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SEROSURVEY OF SELECTED ARBOVIRAL PATHOGENS IN FREE-RANGING, TWO-TOED SLOTHS (*CHOLOEPUS HOFFMANNI*) AND THREE-TOED SLOTHS (*BRADYPUS VARIEGATUS*) IN COSTA RICA, 2005–2007

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ABSTRACT: We screened for antibodies to 16 arboviruses in four populations of free-ranging sloths in Costa Rica. Blood samples were taken from 16 Hoffman's two-toed sloths (HTSs; *Choloepus hoffmanni*) and 26 brown-throated sloths (BTSs; *Bradypus variegatus*) over a 3-yr period. We used serologic assays to detect antibodies against 10 arboviruses previously described in sloths (St. Louis encephalitis [SLEV], Changuinola, Venezuelan equine encephalitis, Ilheus [ILHV], Oropouche, Mayaro, Utinga, Murutucu, Punta Toro, and vesicular stomatitis [VSV] viruses) and six arboviruses not described in sloths (Rio Grande, West Nile [WNV], eastern equine encephalitis, Piry, Munguba, and La Crosse viruses). Overall, 80% of sloths had detectable antibodies to SLEV, 67% had antibodies to ILHV, 32% to Punta Toro virus, 30% to Changuinola virus, 15% to WNV, 14% to VSV, 11% to Venezuelan equine encephalitis virus, and 10% to Rio Grande virus. No samples had detectable antibodies to the remaining eight viruses. We found a significant increase in prevalence of antibody to VSV in HTSs between 2005 and 2007, and for WNV antibody between 2005 and 2006. We found no significant differences in the prevalences of antibodies to the sampled viruses between the two locations. Antibody prevalences were significantly higher in HTSs than in BTSs for SLEV in 2005. Antibody-positive results for ILHV were likely due to cross-reaction with SLEV. The novel finding of antibodies to Rio Grande virus in sloths could be due to cross-reaction with another phlebovirus. These findings might have implications for land management and domestic animal health. Due to the nature of the study, we could not determine whether sloths could represent amplification hosts for these viruses, or whether they were only exposed and could be used as sentinel species. Further studies are needed to fully characterize arboviral exposure in sloths.

Key words: Arbovirus, *Bradypus variegatus, Choloepus hoffmanni,* serology, sloth.

INTRODUCTION

Choloepus hoffmanni and *Bradypus variegatus*, commonly known as the Hoffman's two-toed sloth (HTS) and the brown-throated sloth (BTS), respectively, are hosts for many tropical arboviruses with potential public health implications (Seymour et al. 1983a, b, c; Gilmore et al. 2001). Additionally, sloths are hosts to some viruses often shared between livestock and other domesticated species (Seymour et al. 1983c; Figueiredo 2007). The low metabolism of sloths could be associated with a long-lasting viremia for many viruses, which might increase transmission stability (Seymour et al. 1983a).

We screened for antibody to selected arboviruses in populations of BTSs and HTSs inhabiting two locations in Costa Rica. The viruses included 10 arboviruses previously described in sloths: St. Louis encephalitis (SLEV), Venezuelan equine encephalitis (VEEV), Changuinola (CGLV), Ilheus (ILHV), Oropouche (OROV), Mayaro (MAYV), Utinga (UTIV), Murutucu (MURV), Punta Toro (PTV), and vesicular stomatitis (VSV) viruses (Tesh et al. 1969; Seymour et al. 1983a, b, c; Seymour 1985; Gilmore et al. 2001) and six arboviruses not reported in sloths: Rio Grande (RGV), West Nile (WNV), eastern equine encephalitis (EEEV), Piry (PIRYV), Munguba (MUNV), and La Crosse (LCV) viruses.

Flaviviruses

Among flaviviruses, we investigated exposure to WNV, ILHV, and SLEV, all vectored by mosquitoes (Pauvolid-Correa et al. 2013; Rivarola et al. 2014). Saint Louis encephalitis virus infects a variety of vertebrate hosts, among which sloths, primates, birds, marsupials, and armadillos are considered reservoirs (Seymour et al. 1983a; Figueiredo 2007). Free-ranging BTSs in Panama maintain a high SLEV antibody prevalence of 39% (Seymour et al. 1983b). Experimental infection with SLEV in sloths produced a high-titer and long-lasting antibody response compared to other hosts (Seymour et al. 1983a). Sloths are relatively poor amplification hosts after mosquito inoculation compared to chicks (*Gallus gallus domesticus*) or mice (*Mus musculus*; Seymour et al. 1983a). Despite low transmission, because sloths are relatively common in Central American forests (Eisenberg and Thorington 1973; Montgomery and Sunquist 1978), they are considered important hosts in the transmission of SLEV.

