Retention of *Leishmania (Leishmania) mexicana* in Naturally Infected Rodents from the State of Campeche, Mexico

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In the State of Campeche, Mexico, zoonotic cutaneous leishmaniasis is mainly due to Leishmania (L.) mexicana. *The parasite population is maintained in a mammalian species, a reservoir in which the ideal course of infection should be long and relatively nonpathogenic. The objective of the present study was to document the retention of* L. (L.) mexicana *in 29 naturally infected rodents. These cricetids lived in captivity for up to two years and were tested monthly for the presence of the parasite, by cultures of needle aspirates from the base of the tail.* Peromyscus yucatanicus *and* Ototylomys phyllotis *were incriminated as the primary reservoir hosts. The finding that the multiplication of parasites in* P. yucatanicus *might be triggered by temperature, suggests that this animal would be a good choice for further research on* L. (L.) mexicana.

Key words: *Leishmania (L.) mexicana* - wild rodents - reservoir - retention - Mexico

Leishmaniasis is a complex of human diseases with complicated transmission cycles and the parasite, *Leishmania* Ross, 1903 is distributed throughout most of the tropics and sub-tropics. The leishmaniases are endemic in at least 22 countries of Latin America (WHO 1990). Zoonotic cutaneous leishmaniasis (ZCL) due to *L. (L.) mexicana* has been identified both in Belize and Mexico (Lainson & Strangways-Dixon 1964, Chance et al. 1974, Gardener et al. 1974, Moreno et al. 1986, Barker et al. 1986, Grimaldi et al. 1987, Cupolillo et al. 1994, Chablé-Santos et al. 1995, Canto-Lara et al. 1999). In the latter, leishmaniases have been recorded in 17 states, where localized cutaneous leishmaniasis (LCL) is the most common clinical form (Seidelin 1912, Velasco-Castrejón et al. 1997).

In the State of Campeche, LCL is a wild zoonosis (Andrade-Narváez et al. 1990, Chablé-Santos et al. 1995, Rebollar-Téllez et al. 1996). Four rodent species *Sigmodon hispidus* Say and Ord, 1825; *Oryzomys melanotis* Hooper, 1953; *Ototylomys phyllotis* Merriam, 1901; and *Peromyscus yucatanicus* JA Allen and Chapman, 1897 (Rodentia: Cricetidae) have been reported naturally infected (Chablé-Santos et al. 1995). Most of the infected animals presented dermal lesions on the tail but in some specimens the infection was asymptomatic. *Leishmania (L.) mexicana* Biagi, 1953 *emend.* Garham, 1962 is the main agent of ZCL in the State of Campeche (Pérez-Mutul et al. 1994, Canto-Lara et al. 1998, 1999) and was characterized by using an indirect immunofluorescence assay with monoclonal antibodies in two *S. hispidus*, eight *Or. melanotis*, six *Ot. phyllotis*, and five *P. yucatanicus* (Canto-Lara et al. 1999).

Leishmania needs a mammalian species, a reservoir, to maintain the parasite population (WHO 1984, 1990, Bray 1987). Ideally, the course of infection should be long and relatively nonpathogenic, and the parasites should be available in the skin or the blood in sufficient numbers for the sand fly vector (WHO 1984, 1990, Bray 1987). To document this requirement in the field is nearly impossible due to the small sample-size of infected individuals and the low probability of recapture.

Thus, the objective of the present study was to document the retention of *L. (L.) mexicana* in 29 naturally infected rodents from the State of Campeche, kept in captivity in Mérida, Yucatán, Mexico. These captive cricetids lived for up to two years during which the course of infection was following up by parasitological methods.

MATERIALS AND METHODS

Animal care - Twenty-nine naturally infected rodents (2 *S. hispidus*, 12 *Or. melanotis*, 9 *Ot. phyllotis*, and 6 *P. yucatanicus*) were captured in La Libertad, State of Campeche, Mexico, between

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November 1993 and March 1994 (Chablé-Santos et al. 1995). The animals were housed individually and fed rabbit chow (Provi, Mérida, Yucatán). Corn, sunflower seed, vegetable, or fruit were added at weekly intervals during the period of acclimation to captivity (one month). Air-conditioning was turned on during daytime and a ceiling ventilator during the night. The environmental conditions of the animal-care facilities were not measured and variations might have occurred. In general, the temperatures inside were cooler during the dry season (April to June).

