

Original Contribution

## Surveillance of Arboviruses in Primates and Sloths in the Atlantic Forest, Bahia, Brazil

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**Abstract:** From 2006 through 2014, we conducted seroepidemiological surveys on non-human primates and sloths to investigate the possible circulation of arboviruses in Bahia Atlantic Forest, Brazil. We collected a total of 196 samples from 103 *Leontopithecus chrysomelas*, 7 *Sapajus xanthosternos*, 22 *Bradypus torquatus* and 7 *Bradypus variegatus*. Serum samples were tested using neutralization test and hemagglutination inhibition test to detect total antibodies against 26 different arboviruses. The overall prevalence of arboviruses was 36.6% (51/139), with the genus *Flavivirus* having the highest prevalence (33.1%; 46/139), followed by *Phlebovirus* (5.0%; 7/139), *Orthobunyavirus* (4.3%; 6/139) and *Alphavirus* (0.7%; 1/139). Monotypic reactions suggest that the wild animals were exposed naturally to at least twelve arboviruses. Added results from the neutralization test, animals were exposed to thirteen arboviruses. Most of these viruses are maintained in transmission cycles independent of human hosts, although antibodies against dengue virus serotypes 1, 2 and 3 were found in this study. To our knowledge, this is the first study reporting exposure to arboviruses in *L. chrysomelas*, *S. xanthosternos* and *B. torquatus*. Our results also highlight that the Southern Bahia Atlantic Forest has a variety of vertebrate hosts and potential vectors, which may support the emergence or re-emergence of arboviruses, including those pathogenic to humans.

**Keywords:** Arbovirus, *Leontopithecus* sp., *Sapajus* sp., *Bradypus* sp., Emerging infectious diseases, Atlantic Forest

## INTRODUCTION

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Arboviruses are zoonotic and transmitted among vertebrate hosts by hematophagous vectors (Vasconcelos et al. 2001; Vasconcelos 2010). Due to the emergence and re-emergence of various arbovirus infections in humans (e.g., dengue, chikungunya, Zika, West Nile and yellow fever) (Figueiredo 2015; Gyawali et al. 2016; Heymann et al. 2016), studies focused on identifying these viruses in vectors and hosts are an essential part of active surveillance.

In Brazil, numerous arboviruses have been detected in wild primates: *Ilheus virus* (ILHV) (Laroque et al. 2014), *Saint Louis encephalitis virus* (SLEV) (Lima et al. 2010; Svoboda et al. 2014.), *Rocio virus* (ROCV) (Laroque et al. 2014), *Bussuquara virus* (BSQV) (Moreira et al. 2000), *Mayaro virus* (MAYV) (Mb et al. 2015), *Oropouche virus* (OROV) and *yellow fever virus* (YFV) (Lima et al. 2010; Moreno et al. 2013; Tranquilin et al. 2013; Almeida et al. 2014). Recently, researchers have also discovered *Dengue virus* (DENV) antibodies in Neotropical primates (de Thoisy et al. 2009; Omatsu et al. 2012; Nakgoi et al. 2014; Ferreira et al. 2014). Similarly, several arboviruses were previously described in sloths: SLEV, ILHV, *West Nile virus*, *Utinga virus*, *Venezuelan equine encephalitis virus* (VEEV), MAYV, *Changuinola virus* (CGLV), OROV, *Murutucu virus* (MURV), *Punta Toro virus* (PTV), *Vesicular stomatitis virus* (VSV) and *Rio Grande virus* (Seymour et al. 1983a,b; Gilmore et al. 2001; Medlin et al. 2016).

The diversity of viruses detected in Neotropical mammals raises a conservation concern: What is the threat level of virus infections for endemic and endangered wild populations living in biodiversity *hot spots* (Vasconcelos and Calisher 2016; Althouse et al. 2016; Bueno et al. 2016), such as the Atlantic Forest? These arboviruses have a complex transmission cycle in which vectors, pathogens and animal hosts interact under strong influences of environmental conditions (Vasconcelos 2010; Pautasso et al. 2013; Nakgoi et al. 2014). Anthropogenic activities have a direct influence on the environment, causing rapid changes in habitat available to wildlife, which ultimately enable the emergence of pathogens and zoonotic diseases. The Brazilian Atlantic has been reduced and fragmented due to deforestation (Ribeiro et al. 2009) with many fragments adjacent to villages and agroforest systems which potentially expose animals to transmission of potential pathogens across taxa (Daszak et al. 2004; Engering et al. 2013; Jansen et al. 2015). In addition, arboviruses and competent vectors

(e.g., mosquitoes) may be spread around the world by “anthropogenic activities” (Weaver and Reisen 2010; Weaver 2013), resulting in recent human disease outbreaks (Faria et al. 2016).

Non-human primates and sloths have partially overlapping distribution ranges in the Southern Bahia Atlantic Forest (Oliver and Santos 1991). The golden-headed lion tamarin (*Leontopithecus chrysomelas*), the yellow-breasted capuchin monkey (*Sapajus xanthosternos*) and the maned three-toed sloth (*Bradypus torquatus*) are endemic to the region and categorized as threatened with extinction by the IUCN (2017), while the brown-throated sloth (*B. variegatus*) is not threatened.

In this study, our goals were to evaluate the prevalence of arboviruses in free-living *L. chrysomelas*, *S. xanthosternos*, *B. torquatus* and captive *B. torquatus* and *B. variegatus* and the influence of host species, sex and age as sources of variation in arbovirus diversity.

