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Original

Leishmania in skin of *Rattus rattus* from the urban area in the Corrientes city, Argentina

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ABSTRACT

Objective: the objective of the present work was the detection of *Leishmania sp*. in *Rattus rattus* tail base skin through polymerase chain reaction (PCR) techniques. **Material and Method:** We analyzed 45 *Rattus rattus* skin samples from the urban area of the city of Corrientes in Argentina. The *Leishmania spp* detection was performed by nested PCR technique. **Results**: *Leishmania* DNA was detected in 22 samples out of 45 processed (49%) in the first round of amplification, and in 14 samples (31%) in the second round (31%). **Conclusions**: These results contribute to increase the existing information in our region on the possible relationship between *Leishmania* and *Rattus rattus*, considering the high prevalence found in skin added to the total absence of lesions. Other aspects should be further studied to establish the role of these animals in the epidemiological chain of the disease in an urban area endemic to leishmaniasis in other animal species.

Keywords: *Leishmania* DNA; epidemiological chain; leishmaniasis; prevalence; black rat; polymerase chain reaction (*Sources: MeSH, CAB thesaurus*).

RESUMEN

Objetivo. detectar *Leishmania sp*. en piel de cola de *Rattus rattus* a través de técnicas de reacción en cadena de la polimerasa. **Material y método**. Se analizaron 45 muestras de piel de *Rattus rattus* del área urbana de la ciudad de Corrientes, Argentina. La detección de *Leishmania sp* se realizó mediante técnicas de PCR anidada. **Resultados**. En la primera ronda de amplificación se detectó ADN de *Leishmania* en 22 muestras de 45 procesadas (49%) y en 14 muestras en la segunda ronda (31%). **Conclusiones**. Estos resultados contribuyen a aumentar la información existente en nuestra región sobre la posible relación entre *Leishmania* y *Rattus rattus*, teniendo en cuenta la alta prevalencia encontrada en piel sumado a la total ausencia de lesiones. Otros aspectos deberán seguir estudiándose para establecer el rol de estos animales en la cadena epidemiológica de la enfermedad en una zona urbana endémica a leishmaniasis en otras especies animales.

Palabras clave: ADN de *Leishmania*; Cadena epidemiológica; leishmaniasis; prevalencia; rata negra; reacción en cadena de la polimerasa (*Fuentes: MeSH, CAB*).

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INTRODUCTION

In the Americas, the epidemiological situation of leishmaniasis is complex due to the great variety of phlebotomine vectors and the more than 40 species of mammals that are capable of harboring the parasite in the different transmission cycles. In Argentina, cutaneous leishmaniasis is considered endemic in the provinces of Salta, Jujuy, Tucumán, Catamarca, northwestern Santiago del Estero, Chaco, Formosa, Misiones and northeastern Corrientes. Regarding visceral leishmaniasis, human and canine cases have been reported in the provinces of Misiones and Corrientes, human cases have been reported in Santiago del Estero and only canine cases have been reported in Formosa (1).

Studies in mammals have shown that several species behave as hosts of the parasite and some play an important role as reservoirs, whether domestic, synanthropic or wild. This is because Leishmania can have a large number of different reservoirs, which contributes to the maintenance of the disease cycle in different environments, from wild to peridomestic (2).

The importance of the relationship between synanthropic rodents and leishmaniasis in Latin America has been demonstrated in several studies. These studies have shown that these rodents behave as a reservoir of several species of Leishmania and present a wide distribution and abundance in urban and peri-urban areas. For this reason, these rodents present a risk to humans, who are more likely to come into contact with them and, therefore, to transmit the parasite (3).

In relation to the role of synanthropic rodents, it was hypothesized that environmental changes and loss of biodiversity lead to a selection of synanthropic animals. The latter increase their distribution and abundance, thereby increasing human exposure to pathogens, as is the case, for example, in the Australasian region. In this region, synanthropic wild animals are 15 times more likely to be a source of emerging and re-emerging zoonotic infectious diseases than wild animals, regardless of species (4). In addition to this theory, it is proposed that anthropization and modification of natural habitats causes a decrease in host diversity and may lead to the selection of more generalist species. These species would become competent reservoirs for some pathogens, a process called "reservoir selection" (5).

