA was transcribed using the MEGAscript T7 kit.

### Nucleic Acid Extraction

RNA and DNA from Trinidad bat tissues were extracted using the Cador Pathogen 96 QIAcube HT Kit and QIAcube robot (Qiagen). The bat tissues were lysed in RLT buffer (Qiagen), followed by incubation in 95%–100% ethanol for 10 minutes before extraction. Extracted RNA from virus stocks of all currently

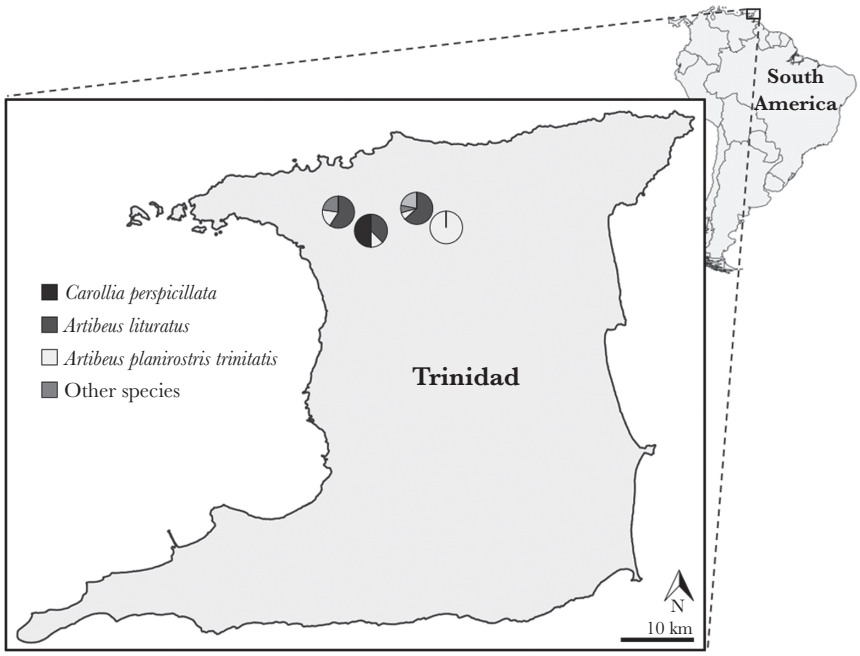


**Figure 1.** Field sites of bat capture. Collections at Mt. Hope were performed during the day at the University of West Indies. Collections at Santa Cruz, Maracas Valley, and Lopinot were performed over 3 nights.

isolated henipavirus and filovirus species were used for assay validation and positive controls. RNA was isolated using the QIAmp Viral RNA Kit (Qiagen) in a biosafety level 4 laboratory, with published modifications appropriate for virus inactivation in biosafety level 4 conditions [43]. Henipaviruses included were NiV, species *Nipah henipavirus*, isolate Malaysia; HeV, species *Hendra henipavirus*, isolate Hendra; and Cedar virus (CedV), species *Cedar henipavirus*, isolate Cedar. Filoviruses included were Ebola virus (EBOV), species *Zaire ebolavirus*, isolate Gabon; Sudan virus (SUDV), species *Sudan ebolavirus*, isolate Boniface; Taï Forest virus (TAFV), species *Taï Forest ebolavirus*, isolate Taï Forest; RESTV, species *Reston ebolavirus*, isolate Pennsylvania; Bundibugyo virus (BDBV), species *Bundibugyo ebolavirus*, isolate Bundibugyo; Marburg virus (MARV), species *Marburg marburgvirus*, isolate Angola; and Ravn virus (RAVV), species *Marburg marburgvirus*, isolate Ravn.

#### Henipavirus, Morbillivirus, and Respirovirus Assay

Complementary DNA (cDNA) was synthesized from 10 µL of RNA using the SuperScript III or IV First-Strand Synthesis System for reverse-transcription PCR (RT-PCR) (Invitrogen). RT-PCR was performed using TopTaq Master Mix Kit (Qiagen) 50-µL reactions, with 25 µL of TopTaq MasterMix, 5 µL of CoraLoad Dye, 1 µL of 10 µmol/L primers (final concentration 1.0 µmol/L), and 5 µL of cDNA template used for each reaction. Previously designed primers targeting a conserved region of the RNA-dependent RNA polymerase gene for henipaviruses, morbilliviruses, and respiroviruses [44] were used for PCR. Thermal cycling conditions were followed, according to the manufacturer's protocol, with an annealing temperature of 50°C. PCR products were analyzed using a 1% agarose gel and SYBR Safe DNA Gel Stain (Fisher Scientific). The expected fragment size based on the position of the second primer set was approximately 600 base pairs.

