CUTANEOUS LEISHMANIASIS OF MAN DUE TO *LEISHMANIA (VIANNIA) SHAWI* LAINSON, DE SOUZA, PÓVOA, ISHIKAWA & SILVEIRA, IN PARÁ STATE, BRAZIL

J. J. SHAW*, E. A. Y. ISHIKAWA**, R. LAINSON*, R. R. BRAGA**, F. T. SILVEIRA**

SUMMARY

Leishmania (Viannia) shawi Lainson, Braga, de Souza, Póvoa, Ishikawa & Silveira, 1989, was originally recorded from monkeys (*Cebus apella* and *Chiropotes satanas*), sloths (*Choloepus didactylus* and *Bradypus tridactylus*) and coatis (*Nasua nasua*) and the sandfly, *Lutzomyia whitmani*. With a panel of *Leishmania* specific monoclonal antibodies, it was found that 30.5 % of the *Leishmania* strains from patients, who had contracted cutaneous leishmaniasis in Pará State, were similar, but not identical to *L. (V.) guyanensis*.

The enzyme profiles of 18 of these strains were determined, and it was found that 12 isolates belonged to the same zymodeme as the type strain of *L. (V.) shawi*. The other 6 belonged to a second *L. (V.) shawi* zymodeme that only differed from the other by having a slightly faster PEP band. These results are the first records of infections of *L. (V.) shawi* in man and suggest that this parasite is probably common in areas of Pará State to the south of the Amazon River.

RÉSUMÉ : Leishmaniose cutanée humaine à *Leishmania (Viannia) shawi* Lainson, Braga, de Souza, Póvoa, Ishikawa & Silveira, 1989 dans l'État du Para, Brésil.

Leishmania (Viannia) shawi Lainson, Braga, de Souza, Póvoa, Ishikawa & Silveira, 1989 a été décrite initialement chez des singes (*Cebus apella* et *Chiropotes satanas*), des paresseux (*Choloepus didactylus* et *Bradypus tridactylus*), des coatis (*Nasua nasua*) et le phlébotome *Lutzomyia whitmani*. L'utilisation d'anticorps monoclonaux spécifiques pour le genre *Leishmania* a montré que 30,5 % des souches de *Leishmania* isolées d'individus ayant contracté la leishmaniose cutanée dans l'État du Pará, sont semblables mais

non identiques à *L. (V.) guyanensis*. Les profils enzymatiques de 18 parmi ces isolats ont été étudiés et 12 se sont révélés identiques à la souche de référence de *L. (V.) shawi*. Six isolats appartiennent au deuxième zymodème de *L. (V.) shawi* avec cependant la bande de l'enzyme PEP légèrement plus rapide. Ce parasite, probablement commun dans les zones de l'État du Pará situées au sud de l'Amazonie, est associé pour la première fois à des infections humaines.

INTRODUCTION

Two new *Leishmania*, *L. (Viannia) naiffi* and *L. (V.) shawi*, were recently described from wild animals by our laboratory (R. Lainson and J. J. Shaw, 1989; R. Lainson *et al.*, 1989). Although 4 human infections of *L. (V.) naiffi* have subsequently been recorded by three different laboratories (R. Lainson *et al.*, 1990), all from the general region of the Trombetas river, Pará, so far, there have till now been no records of infections with *L. (V.) shawi* in man.

Between 1982 and 1985 (J. J. Shaw *et al.*, 1986), we

tested a panel of 6 monoclonal antibodies against 174 *Viania*-like *Leishmania* from wild animals, sandflies and man. At that time, using monoclonal antibodies, it was impossible to identify 34 % of the strains specifically, including those strains of *L. (V.) naiffi* and *L. (V.) shawi* from animals and sandflies previously identified by isoenzymes. The serodemes of an additional 43 strains, isolated from dermal lesions of man in Pará State, were subsequently determined. In the present work the enzyme profiles of 18 of the above mentioned strains, whose serodemes were similar, but not identical to those of *L. (V.) guyanensis*, were compared with the type strain of *L. (V.) shawi*.

MATERIALS AND METHODS

A total of 43 *Leishmania* strains from human cases of dermal leishmaniasis were tested against a panel of 23 monoclonal anti-

* The Wellcome Parasitology Unit, Caixa Postal 3, 66001 Belém, Pará, Brazil.

** Department of Parasitology, The Instituto Evandro Chagas, Fundação Nacional de Saúde, Caixa Postal 1128, Belém, Pará, Brazil.

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TABLE I. — List of strains identified as *Leishmania* (Viannia) *shawi* isolated from patients who became infected in the municipality of Paragominas, Pará State, Brazil.