## MATERIALS AND METHODS

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### Study Sites

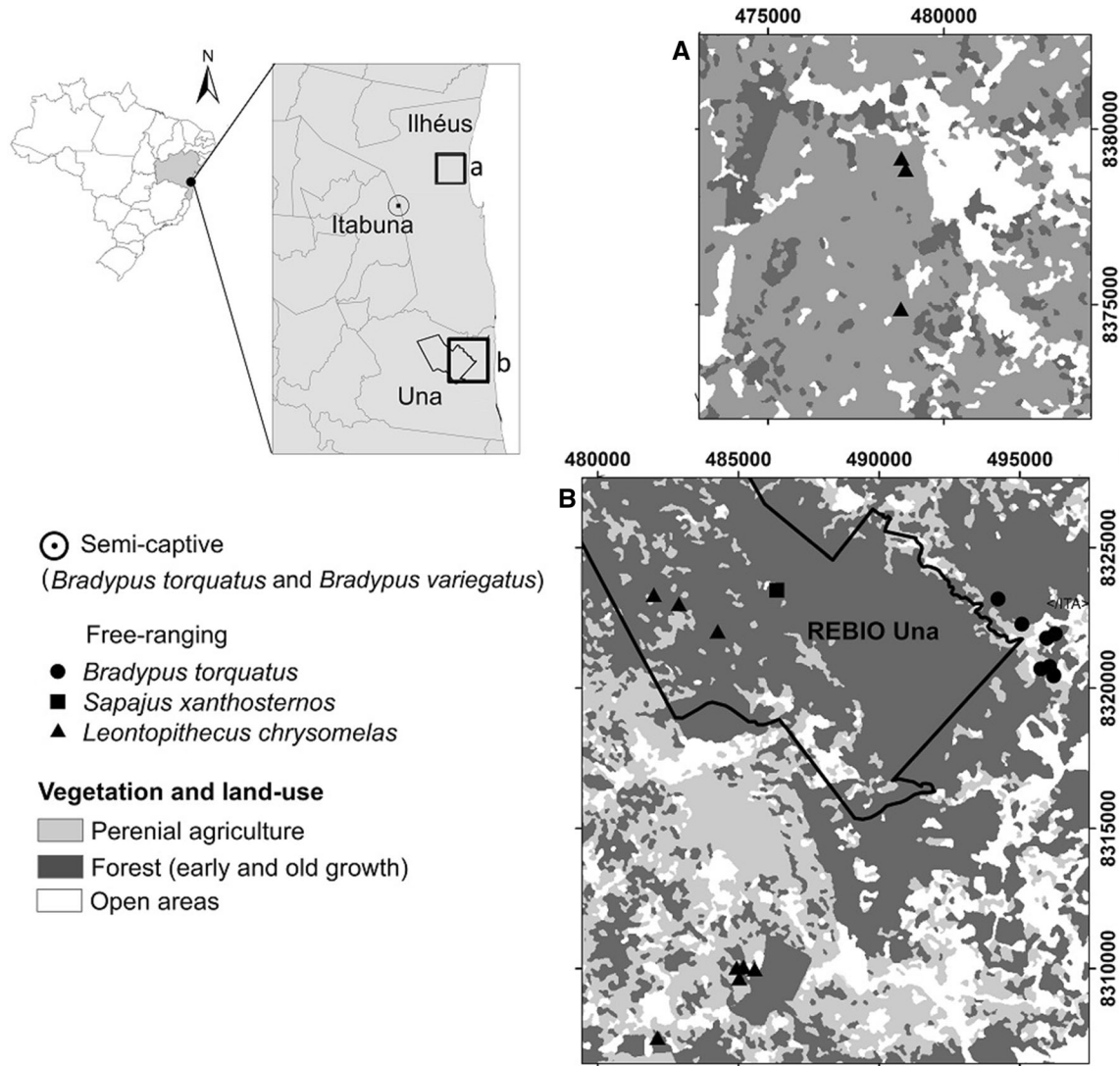
We conducted this study in the Bahia Atlantic Forest, Northeastern Brazil, in the municipalities of Ilhéus and Una (Fig. 1). In the city of Una, the capture sites of free-ranging animals occurred within the largest federal-protected area of the region (Una Biological Reserve—REBIO), as well as in rural private areas around REBIO. The capture sites of Ilhéus city were located only in rural private areas. All the semi-captive animals were captured at the Zoobotanical Reserve Rehabilitation Center in Ilhéus. The predominant land uses in the region of study are cacao agroforests, rubber tree plantations, coconut and cassava plantations (Alger and Caldas 1994; Sollberg et al. 2014).

### Study Group

The samples were collected during health screenings of animals between 2006 and 2014.

### Non-human Primates

One hundred and three lion tamarins and seven capuchin monkeys were captured individually using Tomahawk live traps baited with bananas and placed on platforms above



**Figure 1.** Capture sites of free-ranging and semi-captive species described in this study, in Ilhéus (a) and Una city (b), Bahia, Brazil.

ground in areas used by primate groups (Table 1). Once captured, the animals were maintained overnight in the field laboratory for processing and released the following morning at the site of capture (Miller and Dietz 2006). They were anesthetized with an injection of ketamine hydrochloride (10 mg/kg; i.m.) and midazolam hydrochloride (0.3 mg/kg; i.m.) (Catenacci et al. 2016). During anesthesia, we performed physical examinations and collected biomaterials (e.g., blood). We collected the following data from each animal: sex, age group, animal identification, body weight, biometric data, as well as body temperature and heart and respiratory rate (data not reported). Each animal received a unique tattoo identification number and/or dye mark for subsequent identification in the field.

## Sloths

Seven *B. torquatus* were hand-caught in the tree canopy by a trained climber, placed in a burlap sack and lowered to the forest floor using a rope (Table 1). No anesthesia was administered to any of the sloths. Sloths were weighed, sexed and aged following a previous study (Lara-Ruiz and Chiarello 2005), and morphometric data collected. Animals were handled after capture for 15–30 min and released at the base of the tree where captured.

Additional samples were collected from 15 *B. torquatus* and seven *B. variegatus* housed in a single enclosure (approximately 20 × 50 feet) (Table 1). The samples were collected using the protocol for free-living sloths as described above.

**Table 1.** Antibodies Prevalence Against Arbovirus in Sera From Wild Animals Detected by the Hemagglutination Inhibition Test, with the Results Grouped by Sex, Age and Species, During 2006–2014 at the Southern Atlantic Forest of Bahia State, Brazil.

