

***Leptospira* spp. AND *Toxoplasma gondii* ANTIBODIES IN VAMPIRE BATS
(*Desmodus rotundus*) IN BOTUCATU REGION, SP, BRAZIL**

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ABSTRACT: The destruction of natural ecosystems has caused several problems to humans and other animals; herein we investigate the close relationship among vampire bats, humans and domestic animals. *Toxoplasma gondii* and *Leptospira* spp. infections are two worldwide zoonoses that provoke serious damage to animals. To determine the prevalence of bats seropositive for toxoplasmosis and leptospirosis in the Botucatu region, 204 serum samples of vampire bats (*Desmodus rotundus*) were tested for *T. gondii* antibodies by modified agglutination test (MAT-t) and for *Leptospira* spp. by microscopic agglutination test (MAT-l). No animal was tested positive for *T. gondii* while leptospiral positivity was 7.8% for Pyrogenes, Shermani and Javanica serovars, with titers varying from 100 to 1,600. Thus, it was verified that *D. rotundus* does not play a relevant role in toxoplasmosis epidemiology. However, these bats can be important in the maintenance of *Leptospira* spp. in the environment.

KEY WORDS: vampire bats, zoonosis, serological survey, public health.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Chiropterans are one of the most distinct mammals that play an important role in nature spreading seeds, pollinating flowers and controlling the insect population (1, 2). *D. rotundus* individuals are found in colonies of 20 to 100 that may use caves, tree holes, abandoned mines, house basements, drainpipes, etc. as roosts (3, 4).

The presence of vampire bats near residences and livestock in the Botucatu municipality, favored by the creation of several embankments during the construction of Castelo Branco highway with many areas of water drainage pipes, has contributed to the dissemination of many infectious diseases, including toxoplasmosis and leptospirosis, thus constituting a great public health problem (5).

Toxoplasmosis is a parasitic zoonosis caused by *Toxoplasma gondii* protozoan that presents a worldwide distribution. Moreover, a great part of the human and animal populations is infected by *T. gondii* (6, 7). Chiropterans may be infected by ingestion of oocysts present in the environment, food or water contaminated by feces of infected felids; by consumption of bradyzoites present in tissue of infected intermediate hosts; and also by the transmission of tachyzoites via the transplacental route.

Leptospiral infection is another global zoonosis caused by a spirochaeta, namely, *Leptospira* spp. (8). This bacterium colonizes the kidneys of reservoir animals and is eliminated in their urine. Humans and other animals get infected either directly or indirectly, through skin, oral, nasal or conjunctiva mucosae; however, humans represent a dead-end as accidental hosts (9, 10). The most common transmission routes are water, soil and food contaminated with urine of infected animals.

Contaminated bats constitute a significant infection source since they can carry leptospores (11, 12). Matthias *et al.* (13) reported that renal infection rates may differ among bat genera, and that parasitic burden was most frequent in the kidneys of *Promops*, *Desmodus* and *Myotis* that tested positive by PCR in the following respective percentages: 100%, 50% and 33%. These animals excrete the agent in urine, thus contaminating the environment or even their victims (animals or humans), as they have the habit of urinating while feeding, because wounds caused by vampire bats are a gateway for this bacterium. The possibility that *D. rotundus* may feed on blood of a leptospiremic animal and therefore get infected, should also be considered.

Due to scarce reports on the role of these bats in the epidemiological chain of leptospirosis and toxoplasmosis, we aimed to study the serological profile of both infections among *D. rotundus*, considering their feeding habits and the potential environmental contamination.

A total of 204 vampire bats (*D. rotundus*) were captured from: Bofete (36 animals), Pardinho (10), São Manuel (45), Pratânia (24), Botucatu (16), Itatinga (28) and Anhembi (45). Capture was carried out at night between 6 and 11 p.m. using meshes set up in front of roosts, inside pipes under highways, under railways, in front of caves and tree holes. The animals were sent to the Zoonosis Diagnosis Laboratory, in the Department of Veterinary Hygiene and Public Health, UNESP, Botucatu, SP, Brazil, where they were anesthetized in large-mouthed flasks with cotton soaked in sulfuric ether. Blood was collected via intracardiac puncture, and was centrifuged at 1600 x g for 10 minutes. The resulting serum was frozen at -20°C.