WHO International Code Number	Isolation Date	No. of lesions	Location of lesion(s)	Age Sex	<i>Leishmania</i> test (mm)
MHOM/BR/77/M4984	Dec 05 77	3	Leg	25 F	4.6
MHOM/BR/79/M5561	Apr 04 79	3	Trunk and arm	24 M	0.0
MHOM/BR/82/M6922	Mar 08 82	1	Leg	33 M	9.0
MHOM/BR/82/M7339	Nov 04 82	21	Generalized	30 M	24.0
MHOM/BR/82/M7357	Nov 17 82	1	Leg	27 M	10.0
MHOM/BR/82/M7358	Nov 17 82	9	Trunk and leg	23 M	8.0
MHOM/BR/83/M7528	May 08 83	1	CL	46 M	5.0
MHOM/BR/83/M8020	Dec 26 83	3	Leg	42 M	0.0
MHOM/BR/85/M9222	Jan 07 85	4	Leg	37 M	6.0
MHOM/BR/85/M9491	Mar 05 85	1	Foot	46 M	8.0
MHOM/BR/85/M9507	Mar 12 85	1	Arm	19 M	10.0
MHOM/BR/85/M10066	Nov 12 85	1	Arm	21 M	6.0
MHOM/BR/86/M10813	Sep 29 86	1	Arm	24 M	0.0
MHOM/BR/86/M10851	Oct 14 86	1	Leg	20 M	5.0
MHOM/BR/86/M10877	Nov 03 86	6	Trunk and leg	44 M	6.0
MHOM/BR/87/M11044	Jan 05 87	1	Leg	18 F	0.0
MHOM/BR/87/M11104	Feb 10 87	1	Leg	43 M	11.0
MHOM/BR/87/M11185	Feb 02 87	1	Foot	42 M	11.0

bodies (B2, B5, B11, B12, B13, B18, B19, M2, T3, D13, M11, M12, CO1, CO2, CO3, L1 — donated by Dr. Dianne McMahon-Pratt; WIC.79.3 — donated by Dr. D. Snary; N2, N3, LA2, WH1, WA2, V1 — donated by Dr. C. A. Hanham) using the indirect biotin/Avidin fluorescent antibody method as described by Shaw *et al.* (1989). Eighteen strains (Table I) from the municipality of Paragominas were selected from the group of parasites whose serodemes were different from those of *L. (V.) guyanensis*, but which clearly belonged to the *guyanensis* complex.

The enzyme profiles for ACON, ALAT, ASAT, GPI, G6PD, 6PGDH, MDH, MPI, PGM and PEP of these 18 strains were compared to those of the type strain of *L. (V.) shawi* (MCEB/BR/84 M/8408) and the WHO reference strain of *L. (V.) guyanensis* (MHOM/BR/75/M4147) by the methods described by Miles *et al.* (1981).

During September 1991, in municipality of Dom Eliseu (4° 17' 06"S: 47° 30' 18"W), sandflies attracted to a Shannon trap and man were captured and dissected. Until recently Dom Eliseu was part of Paragominas (2° 59' 43"S: 47° 21' 12"W) municipality.

RESULTS

Of a total 217 *Leishmania* strains from Pará State that were isolated from man, and which includes 43 strains examined in the present work, 47 % could not be identified at the species level with monoclonal antibodies. However, it was possible to group such isolates by their serodeme patterns and it was found that 65 % of the unidentified strains belonged to the *guyanensis* complex. The typical feature of parasites of this complex, including *L. (V.) panamensis* (unpublished observations) and *L. (V.) shawi*, is that variable numbers of promastigotes fluoresce strongly with monoclonal B12 (XIII-3H6-F9). This feature is only detectable when parasites are examined directly and not in tests such as the RIA and ELISA.

All strains of *L. (V.) shawi* belonged to the same serodeme in which epitopes B2, B12, L1, CO1, CO2, CO3

and B13 are expressed. In tests performed on different cultures of the same strain it was noted that the CO1 epitope, and to a lesser extent the CO3 epitope, are variably expressed. Because of this the presence or absence of such epitopes is not very useful for defining serodemes as they are probably stage — rather than species — specific. The CO3 epitope has been found to be expressed by most *Leishmania* species (unpublished observations).

It was noted that unidentified *guyanensis* complex parasites were particularly common in the eastern region of Pará State, composed of the municipalities of Acará, Capitão Poço, Ourém, São Domingues de Capim, Irituia, Viseu, Dom Eliseu and Paragominas. In Paragominas unidentified *guyanensis* complex parasites represented 46 % of the strains isolated from man.

Except for PEP the zymograms of the other 9 enzymes were identical in all 18 strains to those of the reference strain of *L. (V.) shawi*. The PEP of strains MHOM/BR/83/M7528, MHOM/BR/85/MP9222, MHOM/BR/85/M10066, MHOM/BR/87/M11104, MHOM/BR/86/M10877, and MHOM/BR/86/M10813 was slightly faster than that of the *L. (V.) shawi* reference strain. The results for PGM, G6PD and 6PGDH are shown in Figure 1. When the time of the run was 120 mins there was no difference between the G6PD of *L. (V.) shawi* and *L. (V.) guyanensis*, but after 180 mins it was noted that the former was slightly faster than the latter (Fig. 1-C).