Variables	Host species							
	<i>Bradypus torquatus</i>		<i>Bradypus variegatus</i>		<i>Leontopithecus chrysomelas</i>		<i>Sapajus xanthosternos</i>	
	N <sup>a</sup>	Prevalence (CI) <sup>b</sup>	N	Prevalence (CI)	N	Prevalence (CI)	N	Prevalence (CI)
Sex								
Male	11	0.455 (0.213–0.72)	5	0.4 (0.118–0.769)	62	0.371 (0.262–0.495)	4	0 (0–0.49)
Female	11	0.545 (0.28–0.787)	2	0.5 (0.095–0.905)	41	0.268 (0.157–0.419)	3	0.333 (0.061–0.79)
Age								
Non-adult	10	0.4 (0.168–0.687)	1	(0–0.793)	35	0.258 (0.141–0.42)	3	(0–0.56)
Adult	12	0.583 (0.319–0.807)	6	0.5 (0.188–0.81)	68	0.367 (0.263–0.486)	4	0.25 (0.045–0.697)
Overall prevalence	22	0.5 (0.307–0.693)	7	0.43 (0.158–0.75)	103	0.33 (0.247–0.426)	7	0.14 (0.026–0.513)

<sup>a</sup>N number of samples.<sup>b</sup>Confidence intervals.

## Sample Collection

One to three milliliters of blood was collected from the femoral vein of primates and 3–5 ml from the cephalic vein of sloths. Each blood sample was kept at 4°C for 3 h until centrifugation for serum collection. Sera samples were then placed in liquid nitrogen and relocated to a – 70°C freezer at the State University of Santa Cruz, Ilhéus, Bahia, until air-shipped on dry ice to the Division of Arbovirology and Hemorrhagic Fevers at the Evandro Chagas Institute (SAARB-IEC), PA, Brazil. Sera samples were stored at – 70°C until process.

## Serological Test

Sera samples were initially screened by the hemagglutination inhibition test (HI—Clarke and Casals 1958) using a panel containing twenty-six different arboviruses (Table 2). The samples were screened at a dilution of 1:20 against antigens containing four hemagglutination units, and positive sera were titrated (by factors of 2) up to a dilution of 1:1280 (Rodrigues et al. 2010). The positive samples with specific antibodies were considered monotypic when antibodies were detected against only one virus in the same genus. The heterotypic reactions were considered when antibodies for more than one virus were present in a serum sample (Thompson et al. 2012; Casseb et al. 2015).

The heterotypic reactions were confirmed by a separate neutralization test (NT) as described by Beaty et al. (1989). The titer was defined as the logarithmic neutralization in-

dex (LNI), using log 10, and the sample was considered as positive when its LNI was  $\geq 1.7$  (Reed and Muench 1938).

## Statistical Analysis

We stated positive prevalence for any individuals that were positive on any of the serological tests. In the case of multiple captures from the same animal, an individual was considered exposed if it was found to be positive at any point during the sampling interval. Confidence intervals for seroprevalence were calculated in all cases using the “Wilson” method in the “binom.confint” function of the R package “binom” (Lawrence et al. 2001). Additionally, we used a generalized linear model (GLM) with binomial errors to study whether the prevalence was significantly different among virus genera. If the global GLM was significant, we conducted a Tukey post hoc test to identify what pairs of genera were significantly different (function “ghlt” from package “multcomp”; Hothorn et al. 2008).

We also studied whether virus prevalence varied as a function of host characteristics. We constructed a GLM with binomial errors, in which the seroprevalence (i.e., 0 and 1) was the response variable and predictors were host species, sex and age. The significance of each term was tested using the function “step” of the R package “stats” (Crawley 2007). This analysis was repeated to investigate variation in the prevalence of all arboviruses combined, as well as the prevalence of each arbovirus genus separately.

Finally, to investigate the changes in the seroprevalence patterns of arboviruses with host characteristics we con-

**Table 2.** Panel with the Genera and Species of Arbovirus Tested Using the Hemagglutination Inhibition and Neutralization Tests for Blood Samples Collected from Wild Animals During 2006–2014.

Genera	Species virus	Strain virus
<i>Alphavirus</i>	<i>Chikungunya virus</i> (CHIKV)	
	<i>Eastern equine encephalitis virus</i> (EEEV)	AN 7526
	<i>Mayaro virus</i> (MAYV)	AR20290
	<i>Mucambo virus</i> (MUCV)	AN10967
	<i>Western equine encephalitis virus</i> (WEEV)	AN70100
<i>Flavivirus</i>	<i>Bussuquara virus</i> (BSQV)	AN4116
	<i>Cacicapore virus</i> (CPCV)	AR327600
	<i>Dengue</i> serotypes 1, 2, 3 and 4 (DENV-1–DENV-4)	
	<i>Ilheus virus</i> (ILHV)	H7445
	<i>Rocio virus</i> (ROCV)	H34675
	<i>Saint Louis encephalitis virus</i> (SLEV)	AR23379
	<i>Utinga virus</i> (UTIV)	AN84785
	<i>West Nile virus</i> (WNV)	
	<i>Zika virus</i> (ZIKV)	SP0143
	<i>Yellow fever virus</i> (YFV)	H111
<i>Orthobunyavirus</i>	<i>Belem virus</i> (BELV)	AN141106
	<i>Caraparu virus</i> (CARV)	AN3994
	<i>Catu virus</i> (CATUV)	H151
	<i>Guaroa virus</i> (GROV)	H22063
	<i>Maguari virus</i> (MGAV)	AR7272
	<i>Oropouche virus</i> (OROV)	AN19991
	<i>Tacaiuma virus</i> (TCMV)	AN73
	<i>Icoaraci virus</i> (ICOV)	AN24262

structured a species composition matrix where rows were virus species and columns were host species. We extracted two derived axes from this analysis, which summarizes the variation in seroprevalence patterns (virus species) among hosts. We then used analyses of variance (ANOVA) to test whether there were differences among host species, sex and age in values of axes 1 and 2 from the ordination. Significance was determined simply by  $p$  values ( $p < 0.5$ ) of each term in the full model.