Retention of parasite and evolution of lesion - The 29 infected rodents were observed for up to two years (November 1993 to October 1995). Each month, changes in the appearance of the lesion were recorded and needle aspirates made from the skin lesions were cultured in modified Senekjie's medium (Vouldoukis et al. 1987, Weigle et al. 1987). The cultures were kept at 22ºC and checked weekly under a Zeiss light microscope (Standard 25) with a 100/1.25 oil magnifier using culture material stained with Giemsa. An animal was considered positive when at least one promastigote was observed microscopically and negative if no parasites were found within the month. When five or more rodents of the same species were available, non-parametric binomial tests were performed, using the number of positive and negative results of each animal, to find if the distribution of positive cultures was significantly different from the negative ones within each species (Dixon et al. 1990). The survival difference between the heavily and lightly infected animals was estimated using the Kaplan-Meier method with four non-parametric linear rank tests (Mantel-Cox, Tarone-Ware, Wilcoxon/Breslow, Wilcoxon/Peto-Prentice - Dixon et al. 1990). Given the small sample of animals, the results should be interpreted with care.

Systemic dissemination - Five euthanized animals and the ten individuals remaining at the end of the study, were killed with chloroform, weighed, measured, and checked for any external and/or internal signs of infection. In healthy small rodents, popliteal lymph nodes are never visible but they sometimes become apparent when infected. In the latter, impressions of cut lymph nodes were made on microscopic slides, stained with Giemsa, and checked for amastigotes by light microscopy. Smears were also taken from liver and spleen. In ten animals, smears were taken from kidney and heart. Nineteen voucher specimens (skin and skeleton) were prepared (Nagorsen & Peterson 1980) for the museum of the School of Biology of the Universidad Autónoma de Yucatán, Mérida, Mexico and the Royal Ontario Museum, Toronto, Canada.

RESULTS

Animal care - The 29 naturally infected rodents were acclimatised to captivity and lived for up to two years (Table I). No statistical difference was found in the survival rates of heavily and lightly infected animals.

Retention of the parasite - Aspirates were cultured monthly but some of the cultures were contaminated and did not yield any result. No aspirates could be taken during the months of January, April, and August 1994.

Parasites were consistently demonstrated during 15 months in one *S. hispidus* (S-A2) (Table I). On the other hand, the second *Sigmodon* (S-N5) showed parasites irregularly during 21 months. No statistical test could be performed since the number of specimens of hispid cotton-rat was too low.

Oryzomys melanotis consistently retained parasites at the site of the infection (Table I). Individual black-eared rice-rats had a significantly higher number of positive cultures than negative ($Z_{observed}$ = -3.614, $Z_{0.01}$ =-2.58). Both healthy and infected colonies of *Oryzomys* had high mortality rates (100% and 80% respectively).

Four *Ot. phyllotis* (Ot-C9, Ot-K5, Ot-6a, and Ot-C4) were used for the colony and were not sampled during reproduction. The nine big-eared climbing rats retained parasites very irregularly (Table I). Three of them (Ot-K5, Ot-C4, and Ot-S12) were positive at capture and negative afterward. However, three *Ototylomys* (Ot-B4, Ot-S9, and Ot-Q9) retained parasites consistently for at least 18 months. The distribution of positive cultures was not significantly different from the negative ones ($Z_{observed}$ = -0.9267, $Z_{0.05}$ = -1.96).

The retention of parasites was very irregular in the five *P. yucatanicus* studied (Table I). Monthly aspirates gave significantly more negative results than positive ($Z_{observed}$ = -3.5717, $Z_{0.01}$ = -2.58). However, the parasite was found in all infected Yucatán deer-mice in the months of June 1994 and May 1995.

Evolution of the lesion - The clinical signs observed in the two infected *S. hispidus* differed widely. In November 1993, S-A2 presented an ulcer at the base of the tail, similar to the crater-like lesion frequently observed in humans. The ulcer closed during January 1994, but the site of infection remained very swollen and discolored. Metastases were slowly evident in other parts of the body, first in the fleshy area around the anus, then in November 1994, in the depilating rump and swelling ears. In February 1995, this cotton-rat was very weak and was euthanized. On the other hand, the second *Sigmodon* (S-N5) remained very healthy-looking and presented only one white spot of 3 mm in diameter on the proximal third of tail.