#### Panfilovirus Assay

cDNA was synthesized as described above. Nested RT-PCR was performed using TopTaq Master Mix Kit (Qiagen) 50-µL reactions, including 25 µL of TopTaq MasterMix, 5 µL of CoraLoad Dye, 1 µL of 10 µmol/L primers (final concentration 0.2 µmol/L), and 5 µL of cDNA template for each reaction. Previously designed primers targeting a conserved region of the filovirus RNA-dependent RNA polymerase gene [19] was used for nested PCR, with the addition of a modified forward primer for the second reaction (5'-TYTCHVT/ideoxyI/CAAAA/ideoxyI/CAYTGGGG-3'). Thermal cycling conditions for both rounds were as follows: 94°C for 5 minutes; 15 cycles of 94°C, 60.9°C (−1°C/cycle), and 72°C for 1 minute each; 15 cycles of 94°C, 45.9 °C, and 72°C for 1 minute each; and a final extension at 72 °C for 7 minutes. PCR products were analyzed using a 1% agarose gel and GelRed Nucleic Acid Stain (Phenix Research Products) or SYBR Safe DNA Gel Stain (Fisher Scientific). The expected fragment size based on the position of the second primer set was approximately 680 base pairs.

#### RT-PCR Limit of Detection

The genome copy number from the respective henipavirus and filovirus controls was determined using a 1-step protocol

for Droplet Digital PCR (ddPCR) and the Automated Droplet Generator (Bio-Rad), according to the manufacturer's instructions. Eight representative filoviruses (Supplementary Figure 1) and 3 representative henipaviruses (NiV, HeV, and CedV) were used to determine the limit of detection (LOD) for the RT-PCR assay with ddPCR before bat screening. Primers and probes used are listed in Supplementary Table 1. The LOD was determined by means of serial 10-fold dilution of viral RNA–positive controls and further refined with serial 2-fold dilution. The LOD was determined based on the highest dilution from which an observable PCR product was obtained.

## RESULTS

Serum samples from 84 Trinidad bats were screened with MIA for the presence of antibodies reactive to henipavirus or filovirus envelope glycoproteins. The median fluorescence intensity (MFI) cutoff value was set as 3 times the mean MFIs of a naive serum sample from a captive Egyptian fruit bat (*R. aegyptiacus)*. The percentage of bat serum samples reactive against henipavirus- or filovirus-soluble glycoproteins was 3.57% (3 of 84) and 7.14% (6 of 84), respectively. Six serum samples from *A. lituratus* bats were reactive against the soluble glycoproteins of RAVV, SUDV, RESTVp (pig isolate), RESTVm (primate isolate), EBOV, NiV, GhV, or CedV (Table 1). Serum samples from 1 flat-faced fruit bat (*A. planirostris trinitatis)* and 1 greater sac-winged bat (*S. bilineata*) were reactive against RAVV-soluble glycoprotein (Table 1). The highest MFI value relative to negative control was from an *A. lituratus* bat (bat no. 41) against SUDV-soluble glycoprotein (Table 1). Serological reactivity was observed in sample 41 between SUDV, RESTVp, RESTVm, NiV, and GhV and in sample 64 between SUDV and EBOV (Table 1).

Serum samples were also screened by enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies reactive to Nipah F and G glycoproteins. The MFI cutoff value was set as 3 times the standard deviation of the average MFI of naive bat serum from a captive Egyptian fruit bat. The proportions of bat serum samples reactive against NiV G and F at 1:100 dilution were 29.76% (25 of 84) and 19.05% (16 of 84), respectively (Table 1). Only 2 samples were reactive against NiV G and F at dilutions of 1:250 or greater. Twelve samples were reactive against NiV G, but not NiV F, and 3 were reactive against NiV F but not G. All samples that showed reactivity with MIA were reactive to Nipah G at ELISA. However, only 1 sample (bat 41) was reactive to both NiV G and F on ELISA and NiV G on MIA.

