

## ***Trypanosoma (Herpetosoma) lewisi* in *Rattus* spp from La Matica, Lara State, Venezuela and the possible relationship with zoonotic diseases**

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During a study carried out searching reservoir vertebrates for *Leishmania* spp in the area of La Matica, Lara State, Venezuela from March 1997 to October 1998, 40 specimens of *Rattus* spp were captured. Blood was extracted from all of them by means of cardiac puncture. Fresh blood and blood smears stained with Giemsa were examined. Of the 40 specimens studied, 22 (55%) resulted positive for the presence of *Trypanosoma lewisi* in blood. 63.15% of the infected animals were males (12/19), and 47.62% (10/21) females. By age, adult animals were infected in 62.96% of the cases (17/27) while young specimens were infected in 38.46% (5/13). The differences were not statistically significant. Regarding the period of capture almost all the individuals were captured during the raining season; a positive correlation between the average of the monthly rain and the number of individual captured was observed. Finally, we suggest the possible increase of the susceptibility of *Rattus* spp infected with *T. lewisi* to the infection by other species of the family Trypanosomatidae of public health importance.

**Key words:** *Trypanosoma lewisi* - *Rattus* spp - *Trypanosoma cruzi* - *Leishmania* spp - Susceptibility - Venezuela

### INTRODUCTION

*Trypanosoma (Herpetosoma) lewisi* (Kent, 1880; Laveran and Mesnil, 1901) is a specific hemoparasite of the rat (*Rattus rattus* and *Rattus norvegicus*) considered within the Stercoraria group and transmitted through fleas (*Xenopsylla cheopis* in tropical and sub-tropical areas) mainly orally, either by the ingestion of flea feces or the entire flea (Hoare, 1972, Maraghi *et al*, 1995).

Even though some cases have been described in human beings (Johnson, 1933), *T. lewisi* is not considered a pathogen for man. However it has been demonstrated that rat infection by this hemoflagellate increases its susceptibility to other

microorganisms such as *Salmonella typhimurium* (Nielsen *et al*, 1978) and *Toxoplasma gondii* (Guerrero *et al*, 1997).

These two diseases, salmonellosis and toxoplasmosis, represent an important problem of public health worldwide, and rodents (especially of the genus *Rattus*) are good transmitters of these, direct or indirectly (Picco, 2003). Therefore, a high prevalence of *T. lewisi* in a population of *Rattus* spp in a determined geographical area could, increase the incidence of salmonellosis and toxoplasmosis in the human population that lives there.

In addition, the immunological changes generated by *T. lewisi* that determine the increase of susceptibility of *Rattus* spp to *S. typhimurium* and *T. gondii*, could act increasing the susceptibility of these rodents to other parasites.

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## MATERIALS AND METHODS

*Study area* - La Matica is a small village (9° 42' 50" N, 69° 41' 53" W) in the Andrés Eloy Blanco District of the south eastern sector of the Venezuelan State of Lara (Fig. 1). Its average altitude is 1,450 meters above sea level. It has a dry season that extends from December to March, and a rainy season from April to November; the annual average rainfall is approximately 800mm.

*Animal trapping* - The animals were captured five nights per month over a period of a year and a half, from March 1997 until October 1998. They were trapped in home-made Tomahawk-like one door metal traps of two different dimensions, 48x13x13 and 66x23x23 centimeters, with the capacity to capture single small animals or several animals at the same time.

Traps were baited with local fruits (banana, maize, etc.) and placed at 20 or 30 m around and inside the houses in the afternoon and checked early the next morning. Twenty traps were used and all the animals were kept alive until they were studied.

*Diagnostic methods* - The animals were examined for the collection of ectoparasites, specifically fleas, which were meticulously dissected searching for flagellates.

Blood was extracted by means of cardiac puncture and examinations were made in fresh blood and blood smears stained with Giemsa. Xenodiagnosis was carried out using 5 third stage nymphs of *Rhodnius prolixus*. In some specimens a weekly follow-up was made and blood from the tail was used to practice examinations in fresh blood, in order to

Fig. 1. Study area



determine the course of parasitemia regarding its persistence or not. Finally, the rodents were humanely sacrificed and some specimens were preserved for taxonomic purposes.

*Trypanosoma lewisi* parasites were identified based on the following criteria:

- 1) Morphological study by using an immersion objective.
- 2) Study of parasitemia in all positive cases, determined by fresh blood examinations.
- 3) Evolution of parasitemia after a variable follow-up of 7 to 28 days.
- 4) Presence of infected fleas, and
- 5) Xenodiagnosis.

The rodents were classified according to sex and divided in two age groups, in accordance with the characteristics of sexual maturity present, young animals and adults. Monthly prevalences of *T. lewisi* infection were also determined and grouped by seasonal periods.