Alphaviruses

Some alphaviruses are emerging pathogens, including chikungunya virus, VEEV, MAYV, and EEEV (Figueiredo 2007; Cassadou et al. 2014; Fischer and Staples 2014; Horcada et al. 2014). Three of these (VEEV, EEEV, and MAYV) are zoonotic and widespread in South America (Hubalek et al. 2014). Mayaro virus causes epidemics of febrile exanthematous illness in humans in Latin America, often accompanied by arthralgia (Woodall 1967; de Thoisy et al. 2003). It is a major arboviral infection transmitted by mosquitoes and relevant to public health in rural South America (de Thoisy et al. 2003; Figueiredo 2007; Figueiredo and Figueiredo 2014). Sloths with MAYV antibody have been reported in French Guyana (de Thoisy et al. 2003) but not in Panama (Seymour et al. 1983b).

Orthobunyaviruses

La Crosse virus is one of the four primary causes of zoonotic encephalitis in the US (Go et al. 2014). Humans are considered dead-end hosts (Smith et al. 2006; Go et al. 2014) but, when symptomatic, frequently present with influenza-like clinical signs and encephalitis (Go et al. 2014). No disease is documented in animals. Prevalence of LCV antibodies has not been studied in sloths. Rio Grande virus is a member of the phlebotomus fever group. The primary vector is the hematophagous sand fly *Lutzomyia anthophora* (Endris et al. 1983). This virus has not been investigated in sloths.

Oropouche virus is a zoonotic *Orthobunyavirus* causing acute Oropouche fever (Figueiredo 2007). The sylvatic cycle includes sloths as reservoirs and mosquitoes as vectors (Pinheiro et al. 1981; Figueiredo 2007). The urban cycle involves humans with *Culex quinquefasciatus*

and *Culicoides paraensis* midges as vectors (Pinheiro et al. 1981; Figueiredo 2007). Oropouche virus was described in Brazil in 1960, when it was isolated from a pale-throated three-toed sloth (*Bradypus tridactylus*) captured near a forested area during construction of the Belem-Brasilia highway (Woodall 1967).

Utinga virus has been documented in pale-throated three-toed sloth in Utinga forest, Belem, Para, Brazil (Woodall 1967). Punta Toro virus is a rare zoonotic virus causing febrile illness in humans and vectored by sand flies *Lutzomyia* spp. (Seymour et al. 1983c). It was studied as a model for Rift Valley fever infection in laboratory animals (Ashley et al. 2011) and has been used as a vaccine candidate against Rift Valley fever (Lihoradova et al. 2013). Murututcu virus was isolated from a BTS in Para, Brazil (Woodall 1967).

Phleboviruses

Rio Grande virus is a member of the phlebotomus fever group and has not been investigated in sloths. The primary vector is the hematophagous sand fly, *Lutzomyia anthophora* (Endris et al. 1983). A Munguba virus antibody-positive HTS was found in Monte Dourado, Jari, Para, Brazil (Woodall 1967).

Orbiviruses

Changuinola virus is a rare zoonotic orbivirus with only one reported case of febrile human disease (Woodall 1967). The primary vector in Brazil and Panama is the sandfly, *Lutzomyia umbratilis* (Woodall 1967). Antibody-positive sloths have been reported in Panama (Seymour et al. 1983a, b) and Brazil (Gilmore et al. 2001) and are believed to be reservoirs for this virus (Seymour et al. 1983c).

Vesiculoviruses

Vesicular stomatitis is a reportable disease to the Office International des Epizooties. Sand flies (*Lutzomyia* spp.) and black flies (*Simulium* spp.) are vectors (Comer et al. 1990; Cupp et al. 1992; Mead 2006). Virginia opossums (*Didelphis virginiana*) are suspected to be a maintenance host for VSV (Trujillo et al. 2010), and deer mice (*Peromyscus maniculatus*) may represent an amplifying host (Mead 2006). Although VSV may cause a flu-like disease in humans (Mead 2006), the primary concern is to livestock in the agrarian regions of Costa Rica (Atwill et al. 1993). Panamanian sloths have been found to be antibody positive for VSV Indiana serotype by plaque reduction neutralization test, with 32% of *Choloepus* spp. and 15% of *Bradypus* spp. antibody positive (Tesh et al. 1969).