Previously established panviral RT-PCR assays for high-throughput screening of biologically derived samples were used to detect respirovirus, morbillivirus, henipaivirus, and filovirus RNA [19]. The panfilovirus assay was modified by incorporating sequence information for recently identified filoviruses and validated for specificity and sensitivity. Eight representative filoviruses (Supplementary Figure 1) and 3 representative henipaviruses were used to determine the LOD for the assays by means of ddPCR before bat screening. The average LOD for the representative henipaviruses and filoviruses was 3.2 and 1.5 copies/μL, respectively (Supplementary Table 2). The L gene segment of LLOV generated product only at starting concentrations >1000 copies/μL and was considered an outlier for the LOD. Tissue samples from 78 Trinidad bats were screened for respiroviruses, morbilliviruses, henipaviruses, and filoviruses by means of RT-PCR. Tissues screened were lung, liver, kidney, spleen, and brain. No henipavirus or filovirus RNA was detected in this sample set.

## DISCUSSION

Worldwide virus discovery and surveillance efforts have led to the identification of a variety novel European, African, and Chinese henipaviruses and filoviruses [16, 19, 21, 22, 24, 33, 36, 45]. In addition, they have identified potential henipavirus circulation in Latin America [46]. The zoonotic and cross-species spillover potential of these novel viruses is currently unknown. However, these discoveries highlight the importance of virus discovery and surveillance efforts for novel henipavirus and filovirus species given their potential public health, economic, and ecological impacts. Therefore, expanding surveillance efforts beyond the known geographic distributions of henipaviruses and filoviruses may shed further light on the ecology and evolutionary history of these important viruses.

In the current study, we screened phyllostomid and emballonurid bat serum and tissue samples from Trinidad for henipaviruses and filoviruses. Eight of 84 bat serum samples were positive at Luminex serology and reacted to ≥1 of the henipavirus or filovirus glycoproteins. Twenty-eight samples were positive for NiV G, F, or both on ELISA. Of note, the 3 bat species (*A. lituratus, A. planirostris trinitatis,* and *C. perspicillata*) positive for henipavirus-like antibodies on MIA or ELISA are 3 of the 6 species that were positive for henipavirus-like antibodies in a Brazilian study [46]. One bat species sampled in this study, *A. lituratus*, which was found to have antibodies reactive against filovirus-soluble glycoproteins, was among those predicted to be potential hosts of novel filoviruses based on a study by Han et al [40]. Several bats from this species showed reactivity to both filovirus and henipavirus antigens (including an individual with antibodies against both), a phenomenon also observed in pteropodid bats [24, 47, 48].

The serological IgG reactivity observed in our study is likely due to the circulation of viruses that have surface glycoproteins antigenically related to henipavirus and filovirus glycoproteins used in our assays. Similar serological cross-reactivity has been observed in a study of *Rousettus* bats experimentally challenged with filoviruses [49]. We found some discordance between the serological results of the ELISA against Nipah G and the multiplex Luminex assay that includes NiV G, specifically that more

**Table 1. Samples Seropositive Against Henipavirus or Filovirus Glycoproteins with Enzyme-Linked Immunosorbent and Multiplex Luminex Serological Assays**[a]

| | | | ELISA: NiV | | Luminex Multiplex Assay | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bat No. | Sampling Site | Species | G | F | NiV (G) | HeV (G) | GhV (G) | CeV (G) | TAFV (GP) | SUDV (GP) | RAVV (GP) | EBOV (GP) | MARV (GP) | LLOV (GP) | RESTVp (GP) | RESTVm (GP) | BDBV (GP) |
| 12 | UWI Chapel | *Artibeus planirostris trinitatis* | … | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 14 | UWI Chapel | *A. planirostris trinitatis* | 1:100 | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 15 | UWI Chapel | *A. planirostris trinitatis* | … | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 17 | UWI Chapel | *A. planirostris trinitatis* | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 18 | UWI Chapel | *A. planirostris trinitatis* | 1:100 | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 20 | UWI Chapel | *A. planirostris trinitatis* | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 21 | UWI Chapel | *A. planirostris trinitatis* | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 25 | UWI Chapel | *A. planirostris trinitatis* | … | … | … | … | … | … | … | … | 10.6 | … | … | … | … | … | … |
| 30 | Lopinot | *A. lituratus* | 1:1000 | 1:500 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 33 | Lopinot | *A. lituratus* | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 34 | Lopinot | *A. lituratus* | 1:100 | … | … | … | … | … | … | … | 538.1 | … | … | … | … | … | … |
| 35 | Lopinot | *A. lituratus* | 1:100 | … | … | … | … | 183.6 | … | … | … | … | … | … | … | … | … |
| 37 | Lopinot | *A. lituratus* | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 38 | Lopinot | *A. lituratus* | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 41 | Lopinot | *A. lituratus* | 1:100 | 1:100 | 57.3 | … | 319.5 | … | … | 2719.9 | … | … | … | … | 1305.6 | 175.0 | … |
| 42 | Lopinot | *Glossophaga soricina* | 1:100 | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 48 | Lopinot | *G. soricina* | 1:100 | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 53 | Lopinot | *A. lituratus* | 1:100 | | … | | 57.0 | … | … | … | … | … | … | … | … | … | … |
| 55 | Santa Cruz | *A. lituratus* | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 56 | Santa Cruz | *A. lituratus* | 1:100 | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 58 | Santa Cruz | *Sacropteryx bilineata* | … | … | … | … | … | … | … | … | 33.8 | … | … | … | … | … | … |
| 59 | Santa Cruz | *A. lituratus* | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 63 | Santa Cruz | *A. planirostris trinitatis* | 1:100 | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 64 | Santa Cruz | *A. lituratus* | 1:100 | 1:100 | … | … | … | … | … | 306.9 | … | 20.3 | … | … | … | … | … |
| 65 | Santa Cruz | *A. lituratus* | … | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 67 | Santa Cruz | *S. bilineata* | 1:100 | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 75 | Santa Cruz | *A. lituratus* | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 77 | Maracas Valley | *Carollia perspicillata* | 1:100 | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 78 | Maracas Valley | *C. perspicillata* | 1:100 | 1:100 | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 81 | Maracas Valley | *A. lituratus* | 1:250 | 1:250 | … | … | … | … | … | … | … | 626.3 | … | … | … | … | … |

