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The role of capybaras as carriers of leptospires in periurban and rural areas in the western Amazon

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ABSTRACT

Although leptospirosis has been described in capybaras, usually based on serological evidences, bacterial culture of leptospires has been scarcely reported in this species. The western Amazon is a reportedly endemic area where high seroprevalences have been reported in different species of wildlife, domestic animals and in human beings. The present study aimed at investigating the role of capybaras as carriers of leptospires in periurban and rural areas in the western Amazon region. A total of 44 animals were captured, and 41 blood samples (for serology) and 41 urine samples (for PCR and bacterial culture) were obtained. A total of 18/41 (43.9%) of sera were reactive and titers were generally low, indicating chronic infection. PCR was positive in 13/41 (31.7%) samples, isolates were recovered from urine samples belonging to Icterohaemorrhagiae, Grippotyphosa and Shermani serogroups. A high number of carriers (confirmed by PCR) associated to a tendency for harboring Icterohaemorrhagiae serogroup strains could be noticed. Our results suggest that capybaras are massively infected by leptospires. Analogously to Norway rats, capybaras present chronic infection with low titers and long-term bacterial shedding, and may be acting as reservoirs of this bacterium.

1. Introduction

Leptospirosis is an infectious zoonotic disease determined by bacteria of the *Leptospira* genus. This disease appears in well-defined rural and urban cycles (Haake and Levett, 2015). In the disease's urban cycle, rodents mainly, but not exclusively, *Rattus norvegicus*, are known as reservoirs of this bacterium. Other rodents have also been recognized as reservoirs, e.g. *Rattus rattus*, *Mus musculus* (Fortes-Gabriel et al., 2016), and the wild species *Cavia aperea* (Monte et al., 2013), *Arvicola* sp., *Crocidura* sp., *Talpa* sp., *Sorex* sp., and *Microtus* sp. (Obiegala et al., 2016).

The Amazon is the largest rainforest in the world and in the past decades human population has exceedingly increased in this region. The region has an equatorial climate (Af and Am by Koeppen classification) and a close proximity between cities and forest (Saleska et al., 2016). Rio Branco is the biggest city in the western Amazon region (population around 400,000) and is surrounded, on one side, by extensive areas of Amazonian rainforest and, on the other side, by rural cattle breeding areas. Leptospirosis is reportedly endemic in the western Amazon (Chiebao et al., 2015) and high seroprevalences have been reported in different species of wildlife and domestic animals (Jori et al., 2009; Furtado et al., 2015), as well as in human beings (Donaires et al., 2012).

The capybara (*Hydrochoerus hydrochaeris*) is the largest living rodent in the world. It can be found in Latin America, from Panama to Uruguay (García-Esponda and Candela, 2016). This species requires abundant and permanent water supply for its survival (Alho and Rondon, 1987). This rodent has been reported as a reservoir of other pathogens, such as *Toxoplasma* sp. (Abreu et al., 2016), *Trypanosoma* sp. (da Silva et al., 2016), and *Rickettsia* sp. (Monje et al., 2015).

Leptospirosis in capybaras has been described in various regions of Brazil, usually based on serological evidences (Silva et al., 2009; Chiacchio et al., 2014; Langoni et al., 2016). These studies found a seropositivity of 26–41.2% among the studied capybaras, and reported finding Australis, Canicola, Tarassovi, Icterohaemorrhagiae and Pomona serogroups. Nevertheless, serology cannot be considered a reliable tool for diagnosing leptospiral infection, since it may simply indicate exposure to the agent. The gold-standard

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diagnostic method of bacterial isolation has been scarcely reported in capybaras and points out this species as a potential source of infection. The National Collection of Leptospires of Animal Origin (www.labv.uff.br) refers to only ten strains ever recovered from this rodent, of which seven are from the Grippotyphosa serogroup (Marvulo et al., 2002), two from the Shermani serogroup (S.A.Vasconcellos, personal communication) and only one from the Icterohaemorrhagiae serogroup (Jorge et al., 2012). More recently, the isolation and molecular characterization of five strains from capybaras from Brazil have been reported. Curiously, these strains were all identified as *L. santarosai*and belonged to the serogroup Grippotyphosa, serovar Bananal (Moreno et al., 2016).

Considering this, the present study aimed at investigating the role of capybaras as carriers of leptospires in periurban and rural areas in the western Amazon region.

2. Material and methods

Handling procedures in accordance with Ethical Principles in Animal Research adopted by the Animal Ethic Committee of the Federal University of Acre (process number 23107.016723/2014-41) and in full compliance with federal permits issued by the Brazilian Ministry of the Environment (License SISBIO number 44791-1) were followed.

