

Evidence of leptospiral exposure in neotropical primates rescued from illegal trade and a Zoo in Bahia, Brazil¹

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ABSTRACT- Almeida D.S, Santos A.C., Silva C.L.R., Oriá A.P., Oliveira A.V.D., Libório F.A., Athanazio D.A. & Pinna M.H. 2016. **Evidence of leptospiral exposure in neotropical primates rescued from illegal trade and a Zoo in Bahia, Brazil.** *Pesquisa Veterinária Brasileira* 36(9):864-868. Departamento de Patologia e Medicina Legal, Praça XV de Novembro s/n, Largo do Terreiro de Jesus, Salvador, BA 40025-010, Brazil. E-mail: daa@ufba.br

Few studies have compared the seroprevalence of antileptospiral agglutinins with the demonstration of urinary shedding of leptospires or evidence of active infection in the bloodstreams of non-human primates. The study population consists of 58 animals, including 42 monkeys from the Zoological Park of Salvador (*Parque Zoobotânico Getúlio Vargas*), Bahia, Brazil. The study also evaluated 16 primates (*Cebus* sp.) rescued from illegal trade that were housed in the Wildlife Rehabilitation Center of Salvador (CETAS), Bahia, Brazil. The seroprevalence of antileptospiral antibodies was low (2%) in the animals from the Zoo. A higher rate (31%) was observed among the animals that were rescued from illegal trade in the state of Bahia. Even if all the blood and urine samples were negative for leptospiral DNA fragments, the high frequency of serological evidence of exposure suggests a potential risk of leptospirosis transmission when keeping these animals as pets.

INDEX TERMS: Leptospirosis, *Leptospira*, Primates.

RESUMO.- [Indícios de exposição a leptospiros em primatas neotropicais resgatados do comércio ilegal e de um Zoológico da Bahia.] Poucos estudos compararam a soroprevalência de aglutininas antileptospira com a demonstração de excreção urinária de leptospiros ou evidência de infecção ativa em primatas não humanos. A população

estudada consistiu em 58 animais, sendo 42 primatas do Parque Zoobotânico Getúlio Vargas, Bahia, Brasil. O estudo avaliou ainda 16 primatas (*Cebus* sp.) resgatados do tráfico ilegal e abrigados no Centro de Triagem de Animais Silvestres Chico Mendes, Salvador, Bahia, Brasil. A soroprevalência de anticorpos antileptospira foi baixa (2%) nos animais do Zoológico. Uma taxa mais elevada (31%) foi observada nos animais resgatados do tráfico ilegal. Mesmo que todas as amostras de sangue e urina tenham sido negativas para DNA de leptospiros, a alta frequência de evidência de exposição nos animais de origem selvagem indicam o risco potencial da adoção de primatas como animais de estimação.

TERMOS DE INDEXAÇÃO: Leptospirose, *Leptospira*, Primatas.

INTRODUCTION

Leptospirosis is a widespread zoonosis caused by pathogenic leptospires. Humans acquire infection by direct exposure to contaminated urine from mammalian reservoirs or, more commonly, by exposure to contaminated soil or water. The disease occurs in different settings associated with a wide range of reservoirs, such as field mice in paddy fields, farm animals in occupational exposures, urban rodents

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in large urban areas with poor sanitation, and wild animals in water sports and ecotourism related exposures (Bharti et al. 2003). Most human infections are asymptomatic or cause mild febrile illness that is indistinguishable from diseases caused by other infectious agents. However, 5-10% of human infections will become severe, resulting in the Weil's triad of acute renal failure, hemorrhages, and jaundice (with 5-30% fatality) or severe pulmonary hemorrhagic syndrome (SPHS, with $\geq 50\%$ fatality) (WHO 2003a, McBride et al. 2005, Medeiros et al. 2010).

Non-human primates usually develop a self resolving, mild illness in naturally acquired or experimental infections (Minette 1966, Minette & Shaffer 1968, Hambleton et al. 1980, Marshall et al. 1980), but some species may deve-

lop severe disease. Experimental infection mirrored lethal human cases with pulmonary hemorrhages, liver necrosis, and tubular nephritis in marmoset monkeys (*Callithrix jacchus*), and an outbreak of pulmonary hemorrhage spreaded among naturally infected capuchin monkeys in a wildlife rehabilitation center in Colombia (Pereira et al. 2005). There is a significant public health concern associated with illegal wildlife ownership, as wild animals are estimated to be the source of over 70% of all emerging infections (Chomel et al. 2007). Seroprevalence of antileptospiral antibodies in Neotropical primates from zoos in Latin America or from wildlife is variable, partly due to the diverse species studied and the different cutoffs employed (Table 1 and 2) (Baulu et al. 1987, Kessler & Everard 1988, Perolat et al. 1992, Luna-