### Animal Use

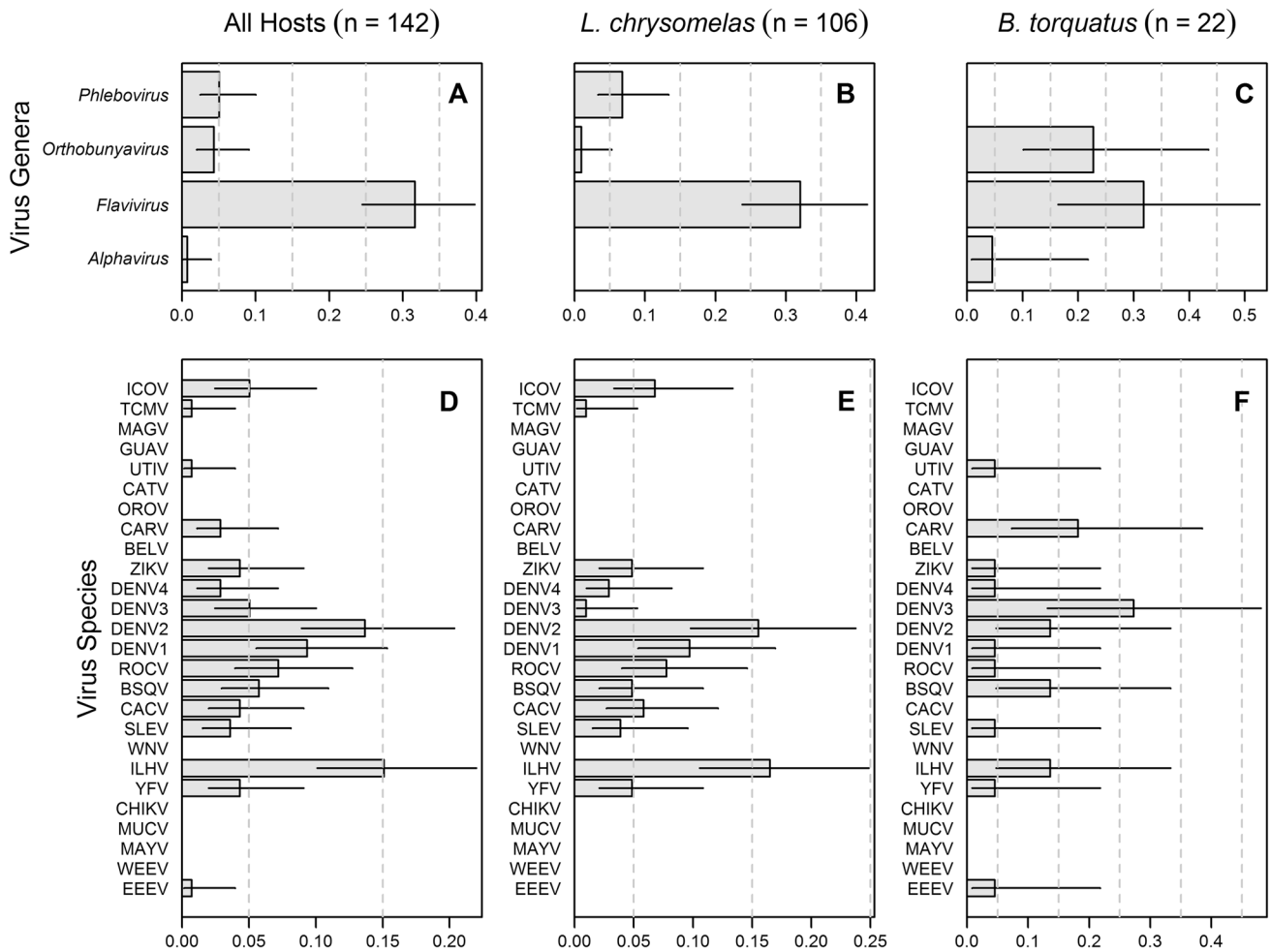
All of the procedures complied with legal requirements as set by the Environmental Services SISBIO permits No. 11885-1, 113/2007, 23069-2, 15025-1, 12787-1, 12787-3, 12787-4, 23457-4, 23457-5, 45513-1 and by the Animal Welfare Committee of Evandro Chagas Institute, under No. 26/2014 and 27/2014.

## RESULTS

### Hemagglutination Inhibition Test Results

From the 139 samples tested 35.2% had arbovirus antibodies (confidence interval—CI 27.8–43.49; Table 1), with the highest prevalence for the sloths *B. torquatus* (50%; CI 30.7–69.3) and *B. variegatus* (42.9%; CI 15.8–75.0) followed by *L. chrysomelas* (33%; CI 24.7–42.6) and *S. xanthosternos* (14.3%; CI 2.6–5.13) (Table 1).

Antibodies against *Flavivirus* were detected in all species tested. *Alphavirus* antibodies were detected only in *B. torquatus*, while *Phlebovirus* antibodies were detected solely in *L. chrysomelas* (Figs. 2, 3). Our statistical analyses indicated significant differences in prevalence levels among the three viral genera. When all hosts were combined and for *L. chrysomelas* alone, we found: (a) *Flavivirus* had significantly higher prevalence than all other virus genera ( $p$  values



**Figure 2.** Patterns of arbovirus prevalence at genus (a–c) and species (d–f) level for viruses found in non-human primates and sloths in Bahia, Brazil. Prevalences are presented for all host ( $n = 139$ ) captures (first column), but also separately for *L. chrysomelas* ( $n = 103$ ) and *B. torquatus* ( $n = 22$ ) (middle and last column, respectively). Lines show 95% intervals estimated using the Wilson method (see “Methods” section for more details).