The chi-square analysis and Spearman rho test were used to determine the degree of association between infection and factors studied.

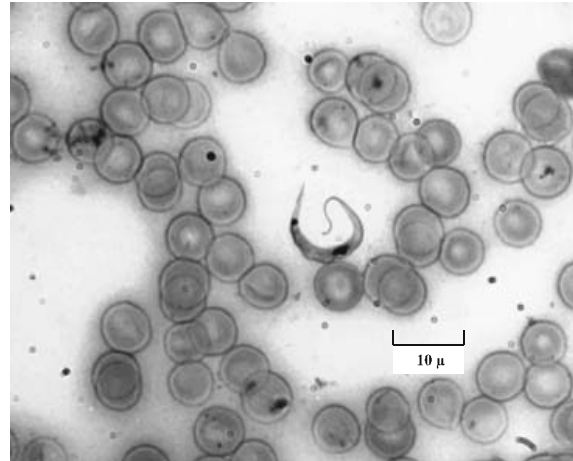
## RESULTS

The morphological study with an immersion objective (100X), revealed some trypanosomes 35 microns long in total, with a very sharp posterior end, a subterminal voluminous kinetoplast, oval nucleus shifted toward the extreme front, a well developed flagellum and a loosely folded undulating membrane (Fig. 2A).

All the specimens evaluated at the time of their capture, with a positive examination in fresh blood for hemoflagellates were characterized by high parasitemias (Fig. 2B), which upon carrying out a weekly follow-up resulted in abrupt and total disappearance of the parasites. Four rodents were kept alive for a period of up to a month, taking blood samples from the tail once a week for examination in fresh blood. It was observed that during a period that

varied from 7 to 28 days, the trypomastigotes present in the blood disappeared completely.

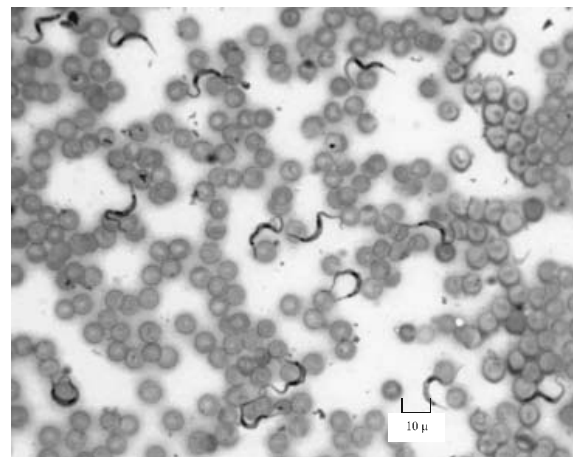
**Fig. 2A** *T. lewisi* in *Rattus* spp. blood smears (1000x)



Thirty-one rodents were studied for the collection of ectoparasites (specifically fleas) that were not taxonomically classified, finding fleas in 13 specimens (41.93%), 7 females and 6 males. A total of 32 fleas were collected (2.46 fleas per individual); 12 fleas were examined under an immersion microscope, revealing flagellated forms compatible with trypanosomes in 8 of them (66.66%).

Xenodiagnosis was carried out on 8 rodents and the faeces examination of the nymphs of *R. prolixus* that was made weekly beginning the second week after feeding, was reported negative for all the cases.

**Fig. 2B** *T. lewisi* in *Rattus* spp. blood smears (400x)



**Table I** Prevalence of *Trypanosoma lewisi* by sex of *Rattus* spp in La Matica, Lara State, Venezuela

Host sex	N° examined	N° infected	% infection
Male	19	12	63.15
Female	21	10	47.62
Total	40	22	55.00