Table 1. Studies in Latin America on the serologic evidence of exposure to leptospires in non-human primates in captivity

Author, year	Site, City, Country	Species	N / n (%)	Predominant serogroup
Baulu et al. 1987	Research Center, Saint Joseph, Barbados	<i>Cercopithecus aethiops sabeaus</i> (Vervet Monkey)	6/24 (25)	Ballum
Perolat et al. 1992	Instituto Pasteur, Caiena, Guiana Francesa	<i>Saimiri sciureus</i> (Common Squirrel Monkey)	24/93 (26)	Icterohaemorrhagiae
Luna-Alvarez et al. 1996	Chapultepec Zoo, Cidade do México, México	<i>Pongo pygmaeus</i> (Bornean Orangutan)	2/3 (66)	Canicola
		<i>Ateles belzebuth</i> (White-fronted Spider Monkey)	2/2 (100)	
		<i>Ateles paniscus</i> (Black Spider Monkey)	3/6 (50)	
		<i>Macaca fuscata</i> (Japanese Macaque)	1/3 (66)	
		<i>Papio ursinus</i> (Chacma Baboon)	1/1 (100)	
Ibanez-Contreras et al. 2010*	Research Center, Cidade do México, México	<i>Macaca mulatta</i> (Rhesus Macaque)	18/59 (30)	Panama, Icterohaemorrhagiae, Australis, and Shermani
Lilenbaum et al. 2002**	Zoo, Rio de Janeiro, Brazil	<i>Ateles belzebuth</i> (White-fronted Spider Monkey)	2/2 (100)	Icterohaemorrhagiae
		<i>Ateles paniscus</i> (Black Spider Monkey)	3/6 (50)	
		<i>Macaca fuscata</i> (Japanese Macaque)	1/3 (66)	
		<i>Papio ursinus</i> (Chacma Baboon)	1/1 (100)	
Corrêa et al. 2004	Zoo, São Paulo, Brazil	<i>Callithrix jacchus</i> (Common Marmoset)	1	Castellonis
			4	
		<i>Callithrix penicillata</i> (Black-tufted Marmoset)	1	
			2	
		<i>Saguinus fuscicollis</i> (Brown-mantled Tamarin)	2	
		<i>Saimiri sciureus</i> (Common Squirrel Monkey)	2	
		<i>Cebus apella</i> (Tufted Capuchin)	4	24/99
			7	(24)
		<i>Ateles paniscus</i> (Black Spider Monkey)	1	
			0	
		<i>Lagothrix lagothricha</i> (Brown Woolly Monkey)	2	
		<i>Cebus nigrivittatus</i> (Capuchin Monkeys)	5	
		<i>Aotus trivirgatus</i> (Three-striped Night Monkey)	1	
		<i>Alouatta fusca</i> (Brown Howler Monkey)	4	
Lilenbaum et al. 2005	Primate center, Rio de Janeiro, Brazil	<i>Leontopithecus</i> sp. (Lion Tamarin)	26/73 (36)	Icterohaemorrhagiae
Pimentel et al. 2009	Zoo, Aracaju, Brazil	<i>Cebus libinosus</i> (Black-striped Capuchin)	1/14 (7)	Icterohaemorrhagiae
		<i>Cebus xanthosternus</i> (Yellow-breasted capuchin)	1/4(25)	Icterohaemorrhagiae
Szonyi et al. 2010	Rehabilitation center, Valle de Aburrá, Colombia	<i>Cebus</i> sp. (Capuchin)	37/52 (71)	Icterohaemorrhagiae
Romero et al. 2011	Mantecaña Zoo, Cidade Pereira, Colombia	<i>Ateles fusiceps</i> (Black-headed Spider Monkey)	5/19 (33)	Icterohaemorrhagiae
		<i>Cebus apella</i> (Tufted Capuchin)	1/14 (7)	
		<i>Cebus capucinus</i> (White Faced Capuchin)	1/3 (33)	
		<i>Cebus albifrons</i> (White-fronted capuchin)	5/6 (83)	
		<i>Ateles hybridus</i> (Brown Spider Monkey)	1/6 (17)	
		<i>Saguinus leucopus</i> (White-footed Tamarin)	2/5 (40)	
Ferreira et al. 2011	CETAS, Northeast Brazil	<i>Cebus</i> sp. (Capuchin)	9/139 (6,5)	Semaranga
Pinna et al. 2012	CETAS, Salvador, Brazil	<i>Callithrix jacchus</i> (Common Marmoset)	16/28 (57)	Icterohaemorrhagiae
		<i>Callithrix penicillata</i> (Black-tufted Marmoset)	4/8 (50)	
		<i>Cebus</i> sp. (Capuchin)	8/5 (62,5)	
Ullman et al. 2012	Zoo, Sorocaba, Brazil	<i>Alouatta caraya</i> (Black howler)	1/3(33)	Icterohaemorrhagiae
		<i>Ateles marginatus</i> (White-cheeked Spider Monkey)	2/6 (33)	
		<i>Brachyteles arachnoides</i> (Southern Woolly Spider Monkey)	1/9 (11)	