On the other hand, the diagnostic techniques used for the detection of the Leishmania parasite are important. In this respect, polymerase chain reaction (PCR) molecular techniques allow detection of Leishmania DNA without the need for prior parasite isolation or microscopic observation and are of particular importance in wild or synanthropic rodents. In the latter, parasites rarely cause skin lesions, are very mild or can even be detected in rodents without any apparent lesions (6).

In Argentina there are several studies on the distribution of the different Leishmania species and their corresponding vectors. However, there are few studies directly related to the detection of the parasite in wild and synanthropic rodents. The significant prevalence of infection in the spleens of rodents of the species *Rattus rattus* inhabiting the city of Corrientes (6) and the detection of *L. braziliensis* in *Akodon sp. and Euryoryzomys russatus* in Puerto Iguazú, Misiones province (7) are known. However, there is other important data that is unknown or little studied, such as the rodent species and the relationship between them and the *Leishmania* parasite. In the city of Corrientes specifically, the importance of rodents in maintaining the disease in our geographical region is unknown, especially if they could behave as natural hosts, reservoirs or maintenance hosts.

The skin of the ear and tail (8) of rodents has been found to be a good site for *Leishmania* infection. This is due to the fact that the skin is thinner and less densely furred at these sites. Consequently, this facilitates the feeding of the vectors due to their short proboscis, therefore, both locations are sites for settlement and proliferation of the parasite. However, there are no studies that show whether this is the case in our region, nor is it known which species of parasites could be located in these places.

From the data already presented, it is important to know this missing information, which would be fundamental to understand the eco-epidemiology of the disease in our region. The aim of the present study was to detect Leishmania sp. in the skin of the tail base of *Rattus rattus* using polymerase chain reaction techniques. We started from this objective taking into account the studies carried out in skin, where the tail base is one of the main places where the *Leishmania* parasite can be found and replicate in rodents. The abundance of the synanthropic

rodent population in the city of Corrientes was also taken into account.

MATERIALS AND METHODS

Type of study. The study was observational, cross-sectional and descriptive.

Study site. The study was conducted in the urban and suburban area of the city of Corrientes, Corrientes province, Argentina (27° 28' S and 58° 50' W).

Geoclimatic conditions. The climate is subtropical with warm temperatures in summer and frosts in winter. The average annual temperature fluctuates between 19.5°C and 22°C. In terms of precipitation, the average annual rainfall ranges between 1200 and 1400 mm.

Study period: The work was carried out between March 2011 and June 2012.

Rodent trapping: this was carried out using Sherman traps, using pumpkin seeds and/or bovine fat as bait. The traps were delivered to different neighborhoods of the city in a number of 2 to 3 cages per block per day. The location of the cages was based on the identification of rodent traces and information from the inhabitants themselves, data that were taken prior to the delivery of the traps. These were checked daily by the people living in the house, who reported the presence or absence of trapped rodents. The captured specimens were taken to the laboratory of the Public Health Department of the Faculty of Veterinary Sciences of the National University of the Northeast (FCV/UNNE), where they were identified according to Osgood's code (1943), and age and sex data were recorded. In addition, an external clinical inspection was carried out, evaluating the presence of alopecic, scaly areas or lesions on the tips of the ears, muzzle, tail, base of the tail and flews of the limbs.

Study samples. We worked with 45 samples of skin from the base of the tail of *Rattus rattus*, sized 1 cm long by 0.2 cm wide. These were obtained previously, identified in eppendorf tubes, stored and preserved in a freezer at -20ºC.

Sample processing was carried out at the Laboratory of the Veterinary Service of Molecular Biology of the Faculty of Veterinary Sciences, National University of the Northeast (FCV/UNNE).