Antibodies against Piry virus have been identified in a wide variety of mammals (Wilks and House 1984) including *Xenarthra* in Brazil with 8.8% antibody prevalence (CDC 2014).

MATERIALS AND METHODS

We collected blood samples from two sloth species inhabiting two locations in Costa Rica between July and August in 2005–2007. We chose two locations corresponding with ongoing wildlife research projects tracking the movement of free-ranging sloths. The primary location used for capture was Finmac, a 107-ha organic cacao plantation in northeastern Costa Rica (10°20′′N, 83°20′′W), surrounded by banana and pineapple plantations and pastures. The second location, used for collection in 2007 only, was a cacao plantation outside of the community of Upala (10°54′N, 85°2′W), surrounded by pasture, sparse forest, and pineapple plantations. Participants included wildlife biologists, veterinarians, and veterinary students from the Universidad Nacional (Heredia, Costa Rica) and the University of Wisconsin, Madison, Wisconsin. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permits and local permissions were obtained for sample collection. All procedures in this study were approved by the Institutional Animal Care and Use Committee of the University of Wisconsin.

Sloths were captured manually, placed in burlap sacks, and transported to a consistent location before being anesthetized as described by Hanley et al. (2008). Time between capture and release did not exceed 12 h. Capture location was documented for each sloth by GPS to ensure return to the original capture site. After transportation, each sloth was immobilized with medetomidine 0.02 mg/kg (1 mg/mL; Domitor®, Pfizer Animal Health, Exton, Pennsylvania, USA) and

ketamine hydrochloride 2.5 mg/kg (100 mg/ml; Ketavet®, Revetmex, Colonia Prado Churubsco, Deleg, Coyoacan, Mexico). Anesthetic monitoring was performed by one of the investigators (CSH) and included respiratory rate and heart rate via Doppler flow probe and stethoscope. A full physical exam was completed. Blood samples were collected from the cephalic, saphenous, or femoral veins, or the cranial vena cava, and preserved in 2.0 mL heparinized tubes. Blood samples were centrifuged within 60 min of collection, and serum was placed in 1.8 mL plastic microtainers (Nalgene screw-top, Lima, Ohio, USA), and kept in an ice chest until frozen at –4 C. Each sloth had a passive integrated transponder (ID 100US, Trovan Ltd., Santa Barbara, California, USA) implanted subcutaneously in the interscapular region to prevent resampling during the same year.

Prior to recovery, each sloth received 20–100 mL of lactated Ringer's solution (Hospira, Lake Forest, Illinois, USA) subcutaneously. Atipamezole 0.1 mg/kg was administered intramuscularly to antagonize medetomidine. After complete recovery, sloths were released at their capture site. Frozen sera were transported to the University of Texas Medical Branch, Galveston, Texas, USA, for testing.

Serologic testing

Hemaglutination inhibition (HI) test: Sera were tested for antibodies to EEEV, ILHV, MAYV, MUNV, MURV, OROV, RGV, SLEV, UTIV, and VEEV. The HI tests were performed in microtiter plates as described by Shope (1963). Nonspecific inhibitors of sera were acetone extracted by a classical technique (Clarke and Casals 1958). To remove goose cell agglutinins, rehydrated sera (pH 9.0 borate saline) were adsorbed with goose cells. Tests were performed with 4 units of antigen, obtained by sucrose acetone extraction and inactivated by treatment with betapropiolactone, against 1:10 serum dilution used for screening.

Complement fixation (CF) test: Sera were screened for antibodies to six arbovirus antigens: ILHV, SLEV, LCV, PTV, CGLV, and PIRYV. Testing by CF was performed using a microtechnique following Beaty et al. (1989). The highest dilutions giving 3+ or 4+ fixation of complement (scale of 0–4) were recorded as positive, and a positive result for the 1:10 dilution was considered antibody positive.

Plaque-reduction neutralization tests (PRNT): All sera SLEV- and VEEV-positive by HI were tested by PRNT to confirm specificity using strains T35573 and 68U201, respectively. In addition, sera were tested for WNV and VSV using strains NY99 and Mudd-Summers, respectively. Serum samples were serially diluted and incubated with 100 plaque-forming units of virus before inoculation onto confluent Vero cells as described by Seymour et al. (1983c).

