Opsoclonus–Myoclonus–Ataxia Syndrome associated with Chikungunya and Dengue Virus coinfection

Article *in* International Journal of Infectious Diseases · August 2018

DOI: 10.1016/j.ijid.2018.07.019

10 authors, including:

Mateus do Rosario

8 PUBLICATIONS **276** CITATIONS

SEE PROFILE

Marta Giovanetti

Fundação Oswaldo Cruz

86 PUBLICATIONS **1,070** CITATIONS

SEE PROFILE

Nuno Rodrigues Faria

University of Oxford

149 PUBLICATIONS **2,957** CITATIONS

SEE PROFILE

Clayton Pereira Silva de Lima

Instituto Evandro Chagas

47 PUBLICATIONS **689** CITATIONS

SEE PROFILE

EcoHealth
https://doi.org/10.1007/s10393-018-1361-2

EcoHealth

Original Contribution

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

L. S. Catenacci,[1,2,3,8] M. Ferreira,[2] L. C. Martins,[2] K. M. De Vleeschouwer,[3,4] C. R. Cassano,[5] L. C. Oliveira,[4,6] G. Canale,[7] S. L. Deem,[8,10] J. S. Tello,[9] P. Parker,[10] P. F. C. Vasconcelos,[2] and E. S. Travassos da Rosa[2]

[1]*Campus Professora Cinobelina Elvas, Federal University of Piaui State, Rod municipal Bom Jesus Viana, BR135, km 1, Bom Jesus, PI 64900-000, Brazil*
[2]*Virology Graduate Program, Evandro Chagas Institute, Ananindeua, PA 67030-000, Brazil*
[3]*Centre for Research and Conservation, Royal Zoological Society of Antwerp, 2018 Antwerp, Belgium*
[4]*Bicho do Mato Instituto de Pesquisa, Belo Horizonte, MG 30360-082, Brazil*
[5]*State University of Santa Cruz, Ilhéus, BA 45662-900, Brazil*
[6]*Faculdade de Formação de Professores, State University of Rio de Janeiro, Rio de Janeiro, RJ 24435-005, Brazil*
[7]*ICNHS/NEBAM, Federal University of Mato Grosso, Campus Sinop, Cuiabá, MT 78557-000, Brazil*
[8]*Saint Louis Zoo Institute for Conservation Medicine, Saint Louis, MO 63110*
[9]*Center for Conservation and Sustainable Development, Missouri Botanical Garden, St. Louis, MO 63110*
[10]*University of Missouri-St Louis, St. Louis, MO 63105*