Infected *Or. melanotis* always presented some visible signs of leishmaniasis: red, hairless spots and/or swelling on the proximal third of the tail. One of these black-eared rice-rats (Or-C8) developed an ulcer which healed a month later. Two rice-rats (Or-O2 and Or-D9) presented depilation of the rump and died soon after. At the end of the two years, the two remaining *Oryzomys* (Or-F9 and Or-C8) presented discolored areas along the whole tail.

The lesions of both *Ot. phyllotis* and *P. yucatanicus* remained mostly inconspicuous as when first captured (Chablé-Santos et al. 1995). One Yucatán deer-mouse (P-B2) died during manipulation and presented a depilated area on the rump.

Systemic dissemination - Only the euthanized animals could be tested for internal dissemination of the parasites. The popliteal lymph nodes of *S.*

TABLE I

Monthly distribution of *Leishmania (Leishmania) mexicana* (*LLm*) in cultures of tail aspirate of 29 naturally infected rodents from La Libertad, Campeche, Mexico

Genus Species I.D.	Sex	*LLm*	Year																							
			Month																							
Sigmodon			1993		1994													1995								
hispidus			N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O
S-A2	f	*	+	+		+	+		+	+	+		+	+	+	+	+	E								
S-N5	m	*				+	-			+	+		-			-	+	-	-	-		+				
Oryzomys			1993		1994													1995								
melanotis			N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O
Or-14b	f	*	+	P																						
Or-13a	m		+	P																						
Or-K6	f	*	+	+		+	+		+	+	+		-		+	+	-		+	+	+	+	E			
Or-12a	m	*	+	+		+	+			+	-		-	-	-	-			-	-	+	-		-	-	D
Or-N1	m	*	+	+		+	+		+	+	+		-	+	-	-			+	+	+	-	+	+	D	
Or.F5	m			+			-			-				-	D											
Or-O2	m			+			+			+	D															
Or-F9	f	*				+	+			+	-		+			+	-	-	-	+	+	-	+	-	+	+
Or-C8	f	*				+	+		+	+	+		+		+	+	+	+	-	+	-		+	+	+	+
Or-E9	m					+	+		+	+	-		-	-	-	-	D									
Or-D9	m	*				+	+		+	+	+			+	-	D										
Or-C9	m	*				+	+		+	+	-		-	-	-	-	+	-	+	+	-	+	+	D		
Ototylomys			1993		1994													1995								
phyllotis			N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O
Ot-C9	f			+			+							+	D											
Ot-K5	m			+			-			-					-	-					-		-		-	E
Ot-6a	f					+	+												-	+			-		-	E
Ot-11a	m					+	-	D																		
Ot-B4	m	*				+	+		+	+	-		-	+		-	+	-	+	+	+	+	+	-	+	+
Ot-C4	f	*					+		-					-	-	D										
Ot-S12	f									+	-		-		-	-	D									
Ot-S9	f	*								+	+		+	+	-	+	+	-	+	+	-	+	+		-	+
Ot-Q9	m	*								+	-		-			+	+	-	+	+		+			-	+
Peromyscus			1993		1994													1995								
yucatanicus			N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O
P-B2	f			+	D																					
P-15a	f	*				+	-			+	-		-	+	+	+	-	-	-	-	+		-			-
P-1a	f	*					+		-	+	-		-	-	-	-	-	-	-		+	-	-	-		-
P-3b	f	*					-			+	-		-	-	-	-			-	+	+	-	+	-		+
P-O11	f	*								+	-		-		+	-	-	-	-	-	+	-		-		-
P-8	m	*								+	-		-		-	-			-	-	+	-	-	-	D	

I.D.: identification; m: male; f: female; *: characterized by McAbs (Canto-Lara et al. 1999); culture of aspirate: positive +; negative -; D: animal died; E: animal euthanized.

hispidus (S-A2) were black and swollen. Impression smears of liver, spleen, and lymph nodes were found positive for the parasite. In October 1995, when the second cotton-rat (S-N5) was euthanized no popliteal lymph node was visible but amastigotes was found in liver (Table II).