Between 8-13th September, 1991, a total of 210 female sandflies were caught in a Shannon trap, and 34 off man. No flagellate infections were found. The following species were caught in the Shannon trap — the number captured is given in brackets: *Lutzomyia brachypyga* (1), *Lu. campbelli* (2), *Lu. dasypodogeton* (6), *Lu. dendrophyla/shannoni* (78), *Lu. flaviscutellata* (20), *Lu. furcata* (10), *Lu.*

FIG. 1. — The identification of *Leishmania (Viannia) shawi* infections in man by isoenzymes profiles. Electrophoretic patterns of 3 enzymes A and B = PGM, C = G6PD and D = 6PGDH. In A and C, the voltage and time of the runs was 200 V for 180 mins and for B and D, 200 V for 120 mins.

For Fig. 1-A and 1-C, lanes 1 and 12 are *L. (V.) guyanensis* and lanes 2-11 *L. (V.) shawi*. The strains examined, from left to right, are (1): MHOM/BR/68/M4147, (2): MHOM/BR/87/M11185, (3): MHOM/BR/M11104, (4): MHOM/BR/87/M11044, (5): MHOM/BR/86/M10877, (6): MHOM/BR/86/M10851, (7): MCEB/BR/84/M8408, (8): MHOM/BR/86/M10813, (9): MHOM/BR/85/M9491, (10): MHOM/BR/83/M8020, (11): MHOM/BR/82/M7357, (12): MHOM/BR/68/M4147.

For Figs. 1-B and 1-D, lanes 1 and 2 are *L. (V.) naiffi*, lanes 3 and 14 are *L. (V.) guyanensis*, and lanes 4-11 are *L. (V.) shawi*. The strains examined, from left to right, are (1): MDAS/BR/79/M5533, (2): ISQQ/BR/85/M9666, (3): MHOM/BR/68/M4147, (4): MHOM/BR/85/M10066, (5): MHOM/BR/85/M9507, (6): MHOM/BR/85/M9222, (7): MHOM/BR/83/M7528, (8): MHOM/BR/82/M7339, (9): MCEB/BR/84/M8408, (10): MHOM/BR/82/M6922, (11): MHOM/BR/82/M7358, (12): MHOM/BR/79/M5561, (13): MHOM/BR/77/M4984, (14): MHOM/BR/68/M4147.

gomezi (13), *Lu. infraspinoso* (14), *Lu. migonei* (7), *Lu. monstrosa* (8), *Lu. nevesi* (9), *Lu. nordestina* (1), *Lu. pilosa* (1), *Lu. punctigeniculata* (1), *Lu. rorotaensis* (1), *Lu. serrana* (4), *Lu. tuberculata* (4), *Lu. umbratilis* (2), and *Lu. whitmani* (28). The species attracted to man were *Lu. dendrophyla/shannoni* (8), *Lu. gomezi* (18), *Lu. nevesi* (6), and *Lu. infraspinoso* (2).

DISCUSSION

The above findings show that 2 zymodemes of *L. (V.) shawi* are responsible for dermal leishmaniasis of man in the Paragominas municipality of Pará State. Of the *Leishmania* isolated from man from the neighbouring munic-

palities we have so far identified 32 strains as belonging to the *guyanensis* complex, but they did not belong to a *L. (V.) guyanensis* serodeme. The zymodemes of these strains have not yet been determined, but the results of the present work suggests that *L. (V.) shawi* commonly infects man in the eastern region of Pará State south of the Amazon river. It is interesting to note that this parasite has so far not been found amongst *Leishmania* strains isolated from patients who were infected in areas of Pará State to the north of the Amazon River and Amapá State, where *L. (V.) guyanensis* is the commonest parasite found in man.

The number of lesions of infections due to *L. (V.) shawi* ranged from 1 to 21 lesions, but the majority (61 %) were

single sores. The multiple-lesion infection due to strain MHOM/BR/82/M7339 was reminiscent of those caused by *L. (V.) guyanensis*, and suggested spread via the lymphatic system, rather than multiple sandfly bites.

The very small number of sandflies caught and the complete absence of sandflies of the genus *Psychodopygus* in the Dom Eliseu area is very unusual. A possible explanation is that it had not rained in the area for approximately 6 months. The fact that human infections of *L. (V.) shawi* are quite common in the region suggested that the vector probably bit man quite readily. In other localities Lainson *et al.* (1989) have registered massive infections of *L. (V.) shawi* in six *Lu. whitmani*, which they consequently felt to be an important vector of this parasite. It was thus a surprise when we observed although that 28 females of this species were captured in the Shannon trap none were caught off man. Further studies on the sandflies of this region of Pará, particularly at other times of the year, are needed to determine the anthropophilic habits of *Lu. whitmani* and to see if *L. (V.) shawi* occurs in other sandflies species.

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