Serologic test variability depending on the year: Due to test availability and financial limitations, some tests were conducted only in some years. We investigated the presence of antibodies to SLEV, VEEV, ILHV, OROV, MAYV, MURV, and WNV every year. In 2007 the VEEV HI test was refined to detect specific antibodies against the subtype I variant AB, typically responsible for epizootic outbreaks, and subtype II. In 2007 PRNT was performed to detect neutralizing antibodies against VEEV subtype I variant E. The VSV PRNT was conducted in 2005 and 2007. Serologic testing for CGLV, UTIV, PIRYV, and LCV was conducted in 2005 and 2006 but not in 2007. Serologic testing for PTV and MUNV was performed only in 2005, and RGV and EEEV were tested only in 2007.

Statistical analysis

Yearly antibody prevalence for each virus was calculated by dividing the total number of antibody-positive animals by the total number of sloths sampled, including sloths resampled each year. Statistical tests were conducted using GraphPad (GraphPad Software, La Jolla, California, USA). Differences between sampling locations were evaluated with Fisher's exact test. For arboviruses evaluated during multiple years, differences between years were also evaluated using Fisher's exact test. For all tests, statistical significance was set at $P<0.05$.

RESULTS

From 2005 to 2007, we collected 109 blood samples from 94 adult free-ranging sloths, including 26 BTSs and 68 HTSs. We collected 45 samples in 2005, 43 in 2006, and 21 in 2007. During physical examinations of 95 sloths, ticks, flies, and moths were present on most sloths. All sloths recovered from anesthesia uneventfully.

Serologic results for the 109 samples are detailed in Table 1, and antibody titers are summarized in Table 2. Overall, via HI and CF techniques, 87 of the 109 samples (80%) were positive for antibody to SLEV. Prevalences of detectable antibody to other viruses were IHLV: 73/109 (67%), PTV: 12/37 (32%), CGLV: 26/88 (30%), WNV: 12/82 (15%),

TABLE 1. Prevalence of antibody to selected viruses in Hoffman's two-toed (*Bradypus variegatus*) and brown-throated sloths (*Choloepus hoffmanni*) in two sampling locations in Costa Rica. Percentage of animals determined to be antibody positive by tests discussed. Dashes indicate 0%; ND indicates not done.

		Bradypus variegatus			*Choloepus hoffmanni*		
Virus[a]	Test	2005 (n=17)	2006 (n=6)	2007 (n=3)	2005 (n=28)	2006 (n=37)	2007 (n=18)
CGLV	CF	–	–	ND	46%	32%	ND
ILHV	HI	41%	50%	33%	82%	86%	39%
	CF	–	ND	–	–	ND	–
MAYV	HI	–	–	–	–	–	–
MURV	HI	–	–	–	–	–	–
OROV	HI	–	–	–	–	–	–
PTV	CF	–	–	ND	–	32%	ND
SLEV[c]	HI	29%	67%	67%	89%	86%	78%
	CF	–	ND	–	–	17%	–
	PRNT	35%	–	ND	82%	–	ND
UTIV	HI	–	–	ND	–	–	ND
VEEV[c]	HI	–	–	33%	–	16%	6%
	PRNT	ND	–	67%	ND	5%	22%
EEEV	HI	ND	ND	–	ND	ND	–
LCV	CF	–	–	ND	–	–	ND
MUNV	HI	–	–	ND	–	–	ND
PIRYV	CF	–	–	ND	–	–	ND
RGV	HI	ND	ND	33%	ND	ND	6%
VSV	PRNT	–	ND	–	4%	ND	44%
WNV	PRNT	–	–	ND	8%	27%	ND

[a] CGLV = Changuinola virus; EEEV = eastern equine encephalitis virus; ILHV = Ilheus virus; LCV = La Crosse virus; MAYV = Mayaro virus; MUNV = Munguba; MURV = Murutucu virus; OROV = Oropouche virus; PIRYV = Piry virus; PTV = Punta Toro virus; RGV = Rio Grande virus; SLEV = St. Louis encephalitis virus; UTIV = Utinga virus; VEEV = Venezuelan equine encephalitis virus; VSV = vesicular stomatitis virus; WNV = West Nile virus.

[b] HI = hemagglutination inhibition; CF = complement fixation; PRNT = plaque reduction neutralization test.