To investigate *Leptospira* spp. antibodies, the microscopic agglutination test (MAT-I) was performed according to the Ministry of Health guidelines (14). Previously, screening was carried out and positive samples were titred. The following leptospiral serovars were tested: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Djasiman, Sentot, Gryppotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo Prajitno, Hardjo Miniswajezak, Hardjo C.T.G., Hardjo Bovis, Wolffi, Shermani, Tarassovi, Andamana and Patoc, considering titration the greatest serum dilution able to agglutinate 50% or more leptospires, compared to the control. Regarding toxoplasmosis, modified agglutination test (MAT-t) was performed according to Desmonts and Remington (15).

All serum samples were negative to toxoplasmosis and 7.84% reacted to different leptospiral serovars, with titers ranging from 100 to 1600 (Table 1).

Table 1. MAT-I results of the studied *Desmodus rotundus*, with respective serovars and reactive titers

Origin	N. samples	N. reagents (%)	Serovars (titers)
Anhembi	45	6 (13.33)	Pyrogenes (400), Javanica (200) Pyrogenes (100) Shermani (200) Shermani (100) Pyrogenes (200) Pyrogenes (100)
Bofete	36	1 (2.78)	Pyrogenes (800)
Botucatu	16	0 (0.00)	–
Itatinga	28	0 (0.00)	–
Pardinho	10	2 (20.00)	Pyrogenes (1600), Javanica (200) Pyrogenes (400)
São Manuel	45	5 (11.11)	Pyrogenes (800) Pyrogenes (800), Javanica (400) Pyrogenes (100) Pyrogenes (100) Javanica (800), Pyrogenes (100)
Pratânia	24	2 (8.33)	Pyrogenes (200) Pyrogenes (400)
Total	204	16 (7.84)	–

N.: number of animals; MAT-I: microscopic agglutination test for *Leptospira* spp.

The toxoplasmosis results agree with Smith and Frenkel (16), who while studying the occurrence of antibodies against *T. gondii* in insectivorous bats, verified also negative results to Sabin-Feldman dye test in one big brown bat (*Eptesicus fuscus*), one red bat (*Lasiurus borealis*), and two evening bats (*Nycticeius humeralis*). These findings indicate that bats may be infected, but with lower probability than other mammals.

The negative results concerning *T. gondii* were probably due to the fact that vampire bats do not usually have any contact with the soil, where infectious oocysts are found. They only ingest their prey blood and have no contact with bradyzoites

present in cysts. Thus, infection would only be possible by ingesting blood of an animal at an acute stage of the disease with flowing tachyzoites, which rarely occurs because toxoplasmosis is mostly a chronic disease.

In a study conducted by Smythe *et al.* (17) in Australia, 28% of the frugivorous or nectarivorous bats belonging to the genus *Pteropus* exhibited antibodies for *Leptospira* spp., which represents seven different serovars, with Australis (75%) being the most common infectious serovar.

The 7.84% positivity found herein had Pyrogenes (87.5%) as the most prevalent serovar, followed by Javanica (25%), and then by Shermani (12.5%). The great majority of the chiropterans captured were caught in drainpipes and tunnels where a large number of rodents is found, suggesting leptospiral transmission among rodents, bats and humans/domestic and wild animals, mainly livestock, because bats were captured in rural areas only.

Cox *et al.* (18) studying Australian flying foxes (*Pteropus* spp.), found in 11% of kidney samples *Leptospira* spp., which indicates that flying foxes, known as fruit bats, are carriers of this pathogen. The identification of one in ten animals with leptospiral DNA suggests that leptospire are more prevalent in flying fox populations than would be expected for a species that is not a carrier.

Thus, the results allow us to conclude that *D. rotundus*, and probably all other bat species, do not play a leading role in the maintenance or transmission of *T. gondii* to humans and to other animals. Further studies should be conducted to establish the role of these animals as *Leptospira* spp. reservoirs.

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