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

Correspondence to: L. S. Catenacci, e-mail: li_vet@yahoo.com.br

Published online: 16 August 2018

Introduction

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

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

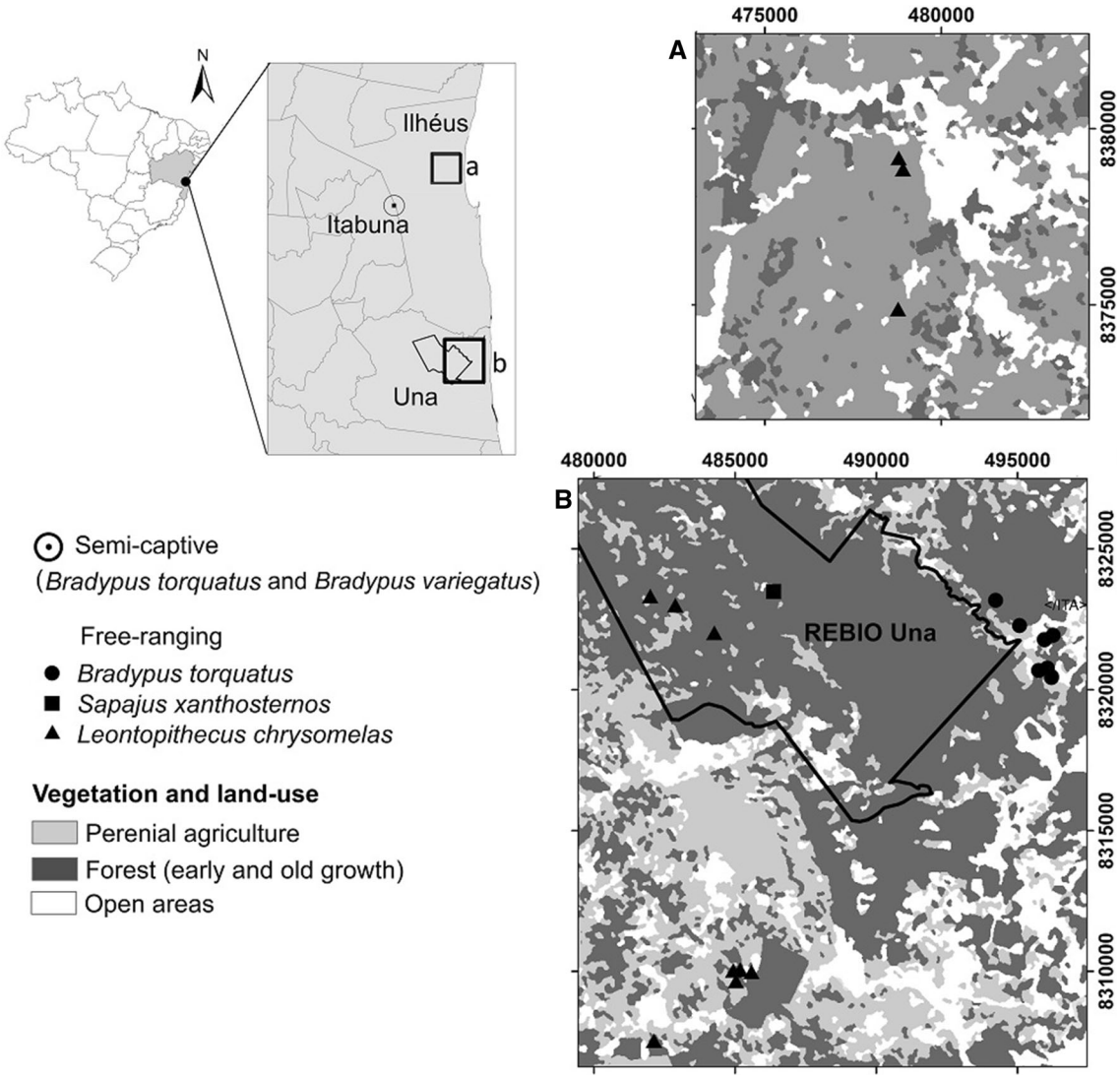


Figure 1. Capture sites of free-ranging and semi-captive species described in this study, in Ilhéus (**a**) and Una city (**b**), Bahia, Brazil.

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							
	Bradypus torquatus		*Bradypus variegatus*		*Leontopithecus chrysomelas*		*Sapajus xanthosternos*	
	N[a]	Prevalence (CI)[b]	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)

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

structed 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

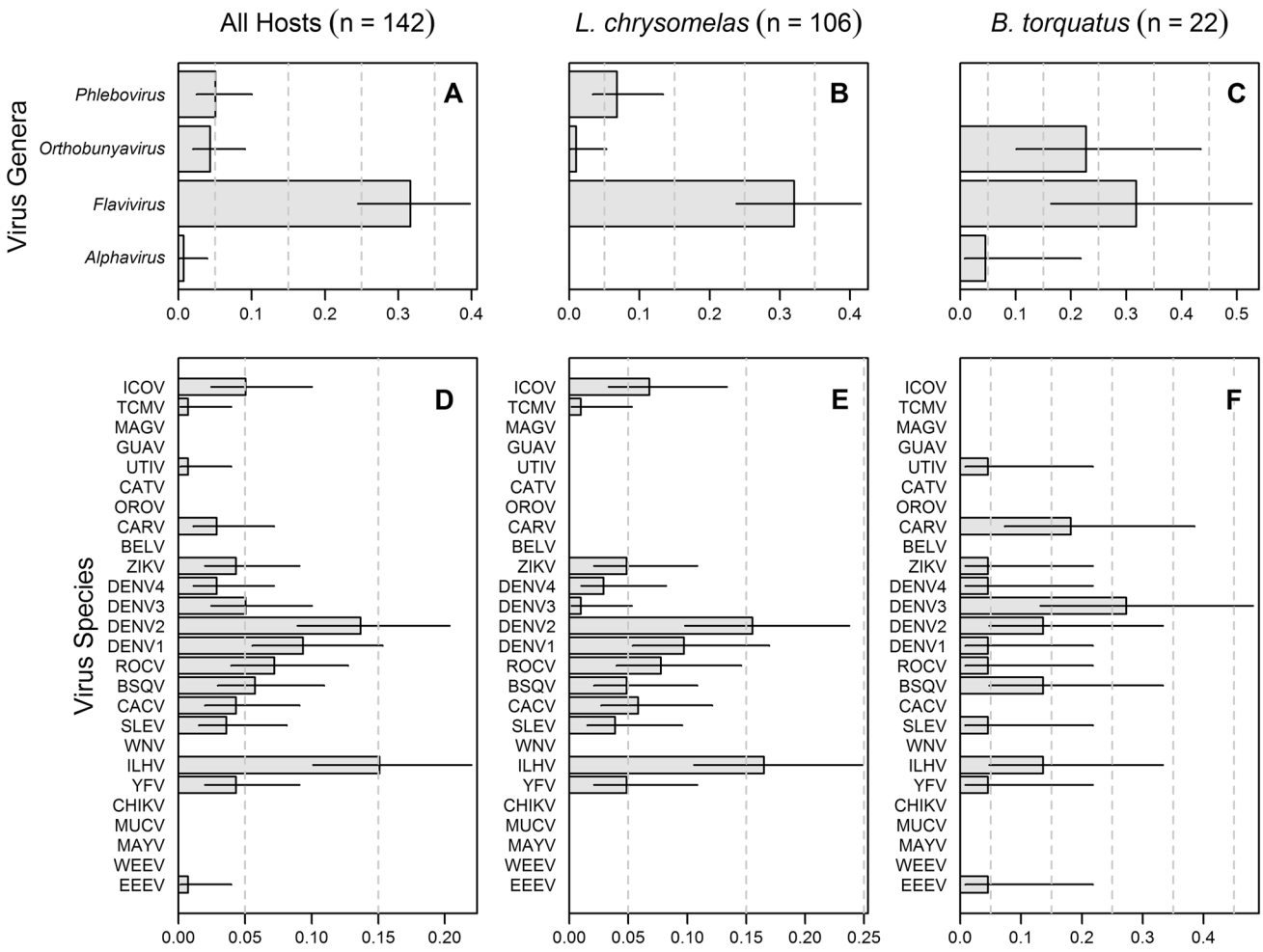


Figure 2. Patterns of arbovirus prevalence at genus (**a**–**c**) and species (**d**–**f**) level for viruses found in non-human primates and sloths in Bahia, Brazil. Prevalences are presented for all host (n = 139) captures (first column), but also separately for *L. chrysomelas* (n = 103) and *B. torquatus*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