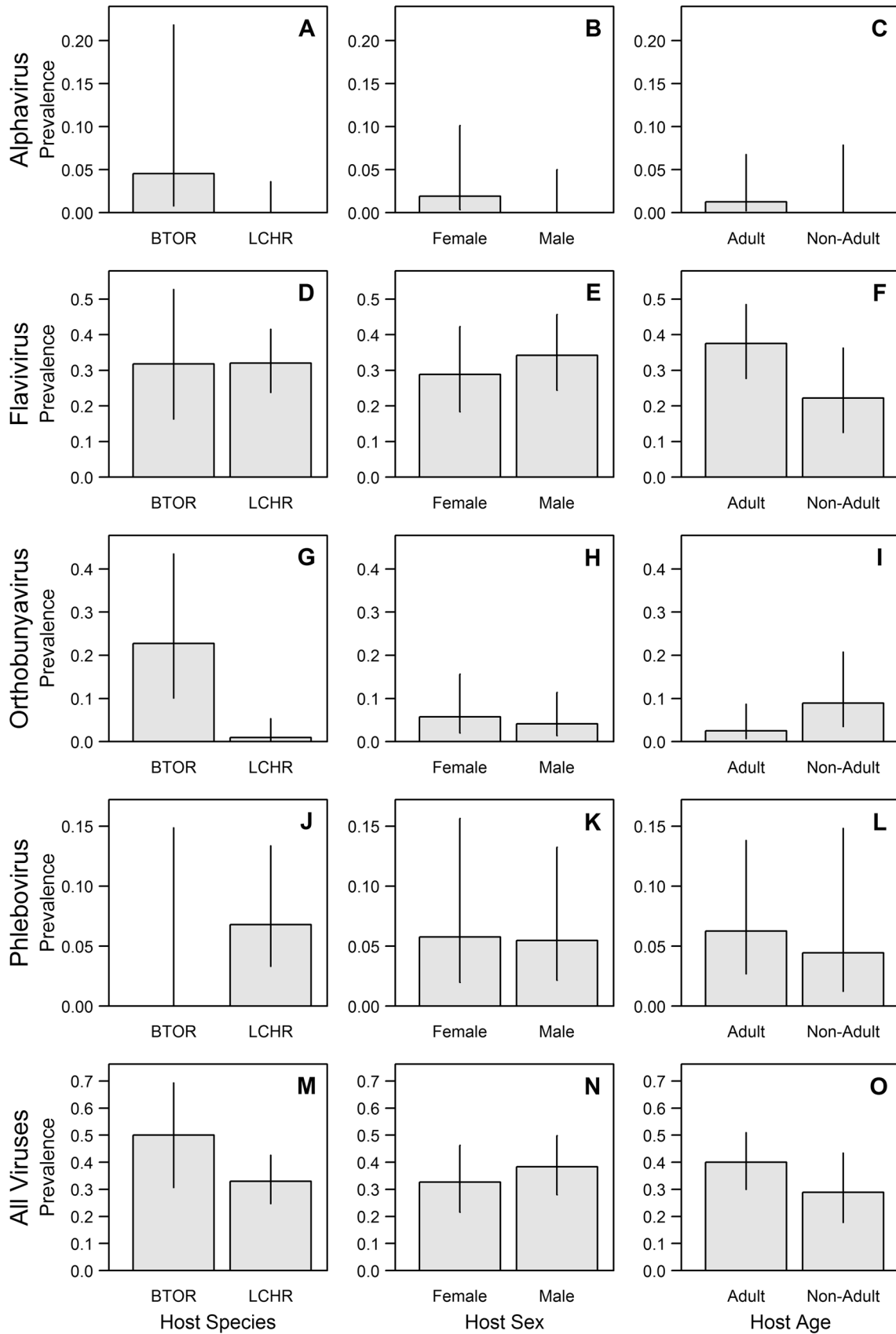
< 0.001), while (b) other virus genera did not differ from each other significantly ( $p$  values > 0.193) (Figs. 2, 3).

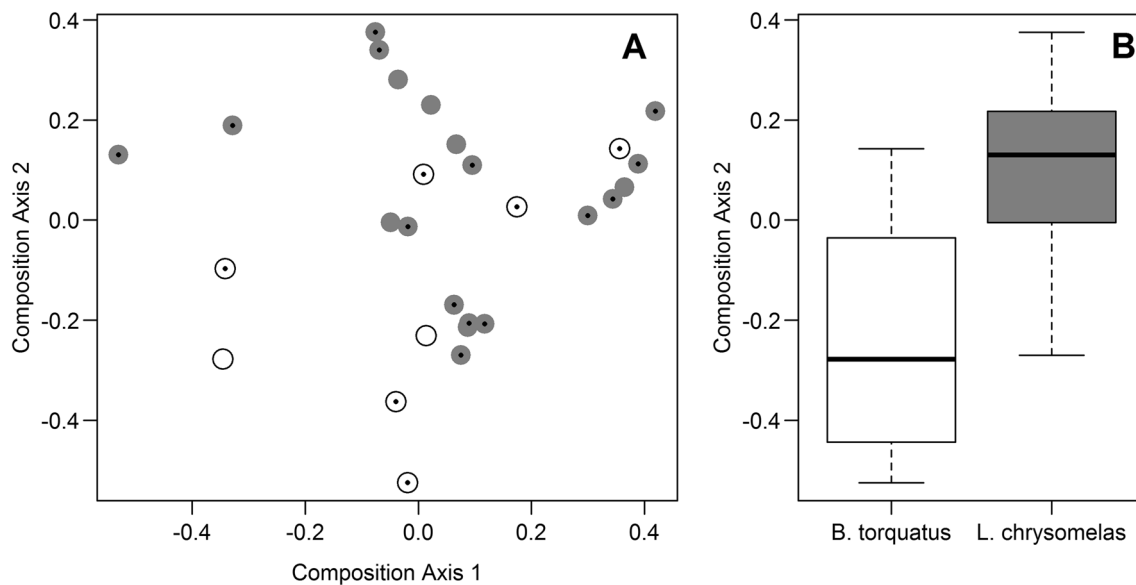
Samples from *L. chrysomelas* showed antibodies against the three viral genera, in which five individuals were seropositive for *Phlebovirus* and *Flavivirus* and one had antibodies against *Phlebovirus*, *Flavivirus* and *Orthobunyavirus*. One *B. torquatus* showed antibodies against *Alphavirus* and *Flavivirus*, and another animal had antibodies against *Flavivirus* and *Orthobunyavirus*.

