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ZOONOTIC CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA (VIANNIA) BRAZILIENSIS* ASSOCIATED WITH DOMESTIC ANIMALS IN VENEZUELA AND BRAZIL

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After outbreaks of cutaneous leishmaniasis in Solano State, Venezuela, 5% of the population had parasitized ulcers while after similar outbreaks in Mesquita, Rio de Janeiro State, Brazil, 9% had the disease. In these foci children, including some under six years of age, were affected. There was no significant difference in the occurrence of the disease according to sex or type of employment. In Solano, 3% of dogs and 28% of donkeys had parasitized lesions, while in Mesquita these indices were 19.8% and 30.8% respectively. The parasite from man, dogs and equines was identified as Leishmania (Viannia) braziliensis, *by zymodeme and serodeme characterization. In these foci there is evidence suggesting that leishmaniasis is a zoonosis, possibly with equines and dogs as reservoirs, although both a wild enzootic cycle and the role of man as a source of infection can not be ruled out. Transmission is assumed to occur peridomestically by sandfly vectors such as* Lutzomyia panamensis *in Venezuela and* Lutzomyia intermedia *in Brazil. Information about the origin of these foci suggests that infected equines may be an important factor in the dissemination of the parasite in a peridomestic situation where these sandflies are abundant.*

Key words: zoonotic cutaneous leishmaniasis – domestic animals – Venezuela – Brazil

After an outbreak of cutaneous leishmaniasis in the rural locality of Las Rosas, in the State of Cojedes, Venezuela (Aguilar et al., 1984), a study of the area and its surroundings was undertaken enabling the detection, diagnosis and treatment of patients and an investigation of their proximity to infected dogs and donkeys. Sporadic cases were recorded in Solano, from 1978, and an outbreak was studied in the same area in 1983. In Brazil a similar study was carried out in April 1986, after an outbreak of leishmaniasis in the peri-urban locality of Mesquita, Rio de Janeiro State (Paes-Oliveira et al., 1985).

Study areas in Venezuela and Brazil – Solano and Valle Hondo are situated in the municipality of San Carlos, in the State of Cojedes, Venezuela (Fig. 1), at a latitude of 9º45'N and a longitude of 68º40'W. Access is by a dirt road which follows the tributaries Solano and Valle Hondo, which form part of the San Carlos River basin (Fig. 1). The region is in the Serrania do Interior, a mountain chain with average altitudes of 160 m above sea level in Valle Hondo, and 300 m in Solano. The primary vegetation is dry forest which has been largely replaced by plantations of yan, manioc and maize. Average temperature is 29 ºC, relative humidity 78% and annual rainfall 1,400 mm. Simple dwellings predominate, often with roofed enclosures nearby which serve as shelters for donkeys. In Solano and Valle Hondo the donkey *(Equus asinus)* is used extensively to transport the harvest to the villages.

In Brazil, the locality of Mesquita, in the municipality of Nova Iguaçu, Rio de Janeiro

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State (Fig. 1), is situated on the Atlantic face of the Massif of Gericinó (altitude 150 m, latitude 23°10'S; longitude 43°34'W). Average temperature is 22,4°C, relative humidity 80% and annual rainfall 1,902 mm. The focus can be divided into two sub-areas: Chatuba, a non-endemic region until 1984, when an outbreak of leishmaniasis began, and Rua da Serra, next to Chatuba where a new outbreak occurred at the end of 1985 and beginning of 1986. In April 1986, after the detection of the outbreak in Rua da Serra, we initiated a study on the distribution of the disease and an examination of equines and dogs. Primary tropical forest exists at the peak of the massif while the slopes are mainly grassland. Houses are concentrated at the foot of the mountains, while on the higher slopes they are scattered in the plantations of mangoes, bananas and other trees. Dogs and equines (horses, donkeys and mules) are abundant and, as in Venezuela, the equines are widely used for transporting material from the plantations.