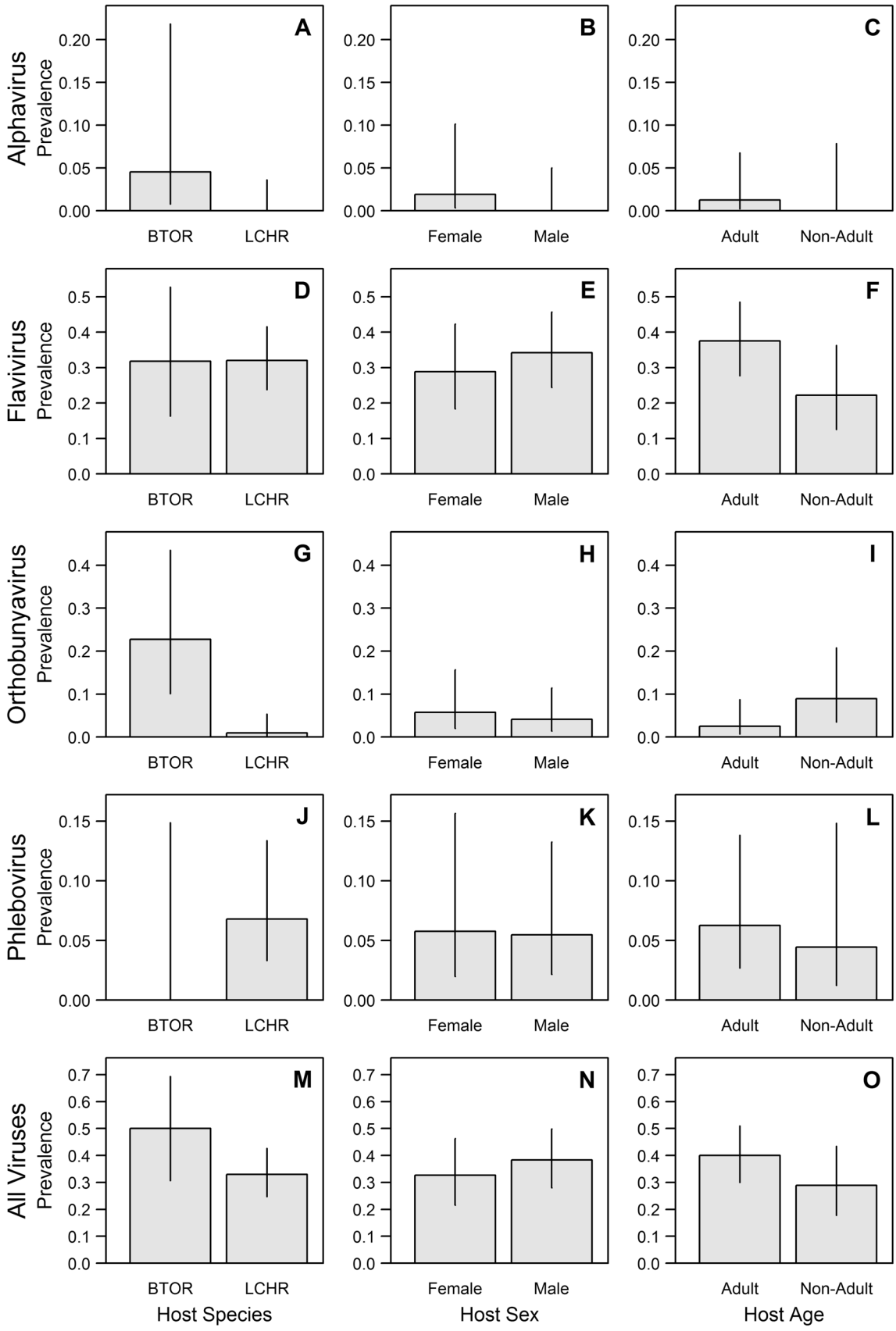
$<$ 0.001), while (b) other virus genera did not differ from each other significantly (p values $>$ 0.193) (Figs. 2, 3).

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


Alphavirus Prevalence
Flavivirus Prevalence
Orthobunyavirus Prevalence
Phlebovirus Prevalence
All Viruses Prevalence
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
BTOR
LCHR
Female
Male
Adult
Non-Adult
Host Species
Host Sex
Host Age