When investigating how virus prevalence changes as a function of host characteristics, we found little evidence for the effects of species, sex or age on seroprevalence (Fig. 3). First, the species effect was retained by the stepwise selection based on AIC, albeit with a nonsignificant  $p$  value ( $p = 0.136$ ) in a model by itself. Secondly, species was marginally significant in a model with only main effects

**Figure 3.** Differences in prevalence among host species (BTOR—*B. torquatus*—and LCHR—*L. chrysomelas*; a, d, g, j, m), age level (c, f, i, l, o) and sex (b, e, h, k, n) for viruses found in wildlife in Bahia, Brazil, during 2006–2014.

( $p = 0.093$ , i.e., with age and sex, but without interactions). For *Orthobunyavirus*, species was more clearly significant in a model with only main effects ( $p = 0.003$ ), and *B. torquatus* had higher prevalence for this genus than the other species (Fig. 3). Finally, our analyses of distribution patterns by virus species showed clear results that different host species get exposure from different “communities” of viruses. In our ANOVA models, species was clearly and strongly significant when using the second axis ( $p < 0.001$ ). It is clear that both host species overlap, but also occupy different areas of the spaces created by the two





**Figure 4.** Differences in seroprevalence patterns (virus species composition) among host species. **a** Distribution of host individuals across two axes derived from non-metric multidimensional scaling ordination. Each axis summarizes variation in the virus species composition (seroprevalence) among hosts. Gray circles represent *L. chrysomelas*; white circles *B. torquatus*. Dots in the center of circles mark adult individuals. **b** There were significant differences in values of composition axis 2 between host species, indicating that, despite some overlap, species of virus found infecting wildlife vary among host species (see also Fig. 2).

ordination axes that reflect the patterns of seroprevalence by virus species (Fig. 4).

We found antibodies against 16 of the 26 viruses, with titers between 20 and 320 (Fig. 2; Table 3). The species *L. chrysomelas* and *B. torquatus* shared antibodies for ten of these viruses. The animals were more likely to be exposed to the ILHV (19.8%,  $n = 24$ ) with titers from 1:20 to 1:320, followed by DENV-2 (18.2%,  $n = 22$ ) with 1:20–1:80, DENV-1 (9.1%,  $n = 11$ ) with 1:20–1:80, and ROCV (8.26%,  $n = 10$ ) with 1:20–1:320 (Fig. 2; Table 3).

A total of 67.4% of positive samples were monotypic reactions (21 for *L. chrysomelas*, eight for *B. torquatus*, three for *B. variegatus* and one for *S. xanthosternos*). Considering only the monotypic reactions, the animals were exposed to 12 arboviruses: YFV, ILHV, ROCV, CPCV, BSQV, ICOV, EEEV, CARV, DENV-1, DENV-2, DENV-3 and UTIV (Table 4). Antibodies against DENV-2 ( $n = 7$ ), ICOV ( $n = 7$ ) and ILHV ( $n = 5$ ) were the most common monotypic reactions in non-human primates, while the CARV was for the sloths ( $n = 3$ ).

### Neutralization Test (NT)

Because of the small aliquot of blood collected, not all samples that showed heterotypic reactions were included in neutralization tests. However, for 63 samples tested,

seroneutralizing antibodies were confirmed in five lion tamarins exposed to ICOV, SLEV, BSQV and ILHV (Table 5).

### Longitudinal Study with Multiple Samples from Recaptures

Thirty-seven lion tamarins were caught multiple times (from 2 to 5) throughout the monitoring period. Of 27 positive *L. chrysomelas*, seven became seropositive for one of the following viruses: ROCV, CPCV, ILHV, ICOV, TCMV, YFV and DENV-2. The *B. torquatus* captured multiple times showed seroconversion to CARV, DENV-2, DENV-3, BSQV, ILHV, UTIV.

The sloth positive for EEEV had the same antibody titers (1:20) across time, starting with the first capture in 2006, followed by three additional captures in 2006 to 2008.

One of the *L. chrysomelas* was captured three times over the period 2008 through 2009. In the first two captures (December 2008 and June 2009), we found antibodies to *Flavivirus*. In a third sample from the same individual, collected in December 2009, in addition to *Flavivirus*, antibodies to *Phlebovirus* were also present. Another individual had antibodies against *Phlebovirus*, *Flavivirus* and *Orthobunyavirus* within a period of 6 months. For this animal, the first sample taken in December 2008 was neg-



**Table 3.** Number of Primates and Sloths Positive Samples with Antibodies According to the Titers by the Hemagglutination Inhibition Test, During 2006–2014 from the Atlantic Forest of Bahia State, Brazil.

Virus	Positive (N*)	Titers (HI)				
		1:20*	1:40*	1:80*	1:160*	1:320*
TCMV	1	1/0	–	–	–	–
ILHV	22/2	19/1	2/1	1	–	–
YFV	4/0	2/0	–	–	–	–
ICOV	6/3	4/1	0/1	1/1	–	0/1
UTIV	0/1	0/1	–	–	–	–
SLEV	7/0	2/0	4/0	1/0	–	–
CPCV	4/0	4/0	1/0	1/0	–	0/1
BSQV	5/1	3/0	1/1	1/0	–	–
ROCV	8/2	6/0	1/0	0/1	0/1	1/0
BELV	1/1	1/1	–	–	–	–
CARV	0/4	0/2	0/1	–	0/1	–
DENV-1	9/2	6/2	2/0	1/0	–	–
DENV-2	17/5	10/2	6/3	1/0	–	–
DENV-3	1/5	0/1	¼	–	–	–
DENV-4	4/2	–	4/2	–	–	–
ZIKV	4/0	4/0	2/0	–	–	–

\*The number before “/” corresponds to the number of primates positive samples, and the number after “/” corresponds to sloths positive samples.

ative for arboviruses. The second sample, collected in July 2009, had antibodies against the three genera.

