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COMMENTARY

Detection of Anti-*Leptospira* Antibodies in Captive Nonhuman Primates From Salvador, Brazil

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Leptospirosis is a widely distributed zoonosis that affects several species of domestic and wild animals. Under captive conditions, Leptospirosis is a potential problem because the physical conditions in most zoos and research centers cannot prevent the captive animals from being exposed to rodents, raccoons, opossums, and other local wildlife that are known carriers. Yet, despite the potential risk, animals that are destined for reintroduction into the wild are not routinely tested for anti-*Leptospira* antibodies before their release. The purpose of this study was to determine the occurrence of anti-*Leptospira* antibodies in captive New World monkeys that were housed in the Wild Animals Screening Center in Salvador, Brazil. Blood samples were collected from 44 monkeys (28 *Callithrix jacchus*, eight *Callithrix pennicilata*, and eight *Cebus* sp.). The animals were screened for antibodies with the microscopic agglutination test. Twenty-five (56.8%) primates were seroreactive, with Icterohaemorrhagiae being the most frequent serogroup. None of the monkeys, however, presented clinical signs of leptospirosis. Thus, seroreactivity with low titers in asymptomatic animals, as observed in this study, suggests exposure to the agent. The unexpected predominance of the serogroup Icterohaemorrhagiae further suggests that exposure to this serogroup occurred in captivity. Therefore, the dangerous possibility cannot be ignored that reintroduced monkeys will carry the leptospiral serovars into wild populations. In conclusion, primates exposed to urban serovars before their release from captivity represent a potentially significant health risk to wild populations. Am. J. Primatol. 74:8–11, 2012.

Key words: leptospirosis; nonhuman primates; diagnosis; reintroduction

INTRODUCTION

Leptospirosis is a widely distributed zoonosis that affects several species of domestic and wild animals, and humans are the endpoint of its epidemiological chain. An infected animal, even when asymptomatic, can intermittently introduce viable leptospires into the immediate environment through its urine [Faine et al., 2000].

Wildlife species are susceptible to infection with a wide variety of serovars, for which they serve as incidental hosts. Acute leptospirosis is similar in all animals. In its most flagrant form, it is manifested by listlessness, loss of appetite, irritability, fever, ruffled fur, red eyes, and sometimes diarrhea, hemorrhage, jaundice, spontaneous abortion, chronic renal failure, and death [Faine et al., 2000].

Although frequently present in Brazilian wildlife, anti-*Leptospira* antibodies are not often described in captive animals [Lilenbaum et al., 2002]. The occurrence of leptospirosis under captive conditions, however, presents a potentially significant risk. Housing conditions in zoos and research centers often cannot fully isolate the captive animals from local wildlife (e.g., rodents, raccoons, opossums, etc.), which may gain entry into these facilities [Baulu et al., 1987; Perolat et al., 1992; Scarcelli et al., 2003; Shive et al., 1969; Szonyi et al., 2011].

Captive nonhuman primates may be exposed to [Lilenbaum et al., 2005] and carry leptospirosis [Baitchman et al., 2006; Palmer et al., 1987; Szonyi et al., 2011]. Thus, projects that return wildlife to

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their natural habitats should implement a strict quarantine—60 days for primates—and veterinary program for these animals before their release. A variety of screening tests are recommended by the International Union for Conservation of Nature [IUCN, 1998], but a test for anti-*Leptospira* antibodies is not currently one of them. Not screening for these antibodies represents a risk, because *Leptospira* is dangerous for humans as well as domestic animals, wildlife, and ecosystems in general [Woodford, 2001]. In an attempt to assess the extent of this risk, this study investigated the occurrence of anti-*Leptospira* antibodies in representatives of New World monkey species that were being held in captivity for health screening, before being returned to their natural habitats.

METHODS

The study was approved by the Research Ethics Committee of Federal University of Bahia (process no 025/09-A) and by the Environmental Department of Brazil (IBAMA–SISBIO no 20.831-1). This research adhered to the American Society of Primatologists principals for the ethical treatment of primates.

In September 2009, blood samples were collected from 44 monkeys (28 *Callithrix jacchus*, 8 *Callithrix pennicilata*, and 8 *Cebus* sp.) held in captivity at the Chico Mendes Wild Animal Screening Center, a facility located near the Federal University of Bahia in Salvador, Brazil. The monkeys had been brought to the Center by the Brazilian legal authorities following the arrest of poachers who had trapped the animals in the forest for the purpose of selling them on the black market. None of the monkeys had been vaccinated against leptospirosis. Before the samples were collected, the animals were anesthetized (Ketamine hydrochloride 10%, 20–30 mg/kg IM) and then given a complete clinical examination.

After separation by centrifuge, the serum was stored in 2 ml aliquots at −20°C for subsequent testing in the Veterinary Bacteriology Laboratory at the Universidade Federal Fluminense in Rio de Janeiro, Brazil. Antibodies were screened at a dilution of 1:50 with the microscopic agglutination test (MAT) for the most frequent serogroups in this area [Lilenbaum et al., 2005]: *L. interrogans* serovars Australis (Ballice), Bataviae (Van Tienen), Canicola (Hond Utrecht IV), Icterohaemorrhagiae (RGA), Copenhageni (M 20), Pomona (Pomona), Pyrogenes (Salinem), and Wolffi (3705); *L. borgpetersenii* serovar Ballum (Mus 127); and *L. kirshneri* serovar Grippotyphosa (Moskva V). The serovars were grown in an Ellinghausen liquid medium, which was free of contamination or autoagglutination. For each sample with agglutinating activity at a 1:50 dilution, the antibody titer was established by preparing additional two-fold serial dilutions according to Lilenbaum et al. [2002]. The antigen that gave the highest titer was considered to be the infective serogroup. Samples were considered to be reactive if the titer value was equal to or greater than 100.