[c] For these viruses the same antibody was detected by multiple methods. In these cases the antibody prevalence is reported as obtained with both techniques.

VEEV: 12/109 (11%), VSV: 10/109 (9%), and RGV: 2/21 (10%). Nine of 66 samples (14%) were positive for VSV by PRNT. Antibodies to WNV, VSV, and CGLV were detected only in BTSs. There were no detectable antibodies found during any sampling period for the remaining eight viruses.

We sampled 13 sloths during 2 yr, and one sloth during all 3 yr. Among the recaptured sloths, 7/13 had a consistent titer for SLEV, three had increasing titers, two had decreasing titers, and one was antibody negative during the 3-yr sampling period.

Discrepancies were found between the results of the HI assays and $PRNT_{80}$ in four of 12 serum samples for VEEV and in 41 of 87 positive samples for SLEV antibody. In all cases, sera were positive for VEEV or SLEV antibodies via HI, but negative via $PRNT_{80}$. Because the PRNT is considerably more specific, the results obtained by HI in these cases could reflect cross-reactions with other, unknown viruses. All of these sloths were negative for WNV and SLEV antibodies by $PRNT_{80}$.

Antibodies to VEEV were found at both locations, and in both sloth species. During 2006 none of the BTSs were antibody positive. During 2007, two of three BTSs from the Upala plantation had antibody to VEEV enzootic subtype IE. Three of four HTSs positive for VEEV-IE antibody during 2007 did not have measurable titers for either subtype IAB or II. The remaining HTS had

TABLE 2. Antibody titers for selected viruses in Hoffman's two-toed (*Bradypus variegatus*) and brown-throated sloths (*Choloepus hoffmanni*), 2005–2007, at two sampling locations in Costa Rica. n/a = not applicable due to low number of positive samples.

			Bradypus variegatus				*Choloepus hoffmanni*			
Virus[a]	Method[b]	Location	Mean titer	Range	SD	*n*	Mean titer	Range	SD	*n*
CGLV	CF	Finmac	16	n/a	n/a	1	68	8–256	62	25
ILHV	HI	Finmac	30	10–160	47	10	82	10–640	138	61
		Upala	n/a	n/a	n/a	1	10	n/a	n/a	1
	CF	Finmac	n/a	n/a	n/a	0	n/a	n/a	n/a	0
PTV[c]	CF	Finmac	n/a	n/a	n/a	n/a	128	64–256	64	7
SLEV[c]	HI	Finmac	137	10–640	175	11	315	20–2560	546	60
		Upala	230	140–320	127	2	224	20–1280	345	14
	CF	Finmac	10	n/a	n/a	1	84	8–512	133	30
	PRNT	Finmac	120	40–160	69	3	275	20–640	239	22
VEEV	HI	Finmac	n/a	n/a	n/a	0	31	10–80	33	7
		Upala	20	n/a	n/a	1	10	n/a	n/a	1
	PRNT	Finmac	n/a	n/a	n/a	0	170	20–320	212	2
		Upala	640	n/a	0	2	170	20–320	212	2
RGV	HI	Finmac	n/a	n/a	n/a	0	20	n/a	n/a	1
		Upala	20	n/a	n/a	1	n/a	n/a	n/a	0
VSV	PRNT	Finmac	n/a	n/a	n/a	0	31	20–80	20	9
		Upala	20	n/a	n/a	1	10	n/a	n/a	1

[a] CGLV = Changuinola virus; ILHV = Ilheus virus; PTV = Punta Toro virus; RGV = Rio Grande virus; SLEV = St. Louis encephalitis virus; VEEV = Venezuelan equine encephalitis virus; VSV = Vesicular stomatitis virus; WNV = West Nile virus.

[b] HI = hemagglutination inhibition; CF = complement fixation; PRNT = plaque reduction neutralization test.

[c] For these viruses, the same antibody was detected by multiple methods. In these cases the antibody prevalence is reported as obtained with both techniques if antibody prevalence was not zero.

a higher titer for VEEV-IE antibody than either VEEV-IAB or VEEV-II antibodies. Antibody to Rio Grande virus was detected in two of 21 individuals via HI in 2007 (Table 2). The positive sloths were one female adult HTS at Finmac and one female adult BTS at Upala.