Note: All studies used the cutoff of 1:100 MAT titers except for * and **, which used 1:20 and 1:200 cutoffs, respectively.

Table 2. Studies in Latin America on the serologic evidence of exposure to leptospires in non-human primates captured from the wild

Author, year	Site, City, Country	Species	N / n (%)	Predominant serogroup
Souza Júnior et al. 2002	Lajeado/ Ipueras, Brazil	<i>Alouatta caraya</i> (Black howler) <i>Cebus</i> sp. (Capuchin)	46/82 (16) 2/286 (2)	Pomona Panama
Baulu et al. 1987	Research Center, Barbados, Caribe	<i>Cercopithecus aethiops sabeaus</i> (Vervet Monkey)	150/501 (30)	Ballum
Kessler & Everard 1988	Caio Santiago, Porto Rico	<i>Macaca mulatta</i> (Rhesus Macaque)	5/169 (3)	Icterohaemorrhagiae

-Alvarez et al. 1996, Lilenbaum et al. 2002, Correa et al. 2004, Lilenbaum et al. 2005, Souza Júnior et al. 2006, Pimentel et al. 2009, Ibáñez-Contreras et al. 2010, Ferreira et al. 2011, Romero et al. 2011, Szonyi et al. 2011, Pinna et al. 2012, Ullmann et al. 2012). Few studies have compared the serological evidence of leptospirosis exposure with the molecular evidence of active infection (detection of DNA fragments in blood samples) or the renal carrier state (detection of DNA in urine) in non-human primates (Ullmann et al. 2012). The aim of this study was to investigate the serological evidence of exposure and the presence of DNA fragments of pathogenic leptospires in the blood and urine samples of Neotropical primates from two sources, a zoological park and a wild life rehabilitation center in Salvador, Bahia, Brazil.

MATERIALS AND METHODS

In the present study, 58 of non-human primates were evaluated. The population included 42 monkeys from the Zoological Park of Salvador (*Parque Zoológico Getúlio Vargas*), Bahia, Brazil. These animals belonged to the following species: *Cebus xanthosternus* (n=23), *Cebus flavius* (n=11), *Alouatta caraya* (n=3), *Aotus* sp. (n=4), and *Saimiri sciureus* (n=1). The study also evaluated 16 primates rescued from illegal trade that were housed in the wildlife rehabilitation center of Salvador (Centro de Triagem de Animais Silvestres Chico Mendes, CETAS), Bahia, Brazil. All these animals were identified as *Cebus* sp., as many of them are hybrids.

Microscopic agglutination tests (MAT) were performed, according to recommended protocols (Faine et al. 1999, WHO 2003), and included twenty-three WHO reference strains and a local isolate (serovar Copenhageni strain L1130) (Ko et al. 1999, Nascimento et al. 2004). Titers $\geq 1:100$ were considered positive. Polymerase chain reaction for the detection of the *lipL32* gene was performed as previously described (Rojas et al. 2010, Chagas-Junior et al. 2012).

The research protocols were approved by the Research Ethics Committee of the Faculdade Franca - SP (025/2009-A). In addition, they were in accordance with guidelines System Authorization and Information on Biodiversity the Ministry of Environment of Brazil (number 20831-1)

RESULTS AND DISCUSSION

In the present study, only one of 42 primates (2%) from the Zoological Park of Salvador had positive serum samples, according to the MAT. This animal was an adult female *Alouatta caraya* (black howler) that had a positive serum sample of 1:100 with mixed reactions for the Bratislava and Icterohaemorrhagiae serogroups. This animal was housed in a cage with two other adults of the same species. The positive animal and one negative black howler were born in the Zoological Park, while the other negative adult came from illegal trade (CETAS). Urine and blood samples were negative for all 42 monkeys evaluated at the Zoological Park.