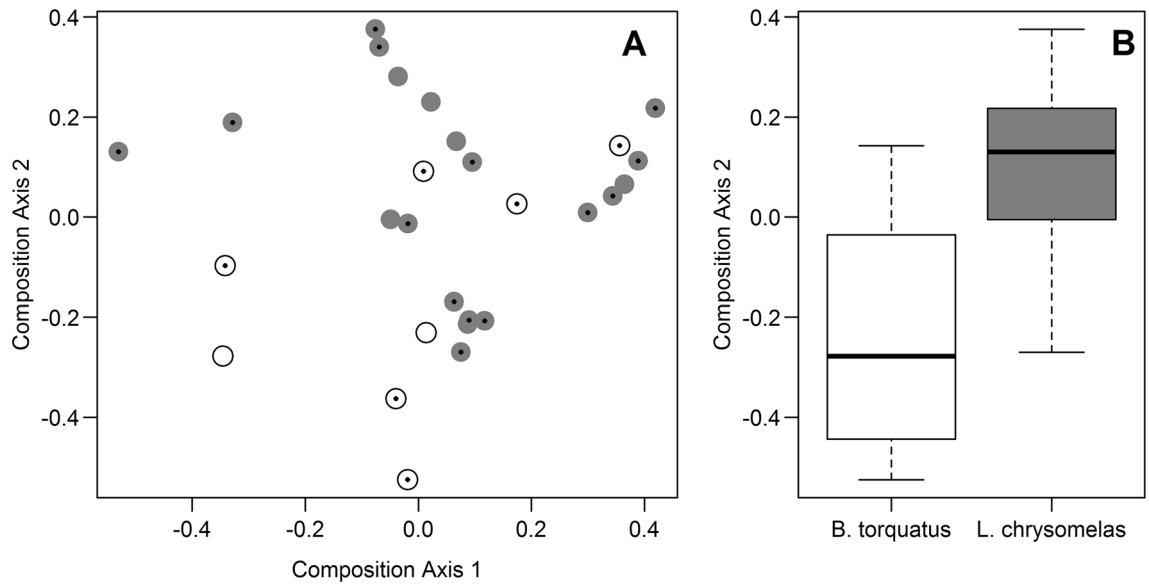


Figure 4. Differences in seroprevalence patterns (virus species composition) among host species. **a** Distribution of host individuals across two axes derived from non-metric multidimensional scaling ordination. Each axis summarizes variation in the virus species composition (seroprevalence) among hosts. 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		
		L. chrysomelas	*S. xanthosternos*	*B. torquatus*
Alphavirus	EEEV	–	–	1
Flavivirus	ILHV	5	–	–
	ROCV	1	1	–
	CPCV	1	–	–
	DENV-1	1	–	–
	DENV-2	7	–	–
	DENV-3	–	–	2
	YFV	2	–	–
Orthobunyavirus	CARV	–	–	3
	UTIV	–	–	1
	TCMV	1	–	–
Phlebovirus	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	*L. chrysomelas*	*Phlebovirus*	*Icoaraci*	2.4
GHLT5098	*L. chrysomelas*	*Flavivirus*	*Saint Louis*	2.9
GHLT35069	*L. chrysomelas*	*Flavivirus*	*Bussuquara*	1.9
GHLT5120	*L. chrysomelas*	*Flavivirus*	*Ilhéus*	1.7
GHLT5194	*L. chrysomelas*	*Flavivirus*	*Ilhéus*	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; Figueiredo1999). 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 spectrum 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 *Dasypus* 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 interepidemic 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.

Acknowledgements

The authors thank the important contributions of the Municipal and Bahia State Health Department and the colleagues from the Evandro Chagas Institute for assistance with the laboratory diagnostics and logistical support in the field. Additional thanks go to Project BioBrasil/Centre for Research and Conservation, ICMBio and the Bicho-da mata NGO for their logistical support and ICMBio for permits to conduct research in the Una Biological Reserve and Una Wildlife Refuge. We also thank the owners of the private areas (Almada, Santa Rita, Ribeiro, Ozawa, Manoel Rosa and São José, Ecoparque de Una, Bonfim) and the Zoobotanical Reserve Rehabilitation Center for permit to conduct the research. We also thank the sponsoring institutions that made this project possible: Saint Louis Zoo WildCare Institute (USA), The Wild Animal Fund, from the American Association of Zoological Veterinarians (USA), CNPq (Brazil), the Center for Research and Conservation of the Royal Zoological Society of Antwerp (Belgium), Lion Tamarins of Brazil Fund, National Lottery of Belgium, Primate Action Fund, Zoological Society of London, Conservação Internacional, Fundação o Boticário de Proteção a Natureza. The Flemish Ministry of Science (Belgium) provided structural support to the Center for Research and Conservation of the Royal Zoological Society of Antwerp.

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.

References

Alger K, Caldas M (1994) The declining cocoa economy and the Atlantic Forest of Southern Bahia, Brazil: Conservation attitudes of cocoa planters. *Environmentalist* 14:107–119. https://doi.org/10.1007/BF01901304

Almeida MAB, Cardoso JC, dos Santos E, da Fonseca DF, Cruz LL, Faraco FJC, Bercini MA, Vettorello KC, Porto MA, Mohrdieck R, Ranieri TMS, Schermann MT, Sperb AF, Paz FZ, Nunes ZMA, Romano APM, Costa ZG, Gomes SL, Flannery B (2014) Surveillance for yellow fever virus in non-human primates in Southern Brazil, 2001–2011: a tool for prioritizing human populations for vaccination. *PLOS Neglected Tropical Diseases*. https://doi.org/10.1371/journal.pntd.0002741

Almeida MAB, Dos Santos E, da Cruz Cardoso J, da Fonseca DF, Noll CA, Silveira VR, Maeda AY, de Souza RP, Kanamura C, Brasil RA (2012) Yellow fever outbreak affecting *Alouatta* populations in southern Brazil (Rio Grande do Sul State), 2008–2009. *American Journal of Primatology* 74:68–76. https://doi.org/10.1002/ajp.21010

Al-Shorbaji F, Roche B, Gozlan R, Britton R, Andreou D (2016) The consequences of reservoir host eradication on disease epidemiology in animal communities. *Emerging Microbes & Infections* 5:e46. https://doi.org/10.1038/emi.2016.46

Althouse BM, Vasilakis N, Sall AA, Diallo M, Weaver SC, Hanley KA (2016) Potential for Zika virus to establish a sylvatic transmission cycle in the Americas. *PLOS Neglected Tropical Diseases* 10:e0005055. https://doi.org/10.1371/journal.pntd.0005055

Azevedo RdoSdaS, Barros VLRdeS, Martins LC, Cruz ACR, Rodrigues SG, Vasconcelos PFdaC (2010) Estudo experimental sobre a patogenicidade do Vírus Ilhéus em hamsters dourados (*Mesocricetus auratus*). *Revista Pan-Amazônica de Saúde* 1:73–80. https://doi.org/10.5123/S2176-62232010000100011