Only positive reactivity with the specific assay and glycoprotein is displayed.

Abbreviations: BDBV, Bundibugyo virus; EBOV, Ebola virus; ELISA, enzyme-linked immunosorbent assay; F, fusion glycoprotein; G, attachment glycoprotein; GhV, Ghana henipavirus; G/GP, glycoprotein; HeV, Hendra virus; LLOV, Lloviu virus; MARV, Marburg virus; NiV, Nipah virus; RAVV, Ravn virus; RESTVm, primate isolate; RESTVp, pig isolate; SUDV, Sudan virus; TAFV, Taï Forest virus; UWI, University of West Indies.

[a]ELISA results are reported as the highest dilution for which each sample was seropositive. We report the mean fluorescence intensity (MFI) of the multiplex Luminex assay after subtracting the value of 3 standard deviations plus the mean MFI of the naive serum sample for each antigen.

samples were positive against NiV G with the ELISA than with the Luminex (Table 1). Most of the samples showing some reactivity against NiV G with ELISA but not the Luminex assay were not seropositive at dilutions above 1:100, with 2 exceptions in which *A. lituratus* bats (bats 30 and 81 [Table 1]) were seropositive by ELISA for both NiV G and F on dilutions >1:100.

The multiplex nature of the Luminex assay complicates interpretation of the serological results, because we detected reactivity

against the glycoproteins of unrelated viruses, including filovirus, SUDV, and Ghana virus, in serum collected from an *A. lituratus* bat (bat 41 [Table 1]). The specific history of viral exposure is inherently unknown in field-collected samples, and polyclonal serum samples are frequently cross-reactive; therefore, the conclusions that we can draw from these data are limited. Further efforts to characterize the viral diversity circulating in South American bats are needed to refine these serological assays and allow for the development of specific target antigens.

Although we improved on the sensitivity and specificity of a previously established panfilovirus RT-PCR assay [50–53], we detected no henipavirus or filovirus RNA. This is not surprising, given our sample sizes and the comparatively low detection rate of virus shedding compared to that of IgG antibodies against these viruses observed in naturally infected bats in field studies and experimentally infected bats in laboratory studies [54–57]. The geographic distributions of several bat species sampled in our study extend as far as Brazil, where bat serum samples were found to be positive for exposure to henipa-like viruses using ELISA and immunofluorescence assays [46], suggesting the possibility of widespread circulation of henipa-like viruses in Central and South America. Here we provide evidence for the potential circulation of henipa-like and filo-like viruses in Trinidad. No viral RNA was detected in this set of bat samples using RT-PCR. However, 35.7% of the samples were serologically positive. A primary limitation of our study is the low sample size; prior surveillance studies have found antibody-positive and PCR-positive prevalences for filoviruses as low as 1.7% and 1.9% respectively [11, 58]. Taken together, our findings provide evidence of more widespread geographic distribution of henipaviruses and filoviruses than previously appreciated.

## Supplementary Data

Supplementary materials are available at The *Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

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