## DISCUSSION

To the authors’ knowledge, this is the first serological survey for 26 arboviral strains in wild populations of these Neotropical species. The animals were classified as healthy based on physical examination findings, although some of the primates had mildly enlarged inguinal lymph nodes.

We hypothesized that the effect of sex and age on seroprevalence would only be associated with age, since both males and female are exposed to potentially infected mosquitoes at a similar rate in the study area. We expected a higher arbovirus prevalence in adults due to their longer life and having more time for exposure to infected mosquitoes (Morales et al. 2017). However, other factors such as changes in the environment might also affect the abundance of vectors over time. Further studies with the entomofauna and viral detection in mosquitoes in the area should be performed to confirm this idea.

The present study supports that arboviruses in sylvatic cycles may be maintained by more than one host species across taxa, as suggested elsewhere (Al-Shorbaji et al. 2016). Previous studies suggest that the low metabolism of sloth

**Table 4.** Monotypic Reactions According to the Host Species and Virus Species During an Arbovirus Serum Survey in Southern Bahia, Brazil, 2006–2014.

Virus genera	Virus species	Host species		
		<i>L. chrysomelas</i>	<i>S. xanthosternos</i>	<i>B. torquatus</i>
<i>Alphavirus</i>	EEEV	–	–	1
<i>Flavivirus</i>	ILHV	5	–	–
	ROCV	1	1	–
	CPCV	1	–	–
	DENV-1	1	–	–
	DENV-2	7	–	–
	DENV-3	–	–	2
	YFV	2	–	–
<i>Orthobunyavirus</i>	CARV	–	–	3
	UTIV	–	–	1
	TCMV	1	–	–
<i>Phlebovirus</i>	ICOV	7	–	–

**Table 5.** Neutralization Test Results According to the Host Species, Virus Genera and Virus Species During an Arbovirus Surveillance During 2006–2014 in Southern Bahia, Brazil.

ID	Host species	Virus genera	Virus species	LNI
GHLT5194	<i>L. chrysomelas</i>	<i>Phlebovirus</i>	<i>Icoaraci</i>	2.4
GHLT5098	<i>L. chrysomelas</i>	<i>Flavivirus</i>	<i>Saint Louis</i>	2.9
GHLT35069	<i>L. chrysomelas</i>	<i>Flavivirus</i>	<i>Bussuquara</i>	1.9
GHLT5120	<i>L. chrysomelas</i>	<i>Flavivirus</i>	<i>Ilhéus</i>	1.7
GHLT5194	<i>L. chrysomelas</i>	<i>Flavivirus</i>	<i>Ilhéus</i>	1.7

LNI logarithmic neutralization index.

species may result in long-lasting viremia for many viruses, increasing transmission capabilities (Seymour et al. 1983a, b). Other species of sloths had high antibody prevalence against the *Alphavirus* MAYV and VEEV (de Thoisy et al. 2004), but we show for the first time *B. torquatus* exposure to EEEV. Sloths are also hosts to some Orthobunyaviruses, including *Oropouche* and *Utinga virus*, which are often shared between livestock and other domesticated species (Seymour et al. 1983b; Figueiredo 1999; Medlin et al. 2016). In this study, we describe antibodies in sloths against CARV for the first time. The CARV has previously been isolated from mosquitoes (*Culex sacchettae*), rodents (*Coendou* spp., *Akodon* spp.), marsupials (*Didelphis marsupialis*) and bats (*Artibeus* spp.) (Figueiredo et al. 1998; Figueiredo 1999). Indeed, an arbovirus with a broad host range might have selective advantages over one capable of causing productive viremia in only a single host and vector (Kading et al. 2013; Caron et al. 2015).

The predominance of the *Flavivirus* genus in Neotropical primates was expected, as they are known to host several strains of this genus (Almeida et al. 2012; Batista et al. 2013; Almeida et al. 2014). However, we expected lower prevalence and circulation in sloths, as few *Flavivirus* had been detected in *Choloepus hoffmanni* and *B. variegatus* (Medlin et al. 2016). The higher prevalence of sloths and non-human primates with antibodies against *Flavivirus* may reflect: (1) a silent virus circulation, on which the virus is maintained in the environment through a low viral load in the vectors enough to expose hosts, but not enough to produce a viremia capable of causing clinical signs or mortality (Teixeira et al. 2002); (2) abundance of vectors, so that there are abundant mosquitoes for the *Flavivirus* present in the area so that infected mosquitoes circulating are sufficient to increase the transmission of the virus between hosts (Teixeira et al. 2002); and/or (3) a wide spec-

trum of vertebrate hosts that are present in the Atlantic Forest on which the arthropod vectors may feed (Al-Shorbaji et al. 2016).

In addition, this long-term monitoring study provided data suggesting viral circulation of *Alphavirus* and *Orthobunyavirus* in sylvatic areas in Bahia. The viral circulation has been confirmed by seroconversion in samples collected from the same animals over time, and added to the presence of antibody titers in juveniles supports that arbovirus infections may last 6 months.

The heterotypic reactions found in this study might be explained by the higher sensitivity than specificity present in the HI test (Zarnke et al. 1983; Kading et al. 2013; Laroque et al. 2014). The HI test is often used in serological surveys because it can detect IgG antibodies over a long period after natural infection. Because of this, the HI test is an ideal method for surveys in situ (Batista et al. 2012). Also, the HI test allows individual samples to be screened against 26 virus antigens, increasing the chance of finding monotypic responses or high differences in titers, and therefore should be enough to identify antibodies at the species level (Tauro et al. 2012; Casseb et al. 2015).