Barreto FR, Teixeira MG, Costa MdaCN, Carvalho MS, Barreto ML (2008) Spread pattern of the first dengue epidemic in the city of Salvador, Brazil. *BMC Public Health* 8:51. https://doi.org/10.1186/1471-2458-8-51

Batista PM, Andreotti R, Almeida PS, Marques AC, Rodrigues SG, Chiang JO, Vasconcelos PFdaC, Batista PM, Andreotti R, Almeida PS, Marques AC, Rodrigues SG, Chiang JO, Vasconcelos PFC (2013) Detection of arboviruses of public health interest in free-living New World primates (*Sapajus* spp.; *Alouatta caraya*) captured in Mato Grosso do Sul, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical* 46:684–690. https://doi.org/10.1590/0037-8682-0181-2013

Batista PM, Andreotti R, Chiang JO, Ferreira MS, Vasconcelos PFdaC (2012) Seroepidemiological monitoring in sentinel animals and vectors as part of arbovirus surveillance in the state of Mato Grosso do Sul, Brazil. *Revista da Sociedade Brasileira de*

Medicina Tropical 45:168–173. https://doi.org/10.1590/S0037-86822012000200006

Beaty BJ, Calisher CH, Shope RE (1989) Arboviruses. Diagn Proced Viral Ricketts Chlamydial Infect 797–855

Brazil (2017) Febre Amarela—Informação e orientação. In: Portal Saúde—Minist. Saúde—www.saude.gov.br. http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/leia-mais-o-ministerio/619-secretaria-svs/l1-svs/27300-febre-amarela-informacao-e-orientacao. Accessed 3 March 2017

Bueno MG, Martinez N, Abdalla L, Duarte dos Santos CN, Chame M (2016) Animals in the Zika virus life cycle: what to expect from Megadiverse Latin American Countries. *PLOS Neglected Tropical Diseases*. https://doi.org/10.1371/journal.pntd.0005073

Canale GR, Kierulff MCM, Chivers DJ (2013) A critically endangered Capuchin Monkey (*Sapajus xanthosternos*) living in a highly fragmented hotspot. In: *Primates in fragments*, Marsh LK, Chapman CA (editors), New York: Springer, pp 299–311

Caron A, Cappelle J, Cumming GS, de Garine-Wichatitsky M, Gaidet N (2015) Bridge hosts, a missing link for disease ecology in multi-host systems. *Veterinary Research*. https://doi.org/10.1186/s13567-015-0217-9

Cassano CR, Kierulff MCM, Chiarello AG (2011) The cacao agroforests of the Brazilian Atlantic forest as habitat for the endangered maned sloth Bradypus torquatus. *Mamm Biol - Z Für Säugetierkd* 76:243–250. https://doi.org/10.1016/j.mambio.2010.06.008

Casseb AR, Silva SP, Casseb LMN, Chiang JO, Martins LC, Vasconcelos PFC (2015) Prevalência de anticorpos contra arbovírus da família Bunyaviridae em búfalos de água Ciênc. *Anim Bras* 16:428–436. https://doi.org/10.1590/1089-6891v16i327208

Catenacci LS, Pessoa MS, Nogueira-Filho SLG, Vleeschouwer KMD (2016) Diet and feeding behavior of *Leontopithecus chrysomelas* (Callitrichidae) in degraded areas of the atlantic forest of South-Bahia, Brazil. *International Journal of Primatology* 37:136–157. https://doi.org/10.1007/s10764-016-9889-x

Clarke DH, Casals J (1958) Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. The American Journal of Tropical Medicine and Hygiene 561–573

Crawley MJ (2007) The R Book. Second Edition. Imperial College London at Silwood Park, UK http://www.bio.ic.ac.uk/research/mjcraw/therbook/index.htm. A John Wiley & Sons, Ltd., 950 pp.

Daszak P, Tabor GM, Kilpatrick AM, Epstein J, Plowright R (2004) Conservation medicine and a new agenda for emerging diseases. *Annals of the New York Academy of Sciences* 1026:1–11. https://doi.org/10.1196/annals.1307.001

de Thoisy B, Dussart P, Kazanji M (2004) Wild terrestrial rainforest mammals as potential reservoirs for flaviviruses (yellow fever, dengue 2 and St Louis encephalitis viruses) in French Guiana. *Transactions of The Royal Society of Tropical Medicine and Hygiene* 98:409–412. https://doi.org/10.1016/j.trstmh.2003.12.003

de Thoisy B, Lacoste V, Germain A, Muñoz-Jordán J, Colón C, Mauffrey J-F, Delaval M, Catzeflis F, Kazanji M, Matheus S, Dussart P, Morvan J, Setién AA, Deparis X, Lavergne A (2009) Dengue infection in neotropical forest mammals. *Vector Borne Zoonotic Dis Larchmt N* 9:157–170. https://doi.org/10.1089/vbz.2007.0280

Deem SL (2016) Conservation medicine: a solution-based approach for saving nonhuman primates. In: *Ethnoprimatology*, Waller MT (editors), Springer International Publishing, pp 63–76

Engering A, Hogerwerf L, Slingenbergh J (2013) Pathogen–host–environment interplay and disease emergence. *Emerging Microbes & Infections* 2:e5. https://doi.org/10.1038/emi.2013.5

