

# The spiny rat *Proechimys guyannensis* (Rodentia: Echimydae) fails to respond to intradermal inoculation with *Leishmania (Viannia) braziliensis*

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## ABSTRACT

Leishmaniasis a disease of worldwide occurrence is caused by protozoa of the *Leishmania* genus. In Brazil, *Leishmania (Viannia) braziliensis* is the main parasite responsible for the American cutaneous leishmaniasis. Main hosts of this protozoa are small wild mammals particularly marsupials and rodents. The aim of this study was to evaluate if spiny rat *Proechimys guyannensis* (Rodentia: Echimydae) has role in the cycle of the American cutaneous leishmaniasis caused by *L. (V.) braziliensis*. Thus, promastigotes (the flagellate stage) of *Leishmania (Viannia) braziliensis* were used to inoculate seven spiny rats (*Proechimys guyannensis*). After inoculated intradermal at the ear pinna, nose and plantar pad, the rats were monitored for 180 days. Tissue samples collected at 90 and 180 days from the rats proved to be negative for the presence of genetic material from the parasite. After euthanasia, the protozoa also failed to growth in culture medium containing tissue samples collected from the rats showing that there was no infection. These results fail to prove that spiny rat has a role in the cycle of the American cutaneous leishmaniasis caused by *L. (V.) braziliensis*.

**KEYWORDS:** Leishmaniasis, American cutaneous leishmaniasis, rodents, reservoirs

## O rato espinhoso *Proechimys guyannensis* (Rodentia: Echimydae) falhou para inoculação intradérmica com *Leishmania (Viannia) braziliensis*.

## RESUMO

A leishmaniose é uma doença de ocorrência mundial causada por protozoários do gênero *Leishmania*. No Brasil, a *Leishmania (Viannia) braziliensis* é o principal parasita responsável pela leishmaniose tegumentar americana. Os principais hospedeiros deste protozoário são pequenos mamíferos selvagens em particular marsupiais e roedores. O objetivo deste estudo foi avaliar o papel do rato espinhoso *Proechimys guyannensis* (Rodentia: Echimydae) no ciclo da leishmaniose tegumentar americana. Para isto, formas promastigotas (estágio flagelado) de *Leishmania (Viannia) braziliensis* foram inoculadas em sete ratos espinhosos (*Proechimys guyannensis*). Após a inoculação intradérmica no pavilhão auricular, focinho e área plantar, os ratos foram monitorizados durante 180 dias. Amostras de tecido colhidas aos 90 e 180 dias dos ratos revelaram-se negativas para a presença de material genético do parasita. Após eutanásia, tecidos coletados dos ratos também falharam para crescimento em meio de cultura demonstrando que não houve infecção. Estes resultados demonstram que o rato espinhoso não tem papel no ciclo da leishmaniose tegumentar americana causada por *L. (V.) braziliensis*.

**PALAVRAS-CHAVE:** Leishmaniose, leishmaniose tegumentar americana, roedores, reservatórios.

Leishmaniasis is a disease of worldwide occurrence, caused by protozoa of the *Leishmania* genus. In Brazil, *Leishmania (Viannia) braziliensis* is the main causative agent of American Cutaneous Leishmaniasis - ACL (Roque *et al.* 2010). *Leishmania* genus exist in two basic body forms: the amastigote, the intracellular form in the vertebrate host, and the promastigote, the extracellular form in the sandfly (*Phlebotomus* spp. and *Lutzomyia* spp.) vector or culture medium. The main hosts of this agent are small wild mammals, especially rodents and marsupials. Spiny rats from the genus *Proechimys* are among the most abundant Amazonian rodents (Eler *et al.* 2012). These rodents have attracted interest in the area of biology and epidemiology of viral and tropical zoonotic diseases because they are often found as hosts of protozoa of the genus *Trypanosoma* and viruses (Carrara *et al.* 2005; Rosa *et al.* 2005; Pinto *et al.* 2006). These rodents are also reported as being reservoirs of *L. (L.) amazonensis* in the Amazon region (Brandão-Filho *et al.* 2003) and studies indicate their potential to act as a host of other species causative of skin disease, such as *L. (V.) panamensis* (Travi *et al.* 2002). *Didelphis marsupialis* and *Proechimys canicollis* are wild mammals with impact on transmission of *L. chagasi* in northern Colombia (Adler *et al.* 2003; Lainson *et al.* 2002). The aim of this study was to evaluate if spiny rat *Proechimys guyannensis* (Rodentia: Echimyidae) has role in the cycle of the American cutaneous leishmaniasis caused by *L. (V.) braziliensis*.