$\chi^2 = 0.97$  df= 1 p=0.32

**Table II** Prevalence of *Trypanosoma lewisi* by age of *Rattus* spp in La Matica, Lara State, Venezuela

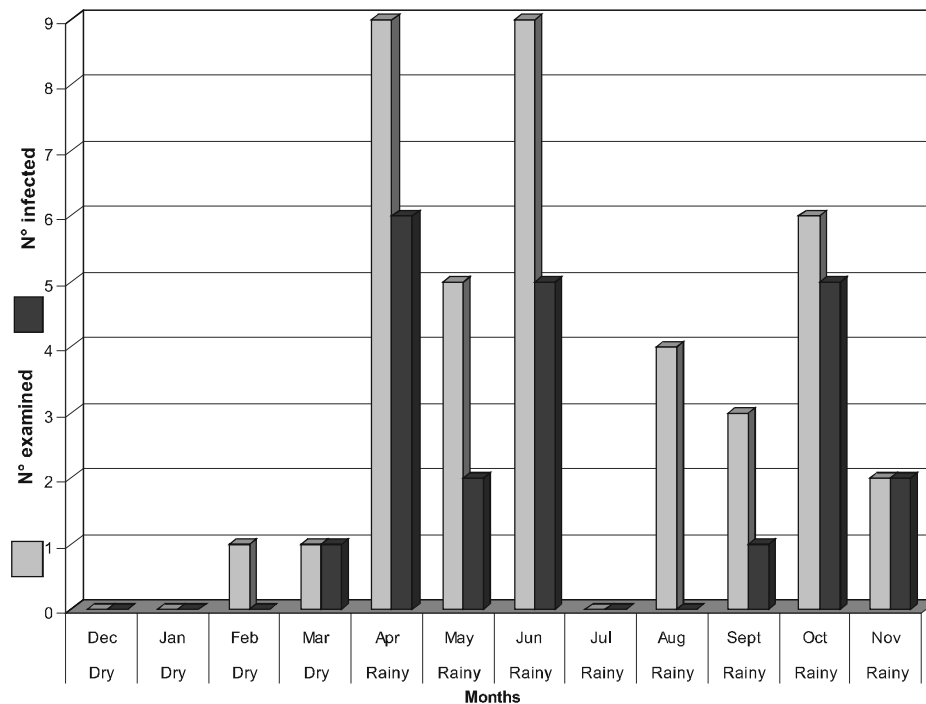
Host age	N° examined	N° infected	% infection
Young	13	05	38.46
Adult	27	17	62.96
Total	40	22	55.00

$\chi^2 = 2.13$  df= 1 p=0.14

**Table III** Co-infections in *Rattus* spp in La Matica, Lara State, Venezuela

N° Individuals examined	Infection with <i>T. lewisi</i>	Infection with <i>Leishmania</i> spp	Infection with <i>T. cruzi</i>
2	+	+	-
3	+	+	-
4	+	+	-
2	-	+	-
25	+	+	-
6	+	-	+
18	+	-	+
19	+	-	+
(a)	+	-	-
(b)	-	-	-

(a) remaining 15 positive cases for *T. lewisi*; (b) remaining 17 negative cases for *T. lewisi*

**Fig. 3** Monthly prevalence values of *Trypanosoma lewisi* in *Rattus* spp in La Matica, Lara State, Venezuela.

Based on these criteria, *T. lewisi* was observed in a total of 22 out of 40 *Rattus* spp examined (55%), but the infection rate was different in percentages, both per sex (Table I) and age (Table II), however the differences were not statistically significant ( $P > 0.05$ ).

The greatest number of rodents was captured during the rainy season, and within this period the driest months (July-August) were the ones that presented the least number of captures and none of the animals were infected. During the dry season captures were very scarce (2). (See Fig. 3 for details).

Of the 40 animals studied, 5 resulted positive for *Leishmania* (3 *L. braziliensis* and 2 *L. mexicana*) (De Lima *et al*, 2002) and 3 resulted positive for *T. cruzi* (data to be published). Of the 5 specimens infected with *Leishmania*, 4 presented simultaneously infection with *T. lewisi*, while the fifth specimen was negative for *T. lewisi*. On the other hand, the 3 specimens infected with *T. cruzi* presented simultaneous infection by *T. lewisi* (Table III). The isolates of *L. braziliensis*, *L. mexicana* and *T. cruzi* were characterized by means of the kDNA restriction pattern and hybridization with species-specific probes.

## DISCUSSION

The infection of *Rattus* spp by *T. lewisi* has been broadly reported throughout the world since the middle of the past century, initially in New Zealand (Laird, 1951), Panama (Calero, 1952), USA (Eyles, 1952), Hawaii (Kartman, 1954), Italy (De Camieri & Castellino, 1964) and Nigeria (Akinboade *et al*, 1981), and more recently in China (Lixian *et al*, 1994), Chile (Franjola *et al*, 1995), Egypt (Abdel-Aal & Abou-Eisha, 1997), India (Laha *et al*, 1997), Nigeria (Ugbomoiko, 1997), Venezuela (Herrera & Urdaneta, 1997) and Brazil (Linardi & Botelho, 2002) finding very important variations as to the prevalence of this infection, fluctuating between 4.60% in New Zealand and 82.30% in India. In this study the prevalence observed was 55% being exceeded only by the observations made in India of 82.30% (Laha *et al*, 1997) and in Nigeria of 75.73% (Akinboade *et al*, 1981). The first report made in Venezuela determined a prevalence of 21.30% (Herrera & Urdaneta, 1997) and the highest reported in South America was of 21.70% in Brazil (Linardi & Botelho, 2002). Nevertheless the present work is the second report from Venezuela about the infection of *Rattus* spp. with *T. lewisi*, this is the first systematic study carried out in this country.