RESULTS

At the time of sampling, none of the 44 monkeys studied presented clinical signs of leptospirosis. Twenty-five (56.8%) were seroreactive (titers $\geq$100). Of this seroreactive group, the serogroup Icterohaemorrhagiae was found most frequently (21 monkeys or 84%), followed by the serogroup Canicola (4 monkeys or 16%).

Seroreactivity was most commonly observed in *Cebus* sp. (62.5%), followed by *C. jacchus* (57.1%) and *C. pennicilata* (50%). Titers ranged from 100 to 200, indicating exposure to the agent. No animal presented titer values greater than 200 (Table I).

DISCUSSION

Seroreactivity was very high in the monkeys investigated in this study (56.8%), higher than that reported in tamarins from Rio de Janeiro, Brazil: 35.6% [Lilenbaum et al., 2005], squirrel monkeys from French Guiana: 26% [Perolat et al., 1992], or vervet monkeys in Barbados: 29.9% [Baulu et al., 1987]. Despite this high seroreactivity, however, none of the monkeys in this study presented clinical signs related to leptospirosis.

The MAT requires significant expertise to perform, and interlaboratory variation in results is high. Despite these limitations, the MAT has epidemiological value, and it is often used to give an indication of the presumptive serovar or serogroup of leptospires involved in an infection [Levett, 2003; Smythe et al., 2009; Sykes et al., 2011].

A nonhuman primate acquiring acute leptospirosis with high titers while living in the wild is uncommon [Minnette, 1966]. As found by others [Szonyi et al., 2011], however, this investigation reveals that monkeys can nevertheless carry the infection at low titers and not present specic signs or symptoms. Such cases suggest that the animals have been exposed to the agent. Moreover, following exposure, some monkeys may shed leptospires through intermittent leptospiruria (i.e., passing

TABLE I. Anti-*Leptospira* Antibodies in 44 Captive Primates From Salvador, Brazil

	Anti-*Leptospira* titers					
Species	<100	100	200	400	≥800	Total
Callithrix jaccus	12	11	5	–	–	28
Callithrix pennicilata	4	4	–	–	–	8
Cebus sp.	3	3	2	–	–	8
Total	19	18	7	–	–	44

leptospires in urine) [Baitchman et al., 2006; Szonyi et al., 2011].

Wildlife populations are frequently seroreactive to the serovars that are prevalent in their environmental surroundings [Lins & Lopes, 1984]. For Brazilian monkeys living in the wild, the most frequent serovars are Javanica, Ballum, Tarassovi, and Grippotyphosa [Correa et al., 1965]. Thus, this study's finding that 84% of the seroreactive monkeys have been exposed to the serogroup Icterohaemorrhagiae is surprising. This serogroup is reported in urban areas worldwide, including Salvador, the city in which the monkeys in this study were housed [Ko et al., 1999]. Although in captivity, animals can be exposed to urban serovars. Compared with their counterparts in the wild, captive monkeys spend more time on the ground, increasing their chances of contact with ground-dwelling urban rodents that are known carriers of leptospirosis, especially of serovars in the serogroup Icterohaemorrhagiae [Lilenbaum et al., 2002].

Considering the above, we suggest that the monkeys were not exposed to Icterohaemorrhagiae in the wild, but in captivity. A similar situation has been recently reported in Colombia [Szonyi et al., 2011], where capuchin monkeys (*Cebus* sp.) in a rehabilitation center were infected with the serovar Copenhageni (a member of serogroup Icterohaemorrhagiae), triggering an outbreak.

Direct transmission of *Leptospira* among monkeys has already been reported [Baulu et al., 1987]. Although not demonstrated in this study, Leptospira-exposed monkeys are known to become carriers [Baitchman et al., 2006; Szonyi et al., 2011]. Nevertheless, studies of the actual risk or frequency of primates serving as permanent or temporary reservoirs for leptospires are lacking. Additionally, once they have recovered from the illness, infected monkeys act as renal carriers, at least for a short time period [Baitchman et al., 2006; Szonyi et al., 2011]. Therefore, the possibility that infected monkeys can carry leptospiral serovars from captivity into the wild cannot be ignored. Although not yet confirmed, these reintroduced animals pose a potential health risk to wild populations [Daszak et al., 2000], particularly in tropical areas where the infection is common.

We recommend that a program be adopted that (1) isolates infected animals and (2) emphasizes the implementation of sanitation initiatives and hygienic conditions (e.g., routinely disinfecting shelters, minimizing exposure to stagnant water, rodent control) [Troedsson, 1997].

In conclusion, primates may be exposed to urban leptospiral serovars (e.g., Icterohaemorrhagiae) and represent a significant health risk to wild populations. To minimize this risk, we recommend that captive monkeys living naturally in those regions where the disease is endemic be tested for anti-*Leptospira* antibodies before being released back into the wild.

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