Faria NR, Azevedo RdoSda, Kraemer MUG, Souza R, Cunha MS, Hill SC, Thézé J, Bonsall MB, Bowden TA, Rissanen I, Rocco IM, Nogueira JS, Maeda AY, Vasami FGdaS, Macedo FLdeL, Suzuki A, Rodrigues SG, Cruz ACR, Nunes BT, Medeiros DBdeA, Rodrigues DSG, Nunes Queiroz AL, da Silva EVP, Henriques DF, Travassos da Rosa ES, de Oliveira CS, Martins LC, Vasconcelos HB, Casseb LMN, Simith DdeB, Messina JP, Abade L, Lourenço J, Carlos Junior Alcantara L, de Lima MM, Giovanetti M, Hay SI, de Oliveira RS, Lemos PdaS, de Oliveira LF, de Lima CPS, da Silva SP, de Vasconcelos JM, Franco L, Cardoso JF, Vianez-Júnior JLdaSG, Mir D, Bello G, Delatorre E, Khan K, Creatore M, Coelho GE, de Oliveira WK, Tesh R, Pybus OG, Nunes MRT, Vasconcelos PFC (2016) Zika virus in the Americas: early epidemiological and genetic findings. *Science* 352:345–349. https://doi.org/10.1126/science.aaf5036

Ferreira MS, de Castro PHG, Silva GA, Casseb SMM, Dias Júnior AG, Rodrigues SG, Azevedo RdoSdaS, Silva MFC, Zauli DAG, Araújo MSS, Béla SR, Teixeira-Carvalho A, Martins-Filho OA, Vasconcelos PFC (2014) *Callithrix penicillata:* A feasible experimental model for dengue virus infection. *Immunology Letters* 158:126–133. https://doi.org/10.1016/j.imlet.2013.12.008

Figueiredo MLG, Gomes CA, Amarilla AA, Leandro SA, Orrico SA, Araujo RF, Castro JSM, Durigon EL, Aquino VH, Figueiredo LTM (2010) Mosquitoes infected with dengue viruses in Brazil. *Journal of Virology* 7:152. https://doi.org/10.1186/1743-422X-7-152

Figueiredo LTM (1999) Vírus brasileiros da família Bunyaviridae. *Med Ribeirão Preto* 32:154–158. https://doi.org/10.11606/issn.2176-7262.v32i2p154-158

Figueiredo LTM, Batista WC, Kashima S, Nassar ES (1998) Identification of Brazilian Flaviviruses by simplified reverse transcription-polymerase chain reaction method using *Flavivirus* universal primers. *Journal of Tropical Medicine and Hygiene* 59(3):357–362

Figueiredo LTM (2015) The recent arbovirus disease epidemic in Brazil. *Revista da Sociedade Brasileira de Medicina* 48:233–234. https://doi.org/10.1590/0037-8682-0179-2015

Gilmore DP, Da Costa CP, Duarte DP (2001) Sloth biology: an update on their physiological ecology, behavior and role as vectors of arthropods and arboviruses. *Braz J Med Biol Res Rev Bras Pesqui Medicas E Biol* 34:9–25

Gyawali N, Bradbury RS, Taylor-Robinson AW (2016) The global spread of Zika virus: is public and media concern justified in regions currently unaffected? *Infectious Diseases of Poverty* 5:37. https://doi.org/10.1186/s40249-016-0132-y

Hanley KA, Monath TP, Weaver SC, Rossi SL, Richman RL, Vasilakis N (2013) Fever versus Fever: the role of host and vector susceptibility and interspecific competition in shaping the current and future distributions of the sylvatic cycles of dengue virus and yellow fever virus. *Infection, Genetics and Evolution*. https://doi.org/10.1016/j.meegid.2013.03.008

Heymann DL, Hodgson A, Sall AA, Freedman DO, Staples JE, Althabe F, Baruah K, Mahmud G, Kandun N, Vasconcelos PFC, Bino S, Menon KU (2016) Zika virus and microcephaly: why is this situation a PHEIC? *Lancet (London, England)* 387:719–721. https://doi.org/10.1016/S0140-6736(16)00320-2

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biometrical Journal* 50:346–363

IUCN. International Union for Conservation of Nature (2017) The IUCN Red List of Threatened Species (cited 2017 Decem-

ber) Accessed on: http://www.iucnredlist.org/details/summary/3036/0.

Jansen AM, Xavier SCC, Roque ALR (2015) The multiple and complex and changeable scenarios of the Trypanosoma cruzi transmission cycle in the sylvatic environment. *Acta Tropica* 151:1–15. https://doi.org/10.1016/j.actatropica.2015.07.018

Kading RC, Borland EM, Cranfield M, Powers AM (2013) Prevalence of antibodies to alphaviruses and flaviviruses in free-ranging game animals and nonhuman primates in the greater Congo basin. *Journal of Wildlife Diseases* 49:587–599. https://doi.org/10.7589/2012-08-212

Lara-Ruiz P, Chiarello AG (2005) Life-history traits and sexual dimorphism of the Atlantic forest maned sloth *Bradypus torquatus* (Xenarthra: Bradypodidae). *Journal of Zoology* 267:63–73. https://doi.org/10.1017/S0952836905007259

Laroque PO, Valença-Montenegro MM, Ferreira DRA, Chiang JO, Cordeiro MT, Vasconcelos PFC, Silva JCR (2014) Epidemiologic survey for arbovirus in galician capuchin monkeys (*Cebus flavius*) free living in Paraíba and captive capuchin monkey (Cebus libidinosus) from northeast Brazil. *Pesquisa Veterinária Brasileira* 34:462–468. https://doi.org/10.1590/S0100-736X2014000500013

Lawrence D, Brown, T, Tony Cai, Anirban DasGupta (2001) Interval Estimation for a Binomial Proportion. In: *Statistical Science*, 2 (may, 2001). pp 101–117