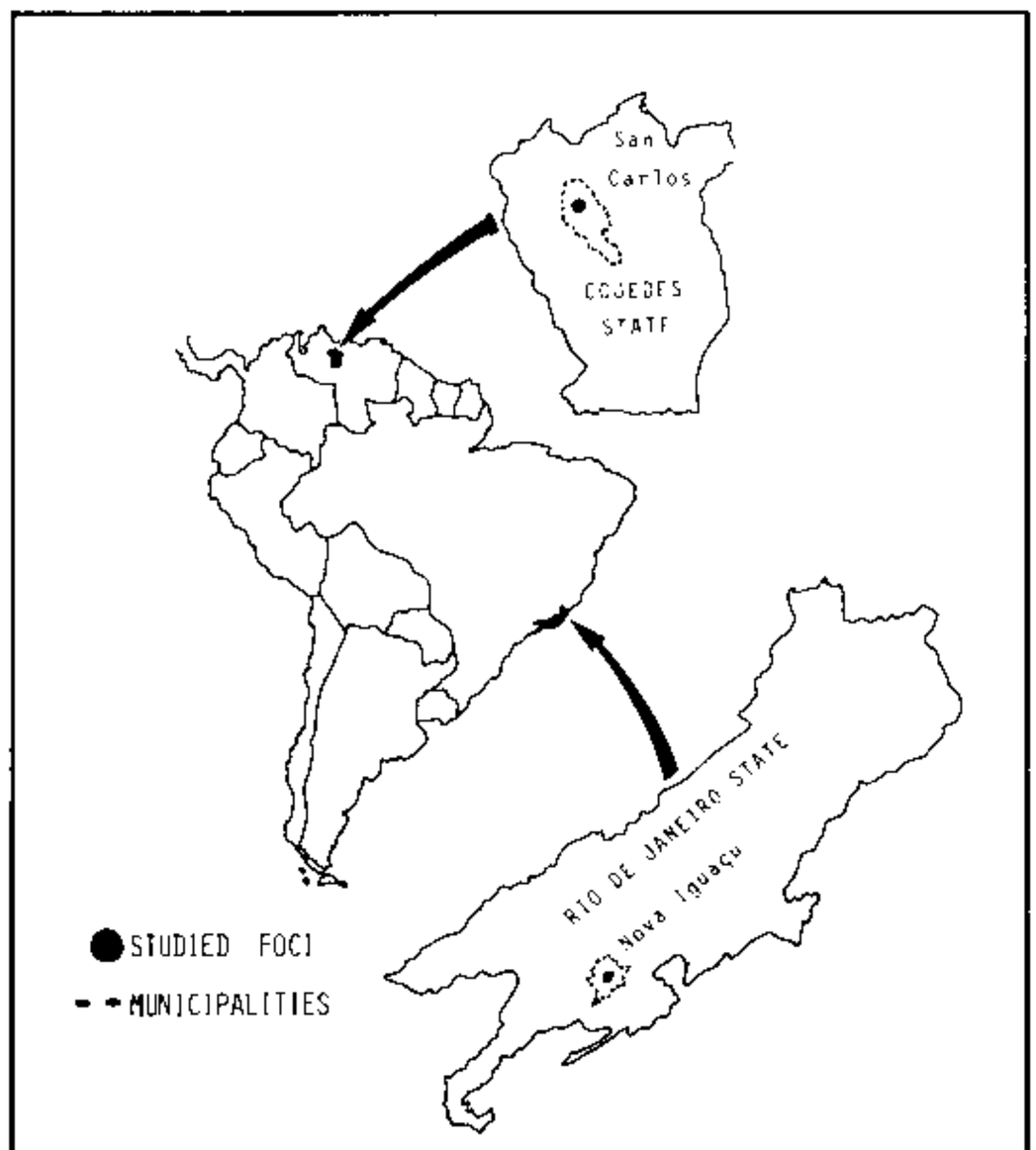

Fig. 1: area studied in Venezuela and in Brazil.

MATERIAL AND METHODS

Study of the human population – A survey of every house and family was made. Parasitological diagnosis was based on the examination of biopsy material from suspected cutaneous lesions. Tissue fragments were taken by scalpel from the border of the lesions and three smears prepared which were fixed in methanol and stained by Giemsa. This was the routine for all cases in Solano. In Mesquita, the number of human cases parasitologically confirmed was not included because when we started the survey many of the patients had been treated, some still showed ulcers, but most had only scars. So, we regarded as cases of leishmaniasis all those patients with ulcers or scars during or after treatment.

Study of the animal population – Examination of dogs and equines began soon after the outbreak in Solano using a questionaire given to the animals' owners and searching for active cutaneous lesions. The presence of parasites was verified using the same procedure as with humans lesions. Tissue fragments were put in test tubes containing 0.8 ml of phosphate buffered saline (pH 7.3) and 1000 μg penicilin + 1000 μg streptomicin and transported to the laboratory where the fragments were homogenized and inoculated subcutaneously into the back of the feet of two hamsters.

Study of the parasites – Fragments from non-ulcerated nodules from infected hamsters, which had been inoculated with biopsied material from 2 dogs (from Solano and Valle Hondo) and 2 donkeys from Solano, were placed in LIT and NNN media and the resulting flagellates grown in Schneider's media with 20% fetal bovine serum at 35 °C. Similar isolates from 1 patient, 4 dogs and 1 equine were obtained from Mesquita and maintained in the enriched media by weekly passage until parasite growth was adequate. The isolates were identified using 30 species-specific monoclonal antibodies for the *Leishmania braziliensis* and *Leishmania donovani* complexes (McMahon-Pratt et al., 1985). Reference strains of *Leishmania* were used as positive controls for the antibodies using a radioimmunoassay (RIA). For zymodeme analysis the following enzymes were used: Proline dipeptidase 3.4.13.9 (PED D), Peptidase 3.4.11 (PEP 1), Nucleoside hydrolase 3.2.2.1 (NH1, NH2, two loci), Malic enzyme 1.1.1.39 (ME), Glicose 6 phosphate dehydrogenase 1.1.1.49 (G6PDH), Glucose phosphate isomerase 5.3.1.9 (GPI), 6 phosphogluconate dehydrogenase 1.1.1.44 (6PGDH), phosphoglucomutase 2.7.7.1 (PGM). Buffers and conditions for staining were as described by Momen et al. (1985). A statistical analysis was carried out on the percentage frequencies from

the study of the human and animal populations. In the evaluation of the differences by sex and profession a unilateral test of differences between proportions was employed assuming a normal distribution and level significance $p = 0.05$.