Such low seroprevalence was not expected. Salvador is the third most populated city in Brazil, with an estimated 2.6 million inhabitants, and 60% of those inhabitants live in slum communities (Riley et al. 2007). It is a large urban center with poor sanitation, and incidence of severe leptospirosis cases peak during rainy seasons and in association with floods (Ko et al. 1999, Costa et al. 2001, Riley et al. 2007). Reports from the Zoological personnel indicated that there is a high population of rodents in the park, and rodents are frequently observed to be in contact with the animals in their cages. In some surveys from Latin American zoos, the seroprevalence of antileptospiral antigens is highly variable among different species (Table 1), and higher rates were attributed to the contact of primates with urban rodents. Additionally, there is a high prevalence of Icterohaemorrhagiae as the predicted infecting serogroup in some of these studies (as implied by MAT highest titers), and this suggests that rodents are the source of non-primate infection because these serovars are known to be selectively carried by urban rodents such as the Brown (*Rattus norvegicus*) and Black rats (*R. rattus*) (Bharti et al. 2003). In a survey of captured rats in Salvador, 80% of a total 142 animals had positive cultures for leptospires from kidney or urine samples, and all 59 serotypes isolated by monoclonal antibodies were characterized as serovar Copenhageni (serogroup Icterohaemorrhagiae) (de Faria et al. 2008), which is the main cause of severe leptospirosis in Salvador and in other large urban Brazilian centers (Ko et al. 1999, Pereira et al. 2000). It is not possible to infer that the single Black Howler with a positive MAT acquired the infection from exposure to rats because it had a mixed reaction with the Bratislava serogroup, which has no known selectivity for rodents.

In summary, the results from the Zoological Park suggest that the Neotropical primates' contact with rodents was not associated with an increased seroprevalence of antileptospiral antibodies. These species may be intrinsically more resistant than others in terms of acquiring leptospiral infection, or conversely, these animals may have had previous exposure in the wild that is associated with a progressive decrease in antileptospiral antibodies during the period of captivity.

In 2009, a previous survey performed in the wildlife rehabilitation center of Salvador (CETAS) found 57% (25/44) seropositivity by MAT. In that study, marmosets were mainly affected, and positive samples were distributed as follows: *Callithrix jacchus* (common marmoset, 16/28: 57%), *Callithrix pennicilata* (Black-tufted marmoset, 4/8: 50%), and *Cebus* sp. (capuchin monkeys, 5/8: 63%) (Pinna et al. 2012). In this study were found 5/16 (31%) positive serum samples by MAT from *Cebus* sp.

Thus, the high rate of serological evidence of *Leptospira* exposure seems to be a consistent finding among animals from the wild that are rescued from illegal trade in the state of Bahia. It is important to note, however, that the predicted infecting serogroup inferred by MAT highest titers was *Icterohaemorrhagiae* in 84% of the cases in a former study. In this study, the five positive samples were distributed in the following predicted serogroups: Ballum (1:100), Semarang (1:200), Grippotyphosa (1:100), Cynopeteri (1:100), and a mixed reaction of Tarassovi/Autumnalis (1:100). Thus, while the high frequency of *Icterohaemorrhagiae* indicated that monkeys could have acquired infection after they were caught in the wild, the wide range of predicted serogroups in the present study indicates that these animals may have been exposed to many potential sources of infection in the wild. In addition to conservation concerns, these data point toward the potential risk of keeping these animals as pets as it appears that they may be exposed to leptospiral infection in the wild or after entrapment.

This study also evaluated the possible association of serological evidence of exposure with active infection and renal carriage using PCR detection of leptospiral DNA in blood and urine samples, respectively. None of the evaluated samples yielded positive results. Further studies with larger sample groups of animals rescued from illegal trade are warranted to estimate the risks of infection from intimate contact with exotic animals, including Neotropical primates, when kept as pets.

CONCLUSIONS

The seroprevalence of antileptospiral antibodies was low (2%) in the Zoological Park of Salvador, Brazil, despite the high frequency of rodents in the area and the endemicity of human leptospirosis in Salvador.

A higher rate (31%) was observed among the animals rescued from illegal trade in the state of Bahia.

Serological evidence of exposure does not predict an active infection or the renal carrier state in non-human primates.

Even if all the blood and urine samples were negative for leptospiral DNA fragments, the high frequency of serological evidence of exposure suggests a potential risk of leptospirosis transmission when keeping these animals as pets.

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