We analyzed changes in antibody prevalence for a few viruses. Between 2005 and 2007 there were no significant variations in prevalence of antibodies to ILHV or SLEV in either sloth species (P=0.16–1.0). Between 2005 and 2007, a significant increase in VSV antibody prevalence was determined in HTSs, from 4% to 44% (P=0.001). Between 2005 and 2006, a significant increase in WNV antibody prevalence from 8% to 27% was measured in HTSs, ($P<0.001$) while no WNV antibody-positive BTSs were detected in either year.

Regarding variations between sloth species, antibody prevalence was significantly higher in HTSs compared to BTSs for SLEV and ILHV in 2005 (P=0.008), whereas in 2006 and 2007, prevalence was not significantly different between sloth species for any of the screened antibodies. For VEEV and RGV antibodies, no significant difference was detected between sloth species. When comparing locations, there were no significant differences in prevalence for any antibodies.

DISCUSSION

We conducted comprehensive serologic testing in sloths in Costa Rica, confirming exposure of sloth populations to, at least, eight arboviruses.

Rio Grande virus

Rio Grande virus was detected in two of 21 sloths but was evaluated only in 2007. These may represent a cross reaction with a closely related *Phlebovirus* or might be true positives.

This virus cross-reacts only rarely with other Phleboviruses via CF and PRNT (Travassos da Rosa et al. 1983), but we used IH. To date, RGV has been isolated in Texas (Calisher et al. 1977) and Mexico (Deardorff et al. 2011). Sand flies are vectors of RGV and VSV (Mead 2006), so the presence of these vectors in Costa Rica (Jimenez et al. 2000a) might be associated with transmission of both viruses being described in Costa Rican sloths.

Punta Toro virus

We detected antibody to PTV in 12 of 37 HTSs evaluated only in 2006. Antibodies against PTV were found in BTSs (8/106) and HTSs (11/66) in Panama (Seymour et al. 1983c). Unfortunately, PTV and RGV antibodies were not tested during the same years, and it was not possible to determine whether some RGV antibody-positive sloths could also be positive for PTV antibody. This requires further evaluation.

Vesicular stomatitis virus

Costa Rica is endemic for VSV (Jimenez et al. 2000b). Similar to a previous study in Panama (Tesh et al. 1969), we detected sloths with antibody for the VSV Indiana serotype. Production of neutralizing antibodies results in cessation of virus shedding (Mead 2006). Although this has not been investigated specifically in xenarthrans, the antibody-positive sloths we detected are unlikely to represent a source of infection for other animals. In addition, experimental studies have shown that biting insects become infected with VSV when feeding on or near virus-rich vesicular lesions (Mead 2006), none of which were observed on physical examinations of VSV antibody-positive sloths. No sloths had detectable antibody to PIRYV, which rules out a cross-reaction between these two vesiculoviruses. We found that prevalence of antibody to VSV increased significantly in HTSs between 2005 and 2007. In addition, all antibody-positive sloths were from Finmac, which could reflect the involvement of environmental factors. Climatic conditions in between sampling areas are different, and weather variability is also expected in the same area each year due to the El Niño southern oscillation. These environmental parameters could have affected multiple serologic results. Further work is needed to investigate whether VSV can have an impact on free-ranging sloth populations and whether contact with domestic animals is a factor associated with seroconversion.

Venezuelan equine encephalitis virus

Six viral species were described within the VEE complex (Go et al. 2014), and multiple variants were described within VEEV, among which subtypes 1AB and 1C are involved in equine epizootic outbreaks (Kinney et al. 1992). *Bradypus* and *Choloepus* spp. individuals with antibody to VEEV have been described in Panama (Grayson and Galindo 1969; Seymour 1985). Following experimental infection, BTSs develop high titers, similar to rodents, which are considered the most important enzootic hosts (Carrara et al. 2007), and their viremias last 5–6 times longer than in *Choloepus* spp. Seymour (1985) suggested that BTSs could be an amplifying host for VEEV (Seymour 1985). In South America, reservoir and amplifying hosts in the VEEV enzootic cycle are unknown but may include rodents and birds (Arrigo et al. 2010; Go et al. 2014). In 2007, two of three BTSs collected at the Upala plantation had antibody to VEEV-IE. One of these sloths also had a low titer for both VEEV-IAB and VEEV-II. Considering the higher titer for subtype IE, the lower titers for subtype IAB and subtype II were most likely due to cross-reactivity of the antibody responses or to the different serologic test (PRNT versus HI). A similar observation was made on one of the four positive HTSs. The VEEV antibody prevalence did not change significantly between 2006 and 2007 at Finmac, suggesting an enzootic virus or long lasting immunity. It was not possible to evaluate this hypothesis at the individual level as no sloth captured in multiple years was VEEV antibody positive.