Lima MA, Romano-Lieber NS, Duarte AMRdeC (2010) Circulation of antibodies against yellow fever virus in a simian population in the area of Porto Primavera Hydroelectric Plant, São Paulo, Brazil. *Rev Inst Med Trop São Paulo* 52:11–16. https://doi.org/10.1590/S0036-46652010000100002

Mb P, A R, Rocha TC da, Cg E, Silva MAN da, Ks W, N J, Gr S, Oc J, Fern P, Vasconcelos o da C (2015) Serosurvey of Arbovirus in Free-Living Non-Human Primates (*Sapajus* spp.) in Brazil. *Journal of Environmental Analytical Chemistry*. https://doi.org/10.4172/2380-2391.1000155

Medlin S, Deardorff ER, Hanley CS, Vergneau-Grosset C, Siudak-Campfield A, Dallwig R, Travassos da Rosa A, Tesh RB, Pia Martin M, Weaver SC, Vaughan C, Ramirez O, Sladky KK, Paul-Murphy J (2016) Serosurvey of selected arboviral pathogens in free-ranging, two-toed sloths (*Choloepus hoffmanni*) and three-toed sloths (*Bradypus variegatus*) in Costa Rica, 2005–2007. *Journal of Wildlife Diseases* 52:883–892. https://doi.org/10.7589/2015-02-040

Melo MSS, Barreto FR, Costa MdaCN, Morato VC, Teixeira MG (2010) Progression of dengue virus circulation in the State of Bahia, Brazil, 1994–2000. *Revista da Sociedade Brasileira de Medicina Tropical* 43:139–144. https://doi.org/10.1590/S0037-86822010000200007

Miller KE, Dietz JM (2006) Effects of individual and group characteristics on feeding behaviors in wild *Leontopithecus rosalia*. *Int J Primatol* 26:1291–1319. https://doi.org/10.1007/s10764-005-8854-7

Morales MA, Fabbri CM, Zunino GE, Kowalewski MM, Luppo VC, Enri DA, Levis SC, Caldero GE (2017) Detection of the mosquito-borne flaviviruses, *West Nile*, *Dengue*, *Saint Louis Encephalitis*, *Ilheus*, *Bussuquara*, and *Yellow Fever* in free ranging black howlers (*Alouatta caraya*) of Northeastern Argentina. *PLOS Neglected Tropical Diseases*. https://doi.org/10.1371/journal.pntd.0005351

Moreira GV, Peixoto CMS, Ziccardi M, Oliveira RL, Castro MG, Dionisio DF, Pissinatti A (2000) Prevalência de Trypanosoma cruzi, Trypanosoma minasense e de anticorpos contra arbovírus em primatas não humanos (Callithrichidae) em cativeiro. *Revista Brasileira de Medicina Veterinaria* 22:252–254

Moreno ES, Spinola R, Tengan CH, Brasil RA, Siciliano MM, Coimbra TLM, Silveira VR, Rocco IM, Bisordi I, de Souza RP, Petrella S, Pereira LE, Maeda AY, da Silva FG, Suzuki A (2013) Yellow fever epizootics in non-human primates, São Paulo state, Brazil, 2008–2009. *Revista do Instituto de Medicina Tropical de São Paulo* 55:45–50. https://doi.org/10.1590/S0036-46652013000100008

Nakgoi K, Nitatpattana N, Wajjwalku W, Pongsopawijit P, Kaewchot S, Yoksan S, Siripolwat V, Souris M, Gonzalez J-P (2014) Dengue, Japanese encephalitis and Chikungunya virus antibody prevalence among captive monkey (*Macaca nemestrina*) colonies of Northern Thailand. *American Journal of Primatology* 76:97–102. https://doi.org/10.1002/ajp.22213

Oliveira LC, Neves LG, Raboy BE, Dietz JM (2011) Abundance of Jackfruit (Artocarpus heterophyllus) Affects Group Characteristics and Use of Space by Golden-Headed Lion Tamarins (*Leontopithecus chrysomelas)* in Cabruca Agroforest. *Environmental Management* 48:248–262. https://doi.org/10.1007/s00267-010-9582-3

Oliver WLR, Santos IB (1991) Threatened endemic mammals of the Atlantic forest region of south-east Brazil. Jersey Wildlife Preservation Trust

Omatsu T, Moi ML, Takasaki T, Nakamura S, Katakai Y, Tajima S, Ito M, Yoshida T, Saito A, Akari H, Kurane I (2012) Changes in hematological and serum biochemical parameters in common marmosets (*Callithrix jacchus*) after inoculation with dengue virus. *Journal of Medical Primatology* 41:289–296. https://doi.org/10.1111/j.1600-0684.2012.00552.x

Pautasso A, Desiato R, Bertolini S, Vitale N, Radaelli MC, Mancini M, Rizzo F, Mosca A, Calzolari M, Prearo M, Ml Mandola, Maurella C, Mignone W, Chiavacci L, Casalone C (2013) Mosquito surveillance in northwestern Italy to monitor the occurrence of tropical vector-borne diseases. *Transboundary and Emerging Diseases* 60:154–161. https://doi.org/10.1111/tbed.12123

Pereira LE, Suzuki A, Coimbra TLM, de Souza RP, Chamelet ELB (2001) Ilheus arbovirus in wild birds (*Sporophila caerulescens* and *Molothrus bonariensis*). *Revista de Saúde Pública* 35:119–123. https://doi.org/10.1590/S0034-89102001000200003

R Core team R: A language and environment for statistical computing. In: R Found. Stat. Comput. Vienna Austria. Accessed on http://www.R-project.org