2.1. Study area

All the studied regions were located around Rio Branco (9°58′33.0″S 67°49′32.2″W), capital of the state of Acre, in the western Amazon region. Periurban regions were represented by a recently occupied borderline area of the city, contiguous to the Amazon rainforest. Rural regions comprised two different farms located 15 km from the city (farm I: 10°01′41.1″S 67°57′29.3″W; farm II: 10°00′53.0″S 67°59′09.9″W). In both areas, animals could circulate from forest to studied areas.

2.2. Animals

Animals were captured using corral-traps with food offered daily. Upon entry, the trap closed and the animals were mechanically contained in dip nets. After this, the animals were identified by microchips and anesthetized with azaperone (1.0 mg/kg), ketamine (12 mg/kg) and diazepam (0.1 mg/kg) applied intramuscularly (King et al., 2010). A total of 44 capybaras were captured, 21 from rural and 23 from periurban areas. All captured animals were rigorously examined by veterinarians and no symptoms of clinical leptospirosis (acute disease) were observed.

2.3. Sampling

From the 44 animals, three blood samples presented hemolysis and were not included in this study, one from rural and two from periurban areas. Thus, a total of 41 blood samples were studied. Sampling occurred by venopuncture of the femoral vein (Vacutainer®, BD, Franklin Lakes, NJ, USA).Then, samples were transported to the laboratory and centrifuged. Serum samples were labelled and stored in 1.5 mL microtubes (Eppendorf®, São Paulo, SP, Brazil) at −20 °C to be tested as a batch. Three animals had empty bladders at the time of urine collection, one from rural and two from periurban areas; hence, urine sampling was not possible. Thus, we were able to obtain 41 urine samples, collected by cystocentesis, that were chilled and transported to the laboratory in syringes. Aliquots of urine samples were used for bacteriological culturing and PCR.

2.4. Serology (MAT)

For the detection of anti-*Leptospira* antibodies, Microscopic Agglutination Test (MAT) was performed with a complete panel including 28 serovars representing 24 serogroups (from Institut Pasteur, Paris, France), according to international standards (OIE, 2014). The serogroup with the highest titer was considered the infective serogroup and animals with titers ≥100 were considered seroreactive.

2.5. PCR

DNA was extracted from urine using the Promega Wizard SV Genomic DNA Purification System® (Promega, Madison, USA). We targeted the *lipL32* gene, referred to be present only in pathogenic leptospiras (lipL32_45F - 5′AAG CAT TAC TTG CGC TGG TG 3′ and lipL32_286R - 5′TTT CAG CCA GAA CTC CGA TT 3′), following PCR conditions described by Hamond et al. (2014).

2.6. Bacterial culturing and serological characterization of isolates

A few drops of urine were seeded into two tubes containing 5 mL of EMJH (BD Difco, Franklin Lakes, NJ, USA), two tubes with 5 mL of EMJH supplemented with a STAFF antimicrobial cocktail (EMJH-STAFF; Loureiro et al., 2015) and two tubes containing 5 mL of Fletcher (BD Difco, Franklin Lakes, NJ, USA). Cultures were incubated at 28 °C and weekly evaluated with dark field microscopy for 30 weeks.

Obtained isolates were tested by Microscopic Agglutination Test (MAT), against a panel of rabbit antisera of 32 reference serovars representing 24 serogroups (provided by Royal Tropical Institute – KIT, Amsterdam), as recommended by Haake and Levett (2015).

2.7. Statistics

Statistical analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Serological and molecular data were treated using the Chi-square test and Fisher's exact test. A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Serology (MAT)

Reactive serum samples were detected in 18/41 (43.9%) of the studied animals. Reactivity was 10/20 (50%) in rural areas and 8/21 in periurban areas (38.1%); a non-significant difference (Table 1). Seroreactivity was very similar to results described in a recent study (Langoni et al., 2016) conducted in capybaras from commercial and experimental breeding facilities in the southeast region of Brazil (41.3%). In contrast, a study conducted in free-ranging capybaras from a park in São Paulo, southeastern Brazil reported a seroreactivity level of 26% (Chiacchio et al., 2014), while another study reported a 27.3% seroprevalence in capybaras from a slaughterhouse in the south region of Brazil (Silva et al., 2009). It is noteworthy to point out that, due to the paucity of studies regarding leptospirosis in capybaras, a wide comparison with literature on this topic is difficult to perform.

Amazonian environmental conditions are highly favorable for the maintenance of leptospires (Jori et al., 2009). Thus, the high seroreactivity observed in animals of the present study, as well as in other studies conducted on wildlife and domestic species from the Amazon biome, such as the collared peccary (*Tayassu tajacu* – 86.4%), maned wolves (*Chrysocyon brachyurus* – 75.0%), manatees (*Trichechus inunguis* – 31.1%), cattle (73.6%) and dogs (37.5%)(Deem