RESULTS

Human leishmaniasis in Solano, Cojedes, Venezuela – A total of 24 cases were diagnosed from 1978 to 1983, 21 in Solano and 3 in Valle Hondo. In Solano, from 1978, cases were sporadic until an outbreak in 1983. In August, 1983, 48 houses with 293 residents were visited and 242 persons (82.6%) were examined: 14 (5.8%) had active cutaneous lesions suspected to be due to leishmaniasis and parasitological diagnosis was made in 12 (5%) by impression smears (Table I). Of 129 females, 9 had clinical evidence of the disease with parasitological confirmation in 6.2%. Of males, 94.4% has suspected lesions, with parasites in 3.5%. Of the parasitologically diagnosed cases, 93.3% were between 0 to 39 years old, including a girl of two years. The difference in disease by sex in the total population was not significant ($p > 0.05$). Only in one case was there metastasis to the nasal mucosa – approximately two years after the appearance of the initial cutaneous lesions. This case was successfully treated with Glucantime. The greatest number of cases were in residents with a domestic occupation: 10.9% had lesions and 9.4% had parasites in the smears. In farmers, 5.3% had suspected lesions of which 4% had parasites. Only one case was confirmed in children under 7 years. The difference in disease between domestic and agricultural occupation was not significant ($p > 0.05$). In Valle Hondo, the disease was diagnosed among the 416 residents of 73 houses, the patients being two males (53 and 54 years old) and on child of 2 1/2 years. In the child there was metastasis of the nasal mucosa two months after the appearence of the cutaneous lesion. The case was succesfully treated with Glucantime.

Human leishmaniasis in Mesquita, Brazil – A total of 109 houses were visited and 97% of the population were examined. The distribution of cases by sex, age and occupation is given in Table II. The age group from 10 to 19 years had the largest number of cases. No significant difference in the number of cases between sexes and occupations were seen ($p > 0.005$).

Leishmaniasis in domestic animals – The distribution of leishmaniasis among the equines and canine populations of Solano is given in Table III. All the infected dogs had multiple ulcers usually localized on the genitals, nose or ears, with similar frequence of infection between sexes. All the parasitologically confirmed cases among the donkeys were in males. The most frequent lesions were ulcers of different forms and sizes, and occurred predominantly behind the ears and on the genitals. In Valle Hondo, the infected dogs and donkeys were detected after the appearence of the human cases in the same house.

The distribution of the disease in dogs and equines in Mesquita, Brazil, is given in Table IV. As in Venezuela, the lesions of dogs were found in areas with little hair: multiple lesions were found in 43.5%. In equines, lesions were also found on the back and at the base of the neck as well as in the nude areas: multiple lesions were found in 35.7%. In general, impression smears of lesions of dogs and equines showed few parasites, with the exception of one mule which had 8 to 10 parasites per microscope field.

Identification of the parasite – When inoculated into hamsters the parasites isolated from dogs and equines from both Brazil and Venezuela, produced slow growing nodules at the site of inoculation after more than 30 days. Some hamsters infected with Venezuelan isolates showed more severe symptoms in the chronic state, becoming thin and immobile in the cages. On autopsy, parasites were detected in the viscera (liver, spleen and heart). In some hamsters, regression of the small nodules at the site of inoculation occurred in both paws. Growth in cultura media was slow and difficult. The specific identification of the parasites from dogs and equines, in Brazil and Venezuela, and from patients in Brazil was *Leishmania (Viannia) braziliensis*, by zymodeme and serodeme analysis.

DISCUSSION

Until 1978, when we began our studies, little was known about cutaneous leishmaniasis in Cojedes State, Venezuela. Since that time we have noted the spread of the disease from the original focus in Las Rosas (Aguilar et al., 1984), to the neighbouring localities of Solano and Valle Hondo. A similar situation to that

TABLE I

People examined and with clinical diagnosis of cutaneous leishmaniasis and parasitological confirmation according to age and sex in Solano, State of Cojedes, Venezuela, August, 1983