DNA extraction by CTAB digestion technique. Firstly, DNA extraction was carried out using the CTAB digestion technique. For this purpose, the skin samples were conditioned using a technique standardized by our work team, consisting in the reduction of the tissue by means of small cuts, with sterile fine surgical scissors, to obtain tiny fragments.

These fragments were then placed in a DNA-free Eppendorf tube and suspended in 500 µl of physiological solution, vortexed and centrifuged at 12.000 rpm for 4 minutes with subsequent removal of the supernatant. Genetic material was obtained from the samples following the techniques described by Alegre et al (9).

PCR control of genetic material from rats. We performed a simple species control PCR in order to corroborate the presence of genetic material, following the recommendations described (6), using rat DNA as a positive control and sterile distilled water as a negative control.

We used electrophoresis in 1% agarose gels in 1X TBE buffer, which were stained with ethidium bromide, to separate the PCR products. UV transillumination was used for visualization using Ladder 100 (CienMarker) as molecular weight marker.

***Leishmania sp.* detection by nested PCR technique.**
This technique is carried out through two rounds of amplification. The first S4 and S12, which amplify 520pb bands, were used for the first round. The first S17 and S18 that amplify bands of 490 pb were used for the second round following the recommendations described by Ruiz et al (6).

We use DNA from *Leishmania sp.* provided by the National Institute of Parasitology "Dr. Mario Fatala Chaben" (Argentina), and distilled water was used as a negative control.

In order to separate the PCR products, we use horizontal electrophoresis in a 2% agarose gel in TBE (Tris- Boric Acid- EDTA) 1X, which were stained with ethidium bromide. Later, the visualization was carried out by UV transillumination.

A molecular weight marker (Ladder 100) was used to compare the size of the fragments that were amplified.

Ethical aspects. Animal welfare criteria were taken into account to conduct this work. The present activities described arise from a larger project, which was approved by the Ethics Committee of the Faculty of Veterinary Sciences, National University of the Northeast, under File No.: 14-2019-02029, protocol No. 0084.

RESULTS

Among the 45 specimens captured, 40% (18) were males and 60% (27) females. In terms of age, the largest number of rodents corresponded to the age range of 6 to 8 months, with 35.5% (16), followed by specimens from 12 to 14 months of age with 17.77% (8).

None of the specimens clinically showed symptoms compatible with leishmaniasis, nor any type of lesion was observed.

Genetic material was extracted from the 45 skin samples from the base of the Rattus rattus tail. Regarding the control PCR, a correct obtaining of genetic material in all the processed samples was reached. Amplification bands of a 118pb fragment were observed, evidencing the presence and integrity of the DNA of the species studied, as well as the absence of inhibitors of the reaction.

We applied the nested PCR technique to each sample. In the first round of amplification, we observed specific DNA bands of Leishmania sp. in 22 samples out of 45 processed (49%) (Figure 1). In the second round of PCR, the number decreased, detecting specific amplification in 14 samples (31%) (Figure 2). No amplification in negative controls was observed in any of the molecular reactions.

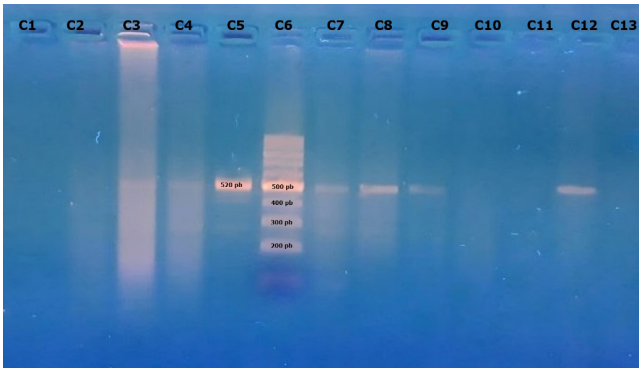

Figure 1. Nested PCR: First round of amplification. C1-4, C10 and C11: *Leishmania sp*. negative tail base skin samples; C7-9, C12; positive samples. C6: molecular weight marker (Ladder 100). C5: positive control and C13 negative control. Amplicon: 520 bp.