Two *Or. melanotis* (Or-14b and Or-13a), checked at the start of the study, did not present any parasite in the cultures of macerated liver and spleen. One rice-rat (Or-K6) euthanized because of respiratory distress and two others (Or-C8 and Or-F9) checked at the end of the research had amastigotes in liver and spleen (Table II). One of those rats (Or-C8) had enlarged liver with granuloma, enlarged spleen, edema in thorax, abdomen and subcutanea.

Amastigotes were found in the liver of only one of the six *Ot. phyllotis* checked (Table II). This climbing rat (Ot-Q9) also presented abdominal and subcutaneous edema.

The four *P. yucatanicus* tested presented amastigotes in every organ (Table II). However, previous to collecting, those Yucatán deer-mice were part of a two-years capture-mark-recapture research. Three of the deer-mice (P-15a, P-1a, and P-3b) were more than three years old.

DISCUSSION

To incriminate a reservoir host, it is neccessary to demonstrate that the parasite population needs that particular mammal for the maintenance of the disease in a given focus. This demands extensive ecological studies. In general, full objective incrimination is not possible and any conclusion depends on the accumulation of evidence on five criteria (WHO 1984, 1990). Two of them have been demonstrated previously (Chablé-Santos et al. 1995, Canto-Lara et al. 1999). The present study presented evidence for the following criteria:

First, the parasites, in a good mammalian reservoir, should be available in the skin or blood in sufficient number to be taken up by the sand fly vector (WHO 1984, Bray 1987). The quantity of parasites needed to infect a sand fly is not known. The proliferation of *Leishmania* in the studied rodents was not quantified. A culture became positive when parasites were aspirated in sufficient quantity to seed the medium. Since the site of infection was very restricted and sometimes asymptomatic, the aspirate was not always successful. Similarly, Herrer et al. (1971) stated that aspirates or smears made some millimeters away, from known leishmanial lesions of *Or. capito* Handley, 1966 found naturally infected, gave negative results. These problems in obtaining the parasite in culture, might also be experienced by the phlebotomine vector.

Most of the year, *P. yucatanicus* showed no presence of parasite. However, since the parasites were encountered in June 1994 and May 1995 in all five mice, *Leishmania* must have been present during the whole research. The presence of cryptic parasites have been indirectly proven in humans (Aragort de Rossell et al. 1992, Alvar et al. 1997) and because of the small quantity, they would not harm the host. The fact that all the Yucatán deer-mice showed the parasites at the same time suggests the existence of a trigger for parasites multiplication. All infected rodents were captured between November 1993 and February 1994, the coolest months of the year in the study site. Thus, the trigger for parasite multiplication in the present deer-mice might be the temperature. In the animal-care facility, the air conditioners were functioning more during the hottest months, May to July. Thus, the peaks of parasite proliferation in captivity did not correspond to the one in the field, but both seemed to be related to temperature. Kerr et al. (1995) suggested that the absence of detectable infections they encounted in summer may be explained by the inhibition of growth of *L. (L.) mexicana* at or above 37ºC.

Second, the course of infection should be long and the reservoir host long-lived to provide a significant source of infection for the sand flies (WHO 1984). In the field, *S. hispidus* does not live for more than six months (Odum 1955, Cameron & McClure 1988) while *Ot. phyllotis* and *P. yucatanicus* live for more than two years (Van Wynsberghe, unpublished data). The high rate of

TABLE II

Distribution of *Leishmania* (*Leishmania*) *mexicana* in smears of organs of naturally infected rodents from La Libertad, Campeche, Mexico

Species	I.D.	Liver	Spleen	Kidney	Heart
Sigmodon hispidus	S-A2	+	+		
	S-N5	+	-		
Oryzomys melanotis	Or-K6	+	+		
	Or-F9	+	+		
	Or-C8	+	-		
Ototylomys phyllotis	Ot-K5	-	-	-	-
	Ot-6a	-	-	-	-
	Ot-B4	-	-	-	-
	Ot-C4	-	-	-	-
	Ot-S9	-	-	-	-
	Ot-Q9	+	-	-	-
Peromyscus yucatanicus	P-15a	+	+	+	+
	P-1a	+	+	+	+
	P-3b	+	+	+	+
	P-O11	+	+	+	+

I.D.: identification; smear: positive +; negative -

mortality in captive *Or. melanotis* demonstrated the sensivity of the black-eared rice-rats to environmental conditions and its probable short life-span.