Age groups	Female					Male					Total				
	Examined	Clinical diagnosis		Parasit. confirm.		Examined	Clinical diagnosis		Parasit. confirm.		Examined	Clinical diagnosis		Parasit. confirm.	
		no.	%	no.	%		no.	%	no.	%		no.	%	no.	%
0- 9	40	1	2.5	1	2.5	42	–	–	–	–	82	1	1.2	1	1.2
10-19	33	5	15.2	4	12.1	24	2	8.3	2	8.3	57	7	12.3	6	10.5
20-39	33	2	6.1	2	6.1	27	3	11.1	2	7.4	60	5	8.3	4	6.7
40-59	18	1	5.6	1	5.6	14	–	–	–	–	32	1	3.1	1	3.1
60 e +	5	–	–	–	–	6	–	–	–	–	11	–	–	–	–
Total	129	9	7.0	8	6.2	113	5	4.4	4	3.5	242	14	5.8	12	5.0
Difference between sexes				DP ($\hat{p}$) = 0.028				Z = 0.046			p > 0.05				Not significant

TABLE II

People examined and with lesions attributed to cutaneous leishmaniasis, according to age and sex, Mesquita, State of Rio de Janeiro, Brazil. August and September, 1986

Age groups	Female			Male			Total		
	Examined	With lesions		Examined	With lesions		Examined	With lesions	
		no.	%		no.	%		no.	%
0- 9	63	7	11.1	71	5	7.0	134	12	9.0
10-19	64	6	9.4	85	13	15.3	149	19	12.8
20-39	74	4	5.4	77	6	7.8	151	10	6.6
40-59	47	4	8.5	45	4	8.9	92	8	8.7
60 e +	15	2	13.7	22	1	4.5	37	3	8.1
Total	263	23	8.7	300	29	9.7	563	52	9.2
Difference between sexes			DP ($\hat{p}$) = 0.024			Z = 0.417		p > 0.05	Not significant

TABLE III

Dogs *(Canis familiaris)* and equines *(Equus asinus)* examined, with cutaneous lesions and parasitological confirmation of cutaneous leishmaniasis in Solano, Cojedes State, Venezuela
July to November, 1983

Animal	Sex	Examined	With lesions		With parasites	
			no.	%	no.	%
Dogs *(Canis familiaris)*	F	33	3	9.1	1	3.0
	M	70	5	7.1	2	2.9
	Both sexes	103	8	7.8	3	2.9
Equines *(Equus asinus)*	F	3	–	–	–	–
	M	29	13	44.8	9	31.0
	Both sexes	32	13	40.6	9	28.1

TABLE IV

Dogs and equines examined, with cutaneous lesions and parasitological confirmation of cutaneous leishmaniasis in Mesquita, Rio de Janeiro State, Brazil
April to October, 1986

Animal	Sex	Examined	With lesions		With parasites	
			no.	%	no.	%
Dogs *(Canis familiaris)*	F	37	13	35.1	11	29.7
	M	44	8	18.2	5	11.7
Total		81	21	25.9	16	19.8
Equines						
Horses *(Equus caballus)*	F	9	4	44.4	3	33.3
	M	2	1	50.0	1	50.0
	Both sexes	11	5	45.5	4	36.4
Donkeys *(Equus asinus)*	F	–	–	–	–	–
	M	1	–	–	–	–
Mules *(Equus caballus X Equus asinus)*	F	8	3	37.5	2	25.0
	M	6	2	33.3	2	33.3
	Both sexes	14	5	35.7	4	28.6
Total	F	17	6	35.3	5	29.4
	M	9	4	44.4	3	33.3
	Both sexes	26	10	38.5	8	30.8

in Las Rosas also occurred in Solano, where sporadic human cases first appeared and were followed by an outbreak in 1983. In Mesquita, Brazil, there were also sporadic cases, followed by an outbreak in 1984 in Chatuba and, soon after, a new outbreak in Rua da Serra.

The appearance of the disease in the human population is apparently not associated with alterations in the environment. The disease was previously unknown to the inhabitants, some of whom are residents of the villages since their foundation at the beginning of the century. The outbreak of leishmaniasis in Solano affected 5% of the inhabitants and in Mesquita 9.2% (Tables I and II). In the foci of both countries, women do not carry out agricultural work and the lack of a significant difference in infection by sex and occupation suggests that farmers and domestic workers are exposed to the same risk of infection.

The disease affected young people of both sexes and children less than 7 years old. This fact, although not denying the existence of the silvatic cycle (Lainson, 1983, 1985), suggests that transmission might be also occurring peridomestically. From the results of Solano it is concluded that the frequency of infection in donkeys (28.1%) (Table III) was greater than that found in dogs and greater than that reported for donkeys in Las Rosas (21.4%). In 8 out of 48 homes in the village there was a coincidence between homes with infected people and infected domestic animals. In one home, humans, dogs and equines were infected, in 7 homes humans and donkeys were infected and in only one home were humans and dogs infected. In Valle Hondo, of the 3 patients, a child of 2 1/2 years lived together with infected donkeys and an infected dog.