On the other hand, regarding the prevalence of the infection by sex, even though the difference found by us between males (63.15%) and females (47.62%) was not, statistically significant ( $p=0.32$ ) (Table I), significant differences by sex have been reported in Brazil (Linardi & Botelho, 2002), where males were more affected. This is attributed to ecological and behavioral factors, such as greater infestation by fleas. Our observations lead us to believe that there should be other more complex factors that determine this situation, since in addition to the existence of other reports where significant differences are not observed regarding sex (Franjola *et al*, 1995, Ugbomoiko, 1997), we found that infestation by fleas in the specimens studied was the same for males and females.

In the two age groups studied, important percentage differences were found between young animals (38.46%) and adults (62.96%) that were not statistically significant ( $p=0.14$ ) (Table II). There are contradictory reports in this sense, some reporting greater prevalence in adults (Ugbomoiko, 1997) and others with greater prevalence in young (Linardi & Botelho, 2002).

Albright & Albright (1991) suggest that the rat develops solid immunity against re-infection. If this is true, we can assume that there are environmental conditions where infection by *T. lewisi* is not present, and this determines the existence of adult specimens susceptible to the infection. Any situation that introduces the parasite in the area would determine a prevalence in adults greater or equal to that in young. The permanent presence of *T. lewisi* in an environment for a considerable time would favor the greatest prevalence in young, since these would be the only ones susceptible to the infection.

Regarding the seasons (Fig. 3), the dry period (December- March) was characterized by the capture of very few rats. We could not determine if this decrease was due to environmental conditions or to flaws in the capturing technique. However, relating the monthly rainfall average of the last five years and captures per month, a significant positive correlation was determined (Spearman  $\rho = 0.78$ ,  $p < 0.05$ ).

We believe that the number of specimens studied in this investigation is not sufficient to determine the degree of association between infection and factors of importance which influence its frequency. A broader study is required to determine these associations in the event they exist.

Just as Nielsen *et al*, (1978) and Guerrero *et al*. (1997) demonstrated that the infection by *T. lewisi* in rats produces immunosuppression that increases the susceptibility of these animals to infection by *S. typhimurium* and *T. gondii*, respectively, we believe that this immunosuppression could also increase the susceptibility of rats to infection by *Leishmania* spp and to *T. cruzi*. Although these are very preliminary results, the importance of this observation lies in that *Rattus* spp is a rodent with intra and peridomiciliary habits, whereby the presence of them in a determined community could determine greater probability of the appearance of outbreaks of leishmaniasis or Chagas disease upon creating a domiciliary cycle. This could help us to explain the appearance of outbreaks in many cases without major environmental modifications.

For this we consider that is necessary the confirmation of the results. We are carrying complementary studies to demonstrate our hypothesis. Additionally the publication of these results will permit to others investigators carried out more careful investigation about the infection by *T. lewisi* witch permit assess this result.

On the other hand, we could think of other control mechanisms such as the elimination of rats, to partially control the transmission of parasites such as *Leishmania* spp and/or *T. cruzi* due to the elimination of the intra and peridomiciliary reservoirs.

## RESUMEN

Durante la realización de un estudio para la investigación de animales reservorios de *Leishmania* spp en la localidad de La Matica, estado Lara, Venezuela en el periodo comprendido entre marzo 1997 hasta octubre 1998, un total de 40 especímenes de *Rattus* spp fueron capturados. Por medio de punción cardiaca se obtuvo sangre, de todos los individuos, para la realización de examen en fresco y extendido coloreado con Giemsa, que permitieran la identificación de *Trypanosoma lewisi*. De los 40 especímenes estudiados, 22 (55%) resultaron positivos para la presencia de *T. lewisi* en sangre. Al categorizar los individuos de acuerdo con el sexo se encontró que 63.15% de los animales infectados eran machos (12/19) y 47.62% (10/21) hembras. Por edad, animales adultos estaban infectados en un 62.96% de los casos (17/27) mientras que los especímenes jóvenes estaban infectados en un 38.46% (5/13). Estas diferencias no fueron estadísticamente significativas. Con respecto al periodo de captura casi todos los ejemplares se

capturaron durante el periodo lluvioso existiendo una correlación positiva entre el promedio de lluvia mensual y el número de ejemplares capturados. Finalmente, se plantea el posible incremento de la susceptibilidad de los *Rattus* spp infectados con *T. lewisi* a la infección por otras especies de la familia Trypanosomatidae de importancia en Salud Pública.

#### ACKNOWLEDGEMENTS

To Mr. Felix Flores and Wilmen Galindo for their technical assistance, and to the community of La Matica for the support given during our field work. Also to Ms. Irene Gamundi for the translation, and Dr. Marian Ulrich for the corrections and critical reading of the manuscript.

Supported by the World Bank/Venezuelan government, projects Ven/96/002/02124 and Ven/96/002/016

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