One *S. hispidus* consistently kept the parasites during 15 months while the second kept the parasites irregularly during 21 months. Similarly, three *Ot. phyllotis* did not retain the parasite, while three others retained the parasite consistently during the two years (Table I). Thus, the sampled area in Campeche might contain mixed populations within each of those two species, resistant and susceptible to *L. (L.) mexicana.*

All the *Oryzomys* retained the leishmanial parasite consistently ($P<0.05$). Because of its good retention of the parasite, the black-eared rice-rat could be an effective reservoir of *L. (L.) mexicana.* However, the low relative abundance of the black-eared rice-rats limited its importance as an effective reservoir host (Chablé-Santos et al. 1995). Herrer et al. (1971) found a similar population decrease after trapping *Or. capito*.

Third, in a reservoir species, the infection should be relatively nonpathogenic (WHO 1984). In general, the clinical signs of leishmaniasis in *Ot. phyllotis* were small or not existent and the infection seemed harmless. Similarly, the infection in black-eared rice-rats and Yucatán deer-mice did not seem to endanger their life in captivity. This asymptomatic infection with little or no pathology probably results from a well balanced and probably ancient host-parasite relationship (Lainson & Shaw 1979).

The finding of systemic dissemination of *L. (L.) mexicana* in naturally infected rodents came as a surprise (Table II). Disney (1964) found visceral involvement probably due to *L. (L.) mexicana* in one *Heteromys desmarestianus* Desmarest, 1817 *emend.* Goldman, 1911 from Belize; however, the Heteromyidae are phylogenetically very different from the Cricetidae here studied. Lainson and Strangways-Dixon (1964), in a study on the reservoir hosts of *L. (L.) mexicana* among the forest rodents carried out in British Honduras, found naturally infected *Ot. phyllotis*, *H. desmarestianus*, *Nyctomys sumichrasti* Saussure, 1860 and the infections were purely cutaneous. Similarly, Herrer et al. (1971) did not find any parasite in liver, spleen, heart blood, and bone marrow of 26 *Or. capito* presenting caudal lesions infected with flagellates causing in golden hamster lesions similar to those of *L. (L.) mexicana.* Lainson and Shaw (1979) stated that, in rodents, the division between cutaneous and visceral leishmaniases is not always clear. However, the occurrence of amastigotes of *L. (L.) mexicana* in organs of the cricetids studied should not be considered as visceralization of the disease, since their effect was non-pathogenic.

In this study, the presence of the parasite in organs could be related to the age of the mice and rats as a consequent depression of the immune response (Bach 1982). The age of the 29 naturally infected rodents was not known at capture, however, all presented adult characteristics such as pelage, weight, and reproductive activity (scrotal - open vagina, pregnant, or lactating). The two *S. hispidus* could be considered very old since this species live up to six months in the wild (Odum 1955, Cameron & McClure 1988). Only two *Oryzomys* from both the healthy (N=20) and the infected (N=8) colonies survived the two-years research. Three of the four *Peromyscus* with amastigotes in the organs were more than three years old as proven by a two-years ecological study, previous to collecting.

The lack of systemic dissemination in three *Ot. phyllotis* (Ot-K5, Ot-6a, and Ot-B4) might be due to the fact that those climbing rats did not retain the parasite (Tables I, II). The last three *Ototylomys* tested for systemic dissemination were not infected except one liver with very few amastigotes. The big-eared climbing rat had very little cutaneous signs of infection and showed the parasites very irregularly, thus demonstrating their relative resistance to *L. (L.) mexicana.*

The results of the present laboratory research in combination with those of the field study (Chablé-Santos et al. 1995) confirms the role of *Ot. phyllotis* as a primary reservoir-host of *L. (L.) mexicana*, and indicates that *P. yucatanicus* is of similar importance in the Campeche focus of infection. The existence of cryptic parasites in *P. yucatanicus* during most of the year and the possible presence of an environmental trigger during the transmission season, makes the Yucatán deer-mouse in the best choice as both a primary reservoir in this area, and an animal model for further research on *L. (L.) mexicana*.

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