In Mesquita (Table IV), the rate of infection (30.8%) in equines was greater than in Solano, and the infection in dogs (19.8%) was greater than that found by Falqueto et al. (1986), 17.2% in the focus of Viana in the State of Espirito Santo (Brazil). These results confirm the high prevalence of infection in dogs, superior to that mentioned by Herrer (1948) for Peruvian "uta", where dogs are considered the reservoir hosts. Of the 116 homes examined in Mesquita, 13 had infected domestic animals. In three homes there was a concidence of humans with lesions and infected dogs and horses; in 7 houses there were infected humans and dogs; in 9 houses only infected people and in 2 houses only infected dogs; in one house there was a dog and an equine with leishmaniasis. In Mesquita as well as in Solano, the distribution of the disease showed a concentration in the houses where there were infected animals or in houses close to these (Figs 2 and 3).

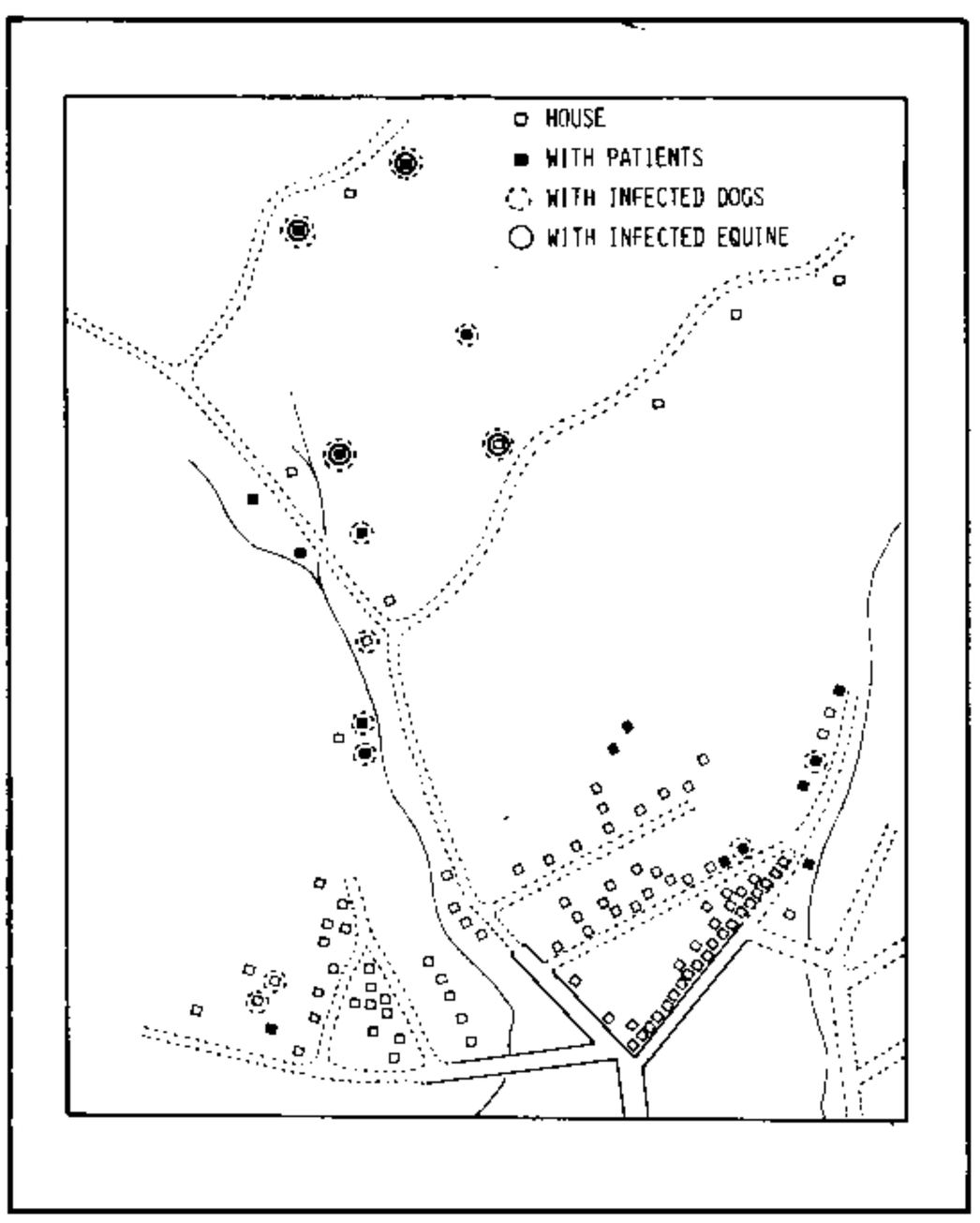

Fig. 2: Mesquita, Rio de Janeiro State, showing the distribution of houses of Rua da Serra with humans cases of cutaneous leishmaniasis, and infected dogs and equines.

