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SEROLOGICAL EVIDENCE OF *LEPTOSPIRA* SPP. IN THE LAMI TUCO-TUCO RODENTS (*CTENOMYS LAMI*)

Simone Tostes Oliveira[1], Gisele Guiomara Stein[1], Andrea Pires Santos[2], Alexander Welker Biondo[1], Joanne Belle Messick[2], Felix Hilario Diaz Gonzalez[3]

[1] UFPR
[2] Purdue University
[3] UFRGS
Correspondência: Simone Tostes Oliveira: tostesimone@gmail.com.br

ABSTRACT: Although rodents are reportedly the major reservoirs of *Leptospira* spp. in the wildlife of Brazil, the role of the widely distributed native tuco-tuco rodent (*Ctenomys lami*) has yet to be determined. Accordingly, a total of 40 serum and eight urine samples from wild *C. lami* were collected from June to September 2008 in the city of Porto Alegre, Southern Brazil. The serum samples were screened using the Microscopic Agglutination Test against 13 *Leptospira* spp. pathogenic serovars. A polymerase chain reaction (PCR) was performed to detect the presence of leptospiral DNA in the urine samples. Five (12.5%) of the serum samples had ≥100 antibody titer levels against one or more of the serovars. None of the urine samples yielded positive PCR amplification. In addition, all animals which had urine samples tested had also negative antibody serum titers. In conclusion, although *C. lami* may be exposed to *Leptospira* spp., infection may be occasional because no detectable leptospiruria was found.
Key Words: leptospirosis; rodent; serology; wildlife; PCR

EVIDÊNCIA SOROLÓGICA DE *LEPTOSPIRA* SPP. EM LAMI TUCO-TUCOS (*CTENOMYS LAMI*)

RESUMO: Apesar dos roedores serem os principais reservatórios de *Leptospira* spp. da fauna silvestre no Brasil, o papel do roedor nativo tuco-tuco Lami (*Ctenomys lami*) ainda não foi determinado. Um total de 40 amostras de soro e oito de urina foram coletados de *C. lami* entre junho e setembro de 2008, no Rio Grande do Sul. As amostras de soro foram testadas através do teste de soroaglutinação microscópica (SAM) contra 13 sorovares de *Leptospira* spp. Reação em cadeia da polimerase (PCR) foi realizada para detectar a presença de DNA de leptospiras patogênicas nas amostras de urina. Cinco (12,5%) das amostras sorológicas tiveram títulos ≥100 contra um ou mais sorovares. Nenhuma das amostras de urina teve amplificação positiva por PCR; no entanto, estes oito animais eram também soronegativos. Apesar da exposição do *C. lami* à *Leptospira spp.,* a infecção pode ser ocasional visto que não foi detectada leptospirúria.
Palavras-chave: leptospirose; roedor; sorologia; PCR; fauna silvestre

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INTRODUCTION

Leptospirosis is a zoonotic bacterial disease that may infect humans and domestic and wild animal species. Rodents have been considered important reservoirs of pathogenic *Leptospira* serovars (LEVETT, 2001). Although the control of rodents may decrease the risk of transmission to incidental hosts and environmental contamination, environmental maintenance of pathogenic *Leptospira* spp. causes the spread of leptospirosis in wild animals (GUERRA, 2009).

The tuco-tucos are fossorial rodents belonging to the genus *Ctenomys* in the family Ctenomyidae, which includes nearly 60 species. They are distributed throughout the southern half of South America (REIG *et al.,* 1990). Lami tuco-tuco (*Ctenomys lami*) is distributed along a sandy area (Coxilha das Lombas) of Southern Brazil, spreading from the northern coast of Lake Guaíba to an area northwest of the Lagoa dos Barros (FREITAS, 2001). Even though some of these areas are environmentally protected, they are under anthropic impact due to the surrounding livestock production. To the authors' knowledge, there are no published data regarding the occurrence of leptospirosis in *C. lami*. Accordingly, the aim of this study was to detect evidence of exposure of wild Lami tuco-tucos to *Leptospira* spp.

MATERIAL AND METHODS

A total of 40 adult Lami tuco-tucos were live-trapped from three areas in Rio Grande do Sul State, Brazil from June to September of 2008. Areas A (Distrito de Itapuã, 30°17'31.2''S, 50°58'31.6''O) and B (Município de Viamão, 30°8'0.8''S, 50°54'38.2''O) are livestock production areas and were affected by human inhabitation. Area C (Município de Viamão, Unidade de Conservação Refúgio de Vida Silvestre Banhado dos Pachecos, Distrito de Águas Claras, 30°5'31.2''S, 50° 50'35''O) was not inhabited by humans. A total of 11, 16 and 13 animals were captured from areas A, B and C, respectively.

The Lami tuco-tucos were weighed and anesthetized with intramuscular ketamine (20 mg/kg of body weight). All animals were evaluated and considered to be clinically healthy. Blood was collected from the cranial vena cava, kept in tubes without anticoagulant at room temperature and subsequently centrifuged to obtain serum. Urine samples were successfully collected from eight animals using gentle abdominal hand pressure. After anesthetic recuperation, all animals were released in their respective native tunnels.

Using the Microscopic Agglutination Test (MAT), the presence of anti-*Leptospira* antibodies was tested in 13 serovars most usually found in Brazil: australis, autumnalis, bratislava, canicola, copenhageni, grippotyphosa, hardjo, hebdomadis, icterohaemorrhagiae, pomona, pyrogenes, tarassovi and wolffi. A titer $\geq$100 was considered positive.

DNA from the urine samples was extracted in duplicate (LUCCHESI *et al.*, 2004) and a conventional polymerase chain reaction (PCR) was performed to detect pathogenic leptospires using the primer sets G1/G2 and B64-I/B64-II (GRAVEKAMP *et al.*, 1993). Positives urines from dogs for both primer sets and DNA extracted from leptospire's cultures were used as positive controls and included in each run.

RESULTS

A total of 5/40 (12.5%) rodents were seropositive for *Leptospira* by MAT, with titers of 100. From area A, two samples were positive for the

serovar wolffi and one sample had equal titers for the serovars wolffi, hardjo and icterohaemorrhagiae. One sample from area B was seropositive for the serovars copenhageni, grippotyphosa, pomona and pyrogenes. From area C, one sample was positive for the serovar pomona.

All urine samples tested negative on the PCR amplification. All eight rodents were seronegative as well.

DISCUSSION

To the authors' knowledge, this is the first report of an investigation of leptospirosis in *C. lami* using serology and PCR. The positive serological results indicated that the *C. lami* had contact with pathogenic leptospires. The source of contamination was unclear, but possible contact with cattle, small mammals or contaminated water should be considered.

Although all of the PCR analyses in urine were negative, the possibility of leptospirosis transmission by *C. lami* cannot be discounted. Moreover, other members of the infraorder Hystricognathi (WILSON & REEDER, 2005) have been reported to have had positive culture/isolation of leptospires in their kidneys and/or urine: the coypu (*Myocastor coypus*) in France (MICHEL *et al.*, 2001) and in Great Britain (WAITKINS *et al.*, 1985) and the capybara (*Hydrochaeris hydrochaeris*) in Brazil (Marvulo *et al.*, 2002, MARVULO *et al.*, 2009, JORGE *et al.*, 2010).

CONCLUSION

Although *C. lami* may be exposed to *Leptospira* spp., infection may be occasional because no detectable leptospiruria was found. Whether the tuco-tuco rodents act as reservoirs of the disease remains to be conclusively established.

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