The monotypic reactions suggest that the wild animals were exposed naturally to at least twelve arboviruses. Adding the results from the NT, the animals were exposed to thirteen arboviruses. Most of these viruses are perpetuated in transmission cycles independent of human hosts (Figueiredo 2015), highlighting the complexity of arbovirus transmission and providing further insight into how arboviruses may be maintained and transmitted in a sylvatic cycle (Kading et al. 2013). We showed that ILHV continues to be widely distributed in Bahia Atlantic Forest, which can lead to outbreaks in livestock and humans. ILHV was discovered in Ilhéus city in 1947 and causes similar symptoms as dengue in humans and presently has been

detected during human surveillance surveys in rural areas in Brazil (Azevedo et al. 2010). The wild birds are the main reservoirs (Pereira et al. 2001) for ILHV, although antibodies and viral isolation also occur in non-human primates, including *Sapajus libidinosus* and *Alouatta caraya* (Laroque et al. 2014; Morales et al. 2017).

Our study also suggests that the YFV is circulating in a sylvatic cycle in this part of the Atlantic Forest, given that two *L. chrysomelas* had YFV antibodies on the same farms (Almada and Bonfim) where four sick Wied's marmosets (*Callithrix kuhlii*) had YFV isolated in the 1940s (Vaz 2005). Furthermore, we showed elevated prevalence for anti-SLEV antibodies when compared to the primates *Sapajus cay* (15.4%; 4/26), *S. nigritus* (6.3%; 4/64) and *A. caraya* (2.3%; 1/43) captured in Southern Brazil (Svoboda et al. 2014). In the state of Mato Grosso do Sul, the CPCV was found in one free-living primate with a titer of 1:20 (Batista et al. 2013), which is similar to that found in the present study. Antibodies against TCMV have been found only in *Cebus apella* originally from Amazonian Forest (Figueiredo 1999). The UTIV-neutralizing antibodies had not been found in *B. torquatus* prior to this study.

Our results also highlight the possibility of viral circulation of dengue virus serotypes 1, 2 and 3 in *B. torquatus*, *B. variegatus* and *L. chrysomelas*. In our study, sampling sites included animal captures inside of agroforestry and agricultural areas, where both human workers and wild animals coexist (Cassano et al. 2011; Oliveira et al. 2011; Canale et al. 2013). This proximity could explain the possibility of transmission and circulation of the DENV to wildlife, although further studies (e.g., viral isolation in wild animals) are necessary to confirm a sylvatic cycle of dengue. Although wild mammals have no confirmed role in the cycle of dengue in South America, serological studies have suggested a possible secondary amplification cycle involving other mammals (de Thoisy et al. 2009; Hanley et al. 2013). In French Guiana, DENV-2 seroneutralizing antibodies were found in *Dasyopus* spp., *Coendou* spp., *Metachirus nudicaudatus*, *Dasyprocta leporine* and *Mazama* spp. (de Thoisy et al. 2004). A few years later, de Thoisy et al. (2009) identified viral RNA in many species of South American bats, rodents and marsupials and provided the first C/prM sequences of strains of DENV-1–DENV-4 circulating in wildlife communities. They may act as an epidemic dead-end host or maintain the virus during inter-epidemic periods or even in virus amplification, with transmission by either populations of other vectors, such as *Aedes albopictus* and *Sabethes* spp. (Caron et al. 2015;

Figueiredo et al. 2010). The ecological dynamics of the mammalian species in relation to the virus should be explored. Extensive research on vectors is required, as *Haemagogus leucocelaenus* infected with DENV-1 has been described in Bahia by RT-heminested-PCR tests (Figueiredo et al. 2010). Finally, in Ilhéus and Una municipalities, all four serotypes are present, and the disease is endemic with sporadic outbreaks (Barreto et al. 2008; Melo et al. 2010).

### Implications for Humans and Wildlife Health

The active surveillance of pathogens in wild animals has the potential to assist public health. This type of epidemiological surveillance may permit early detection of an outbreak in humans and may enable quick establishment of a transmission-blocking vaccination or control of vectors in rural communities (Lima et al. 2010; Tranquilin et al. 2013). The results found in this study were presented to the public and to Federal and State Health Services. This led to arbovirus surveillance in the rural villages nearby where the animals were sampled.

Arbovirus infection could cause wildlife population declines and reduce animal survival, as the ongoing yellow fever outbreaks demonstrate (de Almeida et al. 2012; Tranquilin et al. 2013; Brasil 2017). The exposure to pathogens and potential vectors, in addition to other threats (e.g., deforestation, illegal wildlife trade and introduction of exotic species) may compromise the survival of host populations (Engering et al. 2013; Jansen et al. 2015; Deem 2016; Vorou 2016).

### CONCLUSIONS

The present long-term sera survey suggests that arboviruses are circulating in sloths and primates in the Southern Bahia Atlantic Forest, Brazil, and emphasizes the importance for including sloths in arbovirus surveys. The general low titer of antibodies and the absence of clinical signs in non-human primates and sloths highlight the necessity of further studies to evaluate the role of these species as accidental hosts, bridge hosts (Caron et al. 2015) or competent hosts of arboviruses, and the possibility of isolating the virus and detecting viral sequences by molecular tools.

It is possible that some arboviruses remain silent in the forest, including the dengue virus serotypes 1, 2 and 3. The question of whether mammals maintain the virus in

enzootic cycles and can play a role in its re-emergence in human populations remains to be answered. Considering that areas investigated here harbor some of the few protected populations of threatened wildlife species in Brazil, we recommend long-term monitoring of small populations of these threatened species living close to human villages and households, as well as in more pristine areas. We also suggest improving the long-term entomological studies, which will add to long-term arbovirus surveillance of human populations in these villages and to wildlife health.

The recent invasion of new arboviruses into Brazil, and the ongoing yellow fever epidemics challenge physicians, health professionals, biologists, ecologists and veterinarians. There is a need to intensify active and ongoing investigations on the pathogenesis, etiological agents, laboratory diagnostic capabilities, and wildlife, environmental and social factors that may be associated with arbovirus outbreaks in human populations.

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## COMPLIANCE WITH ETHICAL STANDARDS

**CONFLICT OF INTEREST** The authors declare that they have no conflict of interest.

**HUMAN AND ANIMAL RIGHTS** All applicable institutional and/or national guidelines for the care and use of animals were followed.

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