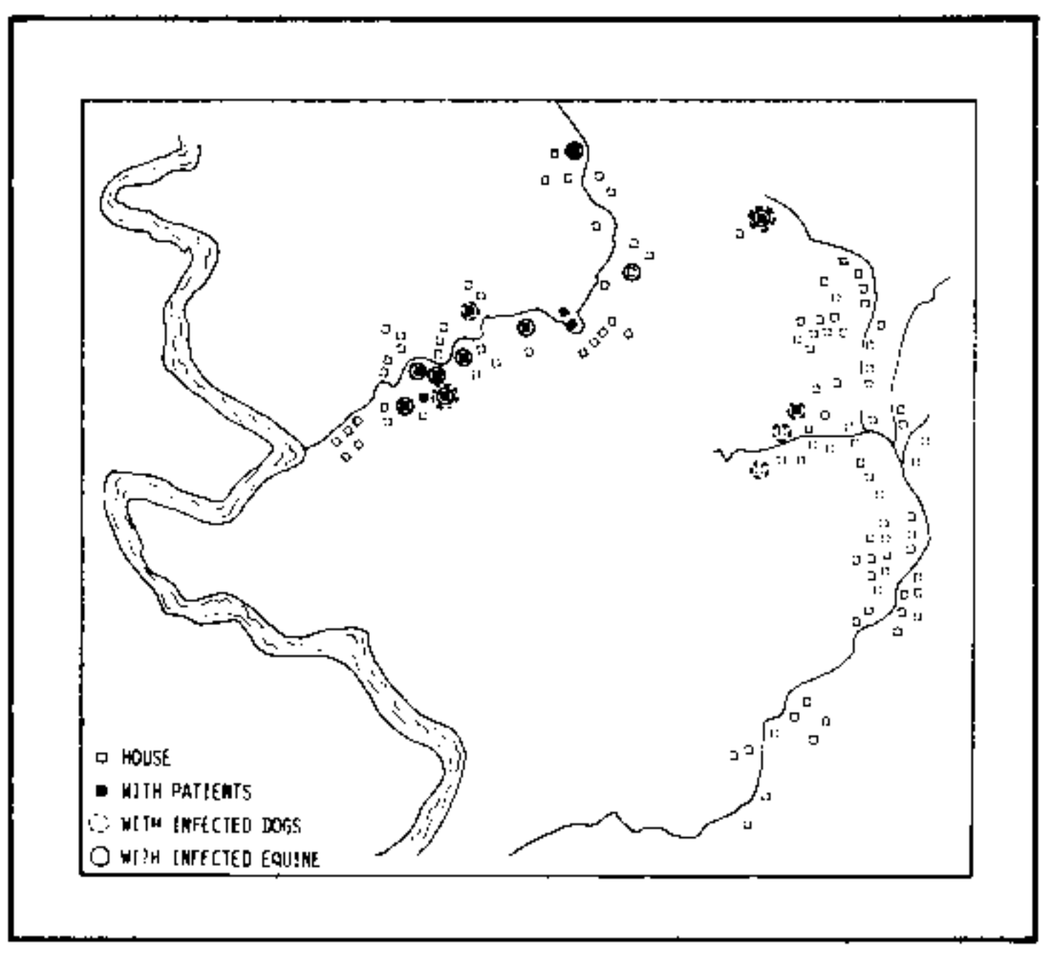

Fig. 3: Solano, Cojedes State, showing houses with humans clinical cases of cutaneous leishmaniasis, and infected dogs and equines.

In Brazil, especially in the South East Region, the occurrence of infected humans and dogs in the same endemic area has already been referred to by other authors (Dias et al., 1977; Araujo Filho, 1979; Souza et al., 1980; Marzochi et al., 1982; Barreto et al., 1982; Paes-Oliveira et al., 1985; Falqueto et al., 1986). In Venezuela, a similar situation involving the occurrence of infections in humans and equines has been reported by us in Cojedes, and by others in the States of Zulia and Lara (Pons & Londres, 1968; Bonfante et al., 1979).

Data on the probable vectors in Solano (Aguilar et al., 1987) have shown that *Lutzomyia panamensis* and *Lu. gomezi* were practically the only species collected by peridomestic nocturnal captures using humans and donkeys as baits. The former in the focus of Las Rosas was considered as the possible vector. It had already been considered as a vector in Venezuela by Pifano et al. (1959). In Mesquita, Rangel et al. (1985), using human and animals baits and CDC light traps, found *Lu. intermedia* to be the most frequent sandfly species inside and outside the houses, and suggest it to probably be the local vector. They concluded that transmission was occurring either intra or peridomestically. Since Aragão (1922) suggested *Lu. intermedia* as the vector of cutaneous leishmaniasis in Rio de Janeiro, Brazil, this species has been found infected in the States of Paraná and São Paulo (Forattini & Santos, 1952; Forattini et al., 1972) adding strength to this suggestion. The parasites were not identified, however. In the State of Rio de Janeiro, it is found in larger numbers in the foci of the disease in the interior of the State as well as in the Capital. The presence of this sandfly is associated with the endemic situation as well as with the appearance of new foci, even in periurban areas where the disease is epidemic. Recently, in Jacarepaguá, on the periphery of the city of Rio de Janeiro, Rangel et al. (1984) found a specimen of *Lu. intermedia* naturally infected with a *Leishmania* species which may have been *L. (V.) braziliensis.*