Fourteen adults males of *Proechimys guyannensis* (10-12 weeks old, 170-180 grams) born in captivity of the Federal University of São João del Rei and as positive control for infection six outbred golden hamsters (*Mesocricetus auratus*), adult males (8-10 weeks old), weighing 90-100 grams (Gomes-Silva *et al.* 2013) were used. The protocols employed for the collection of biological samples for the study of the *Proechimys guyannensis* followed those approved by the ICMBio/SISBIO nº24065 – Ministry of the Environment and Ethics Committee for Animal Experimentation under protocol 017/2010 (CEUA- UFES) registered in the National Animal Control Experimentation (CONCEA, Brazil). The animals were housed in pairs in polycarbonate cages of 41x34x17 cm and under natural conditions. Water and food were provided *ad libitum*. The feed used was Nuvilab CR1 mouse pellets (Nuvalit nutrients SA, Colombo / PR, Brazil), and as bedding sawdust was utilized. The *Proechimys guyannensis* was divided into two groups (n=7); intradermally inoculation with promastigotes of *L. (V.) braziliensis* from of canine lesions for Group 1 and promastigotes standard strain of *L. (V.) braziliensis* (MHOM/BR/75/M2903) for Group 2. The golden hamsters were also divided into two groups of three animals and inoculated with the different *L. (V.) braziliensis* strains. The promastigotes of Group 1 were obtained from an infected dog lesion. The infection of the dog with *L. (V.) braziliensis* was confirmed by culture, polymerase chain reac-

tion (PCR) using primers B1 e B2 (De Bruijn and Barker 1992) and Western Blot (Zanini *et al.* 2010) of a collected fragment from the injured tissue. For sample collection, the dogs were sedated with xylazine (2.2 mg kg<sup>-1</sup>) and ketamine (10 mg kg<sup>-1</sup>) in association. A fragment of 1 cm<sup>2</sup> was collected from the lesion edge. The fragment was divided into two parts - one for performing the PCR, and the other for culture in NNN-LIT (Novy MacNeal Nicolle - Liver Infusion Tryptose) medium, increased 10% Fetal Bovine Serum, which served as a source of promastigotes and culture for the parasite. In their stationary growth phase the two strains (dog strain and standard strain) were then washed in phosphate-buffered saline, 0.15 M, pH 7.2 and adjusted for promastigotes count of 1x10<sup>6</sup> mL<sup>-1</sup>. The count of promastigotes standard strain and of that isolated from the dog was performed with Neubauer hemocytometer (1x10<sup>6</sup> mL<sup>-1</sup>) and 0.05 mL of promastigotes were inoculated intradermally at the right ear pinna, nose and right plantar pad in *Proechimys*. The golden hamsters were inoculated intradermally only at the right plantar pad with 0.05 mL of the suspension 1x10<sup>6</sup> mL<sup>-1</sup> of promastigotes in the stationary growth phase. During inoculation the rodents were sedated with xylazine (4.4 mg kg<sup>-1</sup> IM) and ketamine (72 mg kg<sup>-1</sup> IM) in association. The volume of inoculum used and the concentration of the parasite was used according Roque *et al.* (2010).

After 90 and 180 days, tissue fragments were collected at the site of inoculation. The monitoring of the infected animals was performed for 180 days using PCR and the culture of tissue fragments collected from the site of inoculation, and observation of the development of lesions. From these fragments PCR and culture of microorganisms were performed, using a methodology similar to previously mentioned. For tissues of *P. guyannensis* collected at 90 and 180 days the presence of genetic material of the parasite inoculated were not observed according to PCR assessment or growth in NNN-LIT culture medium. Dissemination of parasites to spleen and other peripheral lymph nodes beyond the inoculation site were evaluated after euthanasia of *P. guyannensis* at 180 days and the result was negative to PCR analysis showing that there was no infection. However all hamsters after 100 days already showed cutaneous ulcerated lesions with elevated erythematous borders or necrotic surface recovered by crusts and culture isolation of *L. (V.) braziliensis*. In other experimental inoculation (data not shown) the isolation of *L. braziliensis* was obtained from two *P. guyannensis* only when intra peritoneal inoculation of the parasite followed by culture of spleen tissue fragments in NNN-LIT was performed although this is not a natural way of infection. As observed, concerning visceral leishmaniasis (Lainson *et al.* 2002), this rodent has not shown itself capable of amplifying the population of *L. (V.) braziliensis* in the skin nor did it allow the isolation of the parasite in

its tissues during the 180 days of monitoring post-infection. Although other authors believe that most rodents are the primary reservoirs of *L. (V.) braziliensis* (Brandão-Filho *et al.* 2003) and particularly the golden hamster, the latter is therefore an appropriate model for immunopathogenesis studies of CL caused by *L. braziliensis* (Gomes-Silva *et al.* 2013). In experimental inoculation the putative role of rodent *Thrichomys laurentius* in the retention of infection and amplification of the transmission cycle of *Leishmania infantum* and *L. braziliensis* (Roque *et al.* 2010) was observed. Also the *Proechimys semispinosus* has been experimentally observed and indicated as a reservoir of *Leishmania (Viannia) panamensis* on the Colombian Pacific coast. While most of the findings of *L. (V.) braziliensis* infection under natural conditions were in *Rattus rattus* (Lima *et al.* 2013), *Thrichomys apereoides* and *Didelphis albiventris* (Quaresma *et al.* 2011) were indicated as natural reservoirs of *L. (V.) braziliensis*. Even though as yet there is no evidence in the literature for the verification of any reported natural infection of *P. guyannensis* by *L. braziliensis*.

The factors like the immunomodulatory properties in the saliva of the insect vector have been studied for vaccine production and may affect the results obtained in experimental infection (Kamhawi 2000; Oliveira *et al.* 2013) but other authors also report success in experimental infection without use of saliva (Gomes-Silva *et al.* 2013; Roque *et al.* 2010). Thus, it seems that *P. guyannensis* does not have an active role in the transmission cycle of ACL caused by *L. (V.) braziliensis* once isolating the parasite or its genome from the inoculated animals has failed.

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