Reed LJ, Muench H (1938) A simple method of estimating fifty percent end points. *American Journal of Hygiene* 27:493–497

Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM (2009) The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation *Biological Conservation* 142:1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021

Rodrigues SG, Oliva OP, Araujo FAA, Martins LC, Chiang JO, Henriques DF, Silva EVP, Rodrigues DSG, Prazeres ASC, Tavares-Neto J, Vasconcelos PFC (2010) Epidemiologia do vírus da Encefalite de Saint Louis na Amazônia brasileira e no Estado do Mato Grosso do Sul, Brasil: elevada prevalência de anticorpos em equinos. *Rev Pan-Amaz Saúde* 1:81–86. https://doi.org/10.5123/S2176-62232010000100012

Seymour C, Peralta PH, Montgomery GG (1983) Serologic evidence of natural togavirus infections in Panamanian sloths and other vertebrates. *The American Journal of Tropical Medicine and Hygiene* 32:854–861

Seymour C, Peralta PH, Montgomery GG (1983) Viruses isolated from Panamanian sloths. *The American Journal of Tropical Medicine and Hygiene* 32:1435–1444

Sollberg I, Schiavetti A, Moraes MEB (2014) Agricultural management in the una's wildlife refuge: a perspective of conservation by agroforestry. *Revista Árvore* 38:241–250. https://doi.org/10.1590/S0100-67622014000200004

Svoboda WK, Martins LC, Malanski LS, Shiozawa MM, Spohr KAH, Hilst CLS, Aguiar LM, Ludwig G, Passos FC, Silva LR, Headley SA, Navarro IT, Svoboda WK, Martins LC, Malanski LS, Shiozawa MM, Spohr KAH, Hilst CLS, Aguiar LM, Ludwig G (2014) Serological evidence for *Saint Louis encephalitis virus* in free-ranging New World monkeys and horses within the upper Paraná River basin region, Southern Brazil. *Revista da Sociedade Brasileira de Medicina Tropical* 47:280–286. https://doi.org/10.1590/0037-8682-0083-2014

Tauro L, Marino B, Diaz LA, Lucca E, Gallozo D, Spinsanti L, Contigiani M (2012) Serological detection of St. Louis encephalitis virus and *West Nile virus* in equines from Santa Fe, Argentina. *Memórias do Instituto Oswaldo Cruz* 107:553–556. https://doi.org/10.1590/S0074-02762012000400019

Teixeira MG, Barreto ML, Costa MCN, Ferreira LDA, Vasconcelos PF, Cairncross S (2002) Dynamics of dengue virus circulation: a silent epidemic in a complex urban area. *Tropical Medicine & International Health* 7(9):757–762

Thompson NN, Auguste AJ, Coombs D, Blitvich BJ, Carrington CVF, Rosa APT, Wang E, Chadee DD, Drebot MA, Tesh RB, Weaver SC, Adesiyun AA (2012) Serological Evidence of Flaviviruses and Alphaviruses in Livestock and Wildlife in Trinidad. *Vector Borne Zoonotic Diseases* 12:969–978. https://doi.org/10.1089/vbz.2012.0959

Tranquilin MV, Lehmkuhl RC, Maron A, da Silva LR, Ziliotto L, Seki MC, Salomon GR, Carrasco AdeOT, Tranquilin MV, Lehmkuhl RC, Maron A, da Silva LR, Ziliotto L, Seki MC, Salomon GR, Carrasco AdeOT (2013) First report of yellow fever virus in non-human primates in the State of Parana, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical* 46:522–524. https://doi.org/10.1590/0037-8682-0106-2013

Vasconcelos PFdaC (2010) Yellow fever in Brazil: thoughts and hypotheses on the emergence in previously free areas. *Revista de Saúde Pública* 44:1144–1149. https://doi.org/10.1590/S0034-89102010005000046

Vasconcelos PFC, Calisher CH (2016) Emergence of Human Arboviral Diseases in the Americas, 2000–2016. *Vector Borne Zoonotic Dis Larchmt N* 16:295–301. https://doi.org/10.1089/vbz.2016.1952

Vasconcelos PFC, Rosa AAPT, Rodrigues SG, Rosa SET, Dégallier N, Rosa SJFT (2001) Inadequate management of natural ecosystem in the Brazilian Amazon region results in the emergence and reemergence of arboviruses. *Cadernos de Saúde Pública* 17:S155–S164. https://doi.org/10.1590/S0102-311X2001000700025

Vaz SM (2005) Mamíferos colecionados pelo Serviço de Estudos e Pesquisas sobre a Febre Amarela nos municípios de Ilhéus e Buerarema, estado da Bahia, Brasil. *Arq Mus Nac* 63:21–28

Vorou R (2016) Zika virus, vectors, reservoirs, amplifying hosts, and their potential to spread worldwide: what we know and what we should investigate urgently. *International Journal of Infectious Diseases* 48:85–90. https://doi.org/10.1016/j.ijid.2016.05.014

Weaver SC (2013) Urbanization and geographic expansion of zoonotic arboviral diseases: mechanisms and potential strategies for prevention. *Trends in Microbiology* 21:360–363. https://doi.org/10.1016/j.tim.2013.03.003

Weaver SC, Reisen WK (2010) Present and future arboviral threats. *Antiviral Research* 85:328–345. https://doi.org/10.1016/j.antiviral.2009.10.008

Zarnke RL, Calisher CH, Kerschner J (1983) Serologic evidence of arbovirus infections in humans and wild animals in Alaska. *Journal of Wildlife Diseases* 19:175–179