In a previous study by zymodeme analysis, isolates from human and donkey in the same foci in Venezuela were shown to be *Leishmania (Viannia) braziliensis* (Aguilar et al., 1982). In Três Braços, State of Bahia, Brazil, the parasite from man and from dogs was also characterized as *Leishmania (Viannia) braziliensis* (Barreto et al., 1982; Cuba et al., 1985), and a similar identification was made in the periurban areas of Jacarepaguá and of Mesquita (Marzochi et al., 1982; Paes-Oliveira et al., 1985). More recently, Vexenat et al. (1986) working in Corte de Pedra, in Bahia, where dogs and humans were also parasitized, found equines infected with *Leishmanis (Viannia) braziliensis.*

These facts indicate that the same parasite is circulating in the human and domestic animal population in foci of *Leishmania (Viannia) braziliensis* in some areas of Venezuela and Brazil. In Venezuela, the equines used in the farms are generally males which originate from other localities of the State of Cojedes or neighbouring States. Therefore most of the animals were imported into the area. In our weekly visits, mainly at harvest time, we were able to observe that some of the animals infected in Las Rosas spend the night in Solano. All this leads us to believe that the activation of the focus and its dissemination is related to the mobility of the infected animals. In Mesquita, according to information given by the owners of the animals, 13 of the 26 equines examined did not originate in the locality, and of the 10 that had suspected lesions, 3 had entered the area with the lesions before the appearence of the outbreak in the humans community. Two of the equines came from Rio da Prata, in Campo Grande, an endemic area for cutaneous leishmaniasis.

With regards to dogs, the introduction of animals from one locality to another is a common occurrence. In Valle Hondo, one of the infected dogs came from Las Rosas, and in Mesquita one of the dogs with lesions came from Jacarepaguá, a periurban area of Rio de Janeiro (endemic for cutaneous leishmaniasis). We, therefore, suggest that *L. (V.) braziliensis* has recently been introduced into the study areas by infected dogs and equines originating from other zoonotic areas. The parasite is disseminated in ecologically receptive areas where vectors allow the transmission to other susceptible hosts. It is possible that what we believe to happen with dogs and equines also occurs with humans (as houses with more than one patient are frequently found in the foci) where there is a high density of vectors. Table V gives the frequency of infection in equines and humans as well as the average capture of those sandfly species considered likely to be vectors in Venezuela and in Brazil (Aguilar et al., 1987; Rangel et al., 1985). It is possible,

therefore, that the equines, dogs and other domestic animals act as "biological barriers", intercepting a large number of sandflies. It is suggested that the keeping of infected domestic animals (particulary equines) near houses may be an important factor in the transmission of the disease in and around dwellings, as sandfly species such as *Lu. panamensis* in Venezuela and *Lu. intermedia* in Brazil, feed preferentially on equines, but may pass the parasite on to other equines, dogs and people near the houses (Fig. 4).

Although cutaneous leishmaniasis has not been well studied in equines, recent observations in dogs have shown that parasites can be found in the visceras (Marzochi et al., 1983), that the parasites can disseminate to give mucosal metastasis (Falqueto et al., 1986; Pirmez et al., 1986) and that it is possible to obtain experimental infection of sandflies by xenodiagnosis on cutaneous lesions produced by *Le. (Viannia) braziliensis* (Vexenat et al., 1986). Deane et al. (1986) have studied the possibility of humans serving as sources of

TABLE V

Comparison of the prevalence of cutaneous leishmaniasis and the number of sandflies bitting equines and humans in the localities studied in Venezuela and Brazil

Country	Locality	Host	% with cutaneous Leishmaniasis	Average number of sandflies for each 10 hours of capture	
				Lu. panamensis	*Lu. intermedia*
Venezuela	Solano	Donkey	28.1	134	–
		Man	5.0	56.6	–
Brazil	Mesquita	Equine	30.8	–	320
		Man*	9.2	–	194

* Lesions attributed to cutaneous leishmaniasis.