Changuinola virus

We found that 30% of HTSs had antibody to CGLV, with prevalences of 46% and 32% in 2005 and 2006 respectively, versus 0% in BTSs. This was similar to the findings of Seymour et al. (1983c) of 42.4% *Choloepus* spp. and 5.2% *Bradypus* spp. CGLV antibody prevalence in Panama. This prevalence supports the theory that the primary hosts for this virus are *Choloepus* spp. sloths.

Ilheus virus

We found 67% of sloths had antibody to ILHV compared to 2% in Panama (Seymour et al. 1983b) and 4% in Brazil (Woodall 1967). These differences could be due to different serologic assays, locations, or years. In our study, individual animals' titers to ILHV antibody were always higher than the ILHV antibody titer using the same samples. In addition, of the 73 samples positive for ILHV antibody, all were also positive for SLEV antibody, in lower titers ($\leq$4), suggesting that HI and CF results may cross-react for SLEV. A cross-reaction between SLEV and ILHV antibodies via HI and CF would also explain that more HTSs than BTSs had detectable antibody to both viruses.

St. Louis encephalitis virus

Annual prevalence of antibodies to SLEV in sloths in Costa Rica using HI ranged 29–67% in BTSs, and from 78 to 89% in HTSs in our study, which appeared higher than those reported from *Choloepus* spp. And *Bradypus* spp. in Panama (Seymour et al. 1983b). However, when considering PRNT as the most specific serologic technique to detect seroconversion to SLEV, only 53% of the positive samples were considered true positives (46 of 87 positive samples by HI), decreasing the antibody prevalence of to 42%. This result is similar to the prevalence of 46% reported by PRNT in *Choloepus* spp. in Panama (Seymour et al. 1983b). For SLEV, our results were consistent with previous studies on antibody prevalences in sloths in Central and South America (Seymour et al. 1983a, b, c; Seymour 1985; Gilmore et al. 2001).

In 2005, antibody prevalences were significantly higher in HTSs than in BTSs, similar to findings in Panama (Seymour et al. 1983b), whereas prevalences were not significantly different between sloth species during the following years. However, the BTS sample sizes in 2006 and 2007 were relatively small in comparison to 2005, which may have decreased the power of the statistical analysis and lead to a type II error. The implications for public health with respect to the high SLEV antibody prevalence detected in sloths is unknown and warrants further monitoring. In previous studies, no flaviviruses were isolated in Vero cells from 59 sloths (Seymour et al. 1983b, c). This could result from sloths having been exposed, but not viremic at the time of sampling, and thus may not represent a source of SLEV transmission (Seymour et al. 1983b, c).

West Nile virus

Our study was performed during the first few years after WNV reached South America (Travis 2008). It was therefore relevant to follow the progression of the number of antibody-positive sloths and the titers of those animals. Although WNV infection has been documented extensively in a wide variety of mammals (Travis 2008), including horses in Costa Rica (Hobson-Peters et al. 2011), antibody prevalence had not been evaluated in sloths. The prevalence of antibody to WNV in our study population of HTSs increased significantly from 2005 to 2006.

Serologic tests for antibodies to other viruses included in this study were consistently negative in these sloth populations. This lack of seroconversion may reflect lack of exposure, resistance to infection, the limited number of individuals sampled, or high case fatality associated with these viruses in sloths. Regardless, we have evaluated exposure of Costa Rican sloths to 16 arboviruses and have now documented seroconversion to VSV and RGV in sloths. RGV infection has possible implications for livestock and human health.

More studies are needed to evaluate the impact of these viruses on free-ranging sloth populations and whether sloths could be incidentally exposed to VSV due to anthropogenic activities involving livestock.

Limitations of this study include the variability associated with viral testing across several years, and lack of serologic assay validation in the two sloth species; hence, the sensitivity and specificity are unknown. For example, cross-reactions between SLEV neutralizing antibodies and ILHV have been previously described in one BTS (Seymour et al. 1983a). Therefore, our results should be interpreted with caution, and further investigations are needed to confirm our findings. Further studies using PCR and viral culture are needed to document the actual presence of these infectious agents, and are required to investigate the role of sloths in arboviral sylvatic cycles.

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