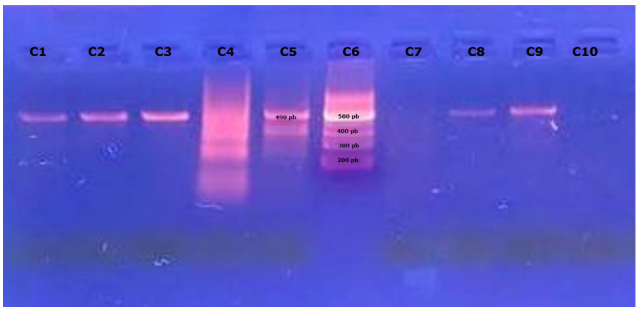

Figure 2. Nested PCR. second round of amplification. positive samples with amplification bands of 490 bp (C1 - C3 and C8). C5: molecular weight marker (ladder 100). C5: positive control and C10: negative control.

DISCUSSION

There are several reasons that make it difficult to understand the epidemiology of leishmaniasis, one of the main ones being the identification of its multiple natural reservoirs. Natural reservoirs are largely unknown due to the difficulties in capturing sufficient numbers of wild animals, the techniques used in the isolation and identification of parasites (2). Furthermore, it is very important to consider that natural reservoirs vary according to the type of parasite, the vector and the geographical area.

In the present study, none of the rodents showed lesions or symptoms compatible with the presence of the parasite. However, a high rate of positive animals was obtained. This demonstrates the importance of applying tools of high sensitivity and specificity, such as the nested PCR technique, especially in epidemiological studies.

The result obtained with a frequency of 31% (14 samples out of a total of 45) is high, compared to other studies carried out on the skin of synanthropic rodents. In Brazil (10) obtained a frequency of 10% (8 samples out of a total of 80) of detection of *Leishmania sp* in *Rattus norvergicus* skin samples using nested PCR. Besides, in *R. norvergicus*, in ear skin samples (11), it was obtained 31% (18 samples out of a total of 57). Also in Brazil, Castro Ferreira et al (12) obtained a 64.9% of prevalence in skin samples and Quaresma et al a 25% in ear skin (13).

In addition to the prevalence of 31% obtained in this study, we conclude that *Rattus rattus* is the synanthropic rodent in which Leishmania has been most frequently detected, compared to other species such as *Mus musculus* or *Rattus norvergicus* (14). This could be due to the fact that *Rattus rattus* is the synanthropic rodent usually found with greater abundance in endemic areas of Leishmaniasis, especially in urban areas (15, 16), as we could also observe in our work, where all the captures were made in the urban area of the City of Corrientes and corresponded to 100% to *Rattus rattus*.

Similar results were obtained by Ruiz and collaborators where 62 specimens out of 63 captured in the urban area of the city of Corrientes corresponded to *Rattus rattus* and only 1 specimen to *Mus musculus* (6).

A very important aspect to take into account is the absence of lesions in the rodents that were analyzed in our study, a fact that coincides with other studies (4,6,15,16,17). This could be due to an adaptation of *Leishmania* to its host, achieving a state of balance resulting from an old host-parasite relationship (18).

The results obtained in the present study contribute to increase the existing information in the city of Corrientes (Argentina) on the possible relationship that could exist between *Leishmania* and a synanthropic rodent such as *Rattus rattus*. In this sense, it is important to take into account the importance of the high prevalence found in skin, with the probability of transmissibility by vectors that this implies, in addition to the total absence of lesions in those specimens, aspects that are essential to determine the role of reservoir in a species.

In any case, other aspects should continue to be studied because there are still many questions to be resolved, especially considering the high prevalence found in the skin of the tail base. It remains to investigate whether this prevalence would correspond to other places, such as ear skin or other tissues such as spleen and bone marrow, studies that will be carried out in other stages of study. And if this prevalence could be relevant in giving *Rattus rattus* an important role in the epidemiological chain of the disease in our region, such as acting as a reservoir.

Conflict of interests.

The authors of this study declare that there is no conflict of interest with the publication of this manuscript.

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