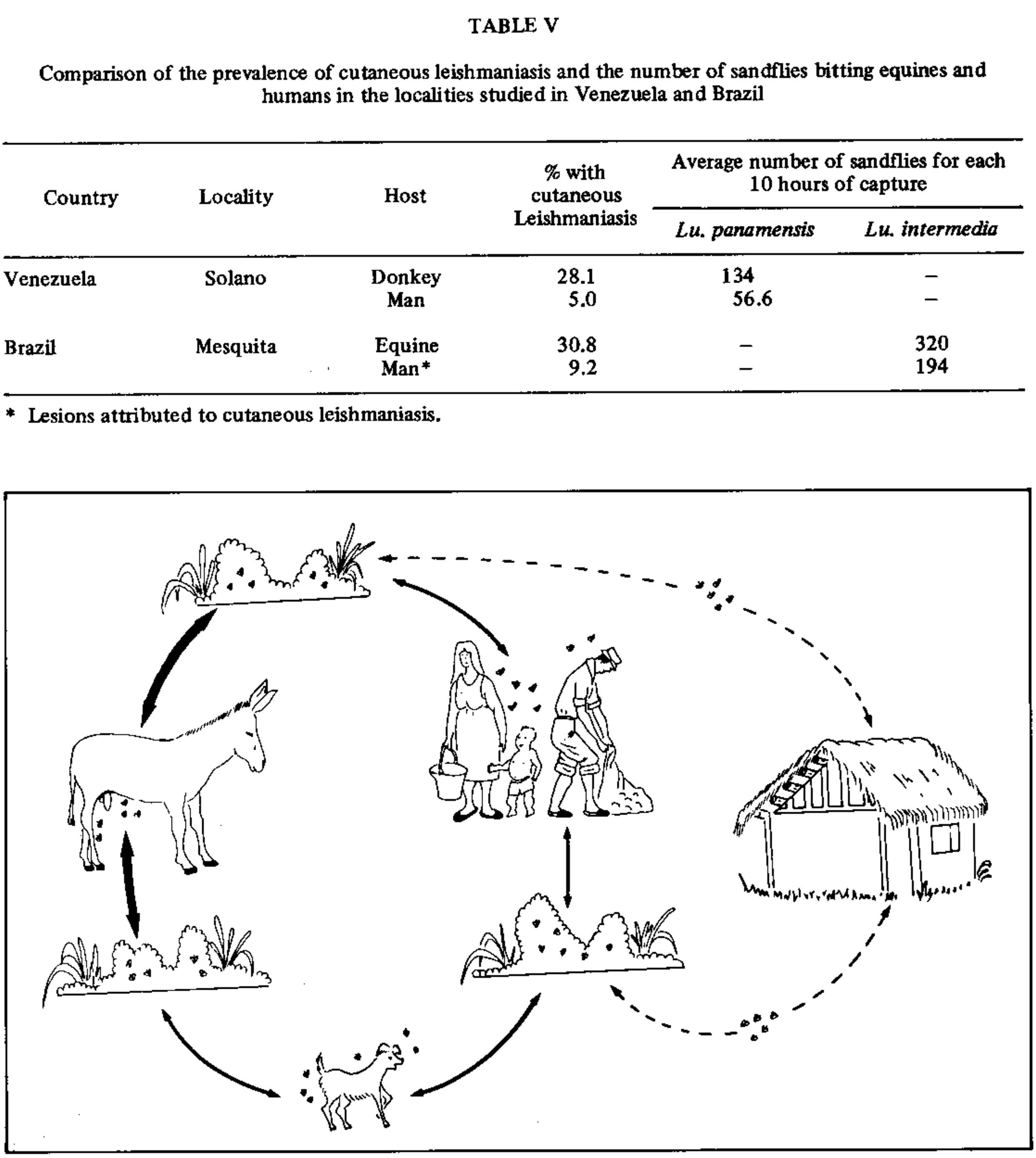

Fig. 4: suggested cycle of peridomestic transmission.

infection. They successfully infected *Lu. longipalpis* by feeding them on the cutaneous lesion of a patient infected with *Le. (Leishmania) amazonensis.*

In addition, the finding of parasites in the bone marrow and in the blood of humans cases (Deane et al., 1966; Bowdre et al., 1981; Ramos et al., 1982), suggests that sandflies might becofne infected after feeding on cutaneous lesions or on blood.

RESUMO

Leishmaniose tegumentar *(Leishmania (Viannia) braziliensis* **associada a animais domésticos na Venezuela e no Brasil** – Após o surgimento do foco de leishmaniose tegumentar em Solano, Estado de Cojedes, Venezuela, 5% da população possuíam úlceras parasitadas, enquanto que em Mesquita, Estado do Rio de Janeiro, Brasil, 9% apresentavam a doença. Nos dois focos, crianças com menos de seis anos eram acometidas. Não existe diferença significativa na ocorrência da doença entre sexos ou entre trabalhadores domésticos e agricultores. Em Solano, 3% dos cães e 28% dos equinos apresentavam lesões parasitadas; em Mesquita estes índices eram de 19,8% e 30,8%, respectivamente. O parasito isolado de humanos, cães e equinos foi identificado como *Leishmania (Viannia) braziliensis,* através da análise de zimodema e serodema. Neste foco existe uma evidência sugerindo que a leishmaniose é uma zoonose, onde os equinos e os cães seriam os prováveis reservatórios. Entretanto, o ciclo enzoótico silvestre e o papel do homem como fonte de infecção não foram devidamente avaliados. A transmissão nestes focos é provavelmente peridomiciliar, envolvendo vetores ecléticos como *Lutzomyia panamensis,* na Venezuela, e *Lutzomyia intermedia,* no Brasil. Dados sobre a origem destes focos sugerem que equinos infectados são importantes na disseminação do parasita em áreas ecologicamente receptivas.

Palavras-chave: leishmaniose tegumentar – animais domésticos – Venezuela – Brasil

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