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STUDY OF AN OUTBREAK OF CUTANEOUS LEISHMANIASIS IN VENEZUELA. THE ROLE OF DOMESTIC ANIMALS

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*During an outbreak of cutaneous leishmaniasis in a locality (Las Rosas, Cojedes State, Venezuela) previously non-endemic, 12.9% of humans, 7% of dogs and 21.4% of donkeys (*Equus asinus*) had lesions with parasites. The agent in the three hosts was identified as* Leishmania braziliensis, *subspecies* braziliensis *at least in man and donkey. The probable vector was* Lutzomyia panamensis.

No infection was found in a small sample of wild mammals examined. The outbreak was apparently linked with the importation of donkeys with ulcers, from endemic areas.

The Authors call attention to the fact that not only in the foci of "uta", but also in areas of the other forms of American cutaneous leishmaniasis, dogs are frequently found infected. They emphasize the necessity of searching for the infection in donkeys and of performing hemocultures and xenodiagnosis with sandflies in human, canine and equine cases, to verify their possible role as sources of infection, and not merely as dead ends in the epidemiological chain of the disease.

In March 1978 the Venezuelan Ministry of Health detected an outbreak of cutaneous leishmaniasis in a small rural settlement, Las Rosas, Cojedes State. Since that part of the country was previously non-endemic we visited the area in April to study the epidemiology of the outbreak. Our attention was soon called to the presence of ulcers in some of the local dogs and donkeys (*Equus asinus*). These species of domestic animals had already been found infected with cutaneous leishmaniasis in other countries, and since in Venezuela similar findings have been recorded in connection with epidemics of leishmaniasis we thought it pertinent to invetigate the possible participation of these domestic animals in the spread of the disease.

MATERIAL AND METHODS

The study area (Map 1). Las Rosas stands on a forested hilly terrain, about 14 kilometers from the capital of the municipality of San Carlos, at 68° 36' longitude West and 9° 41' latitude North, altitude of 200 to 400 meters, average yearly rainfall of about 1300 millimeters, average temperature of 27°C and average humidity of 84%. The vegetation is tropical deciduous gallery forest, of secondary growth around the settlement. Houses are of very primitive types. There are no latrines, electricity or piped water supply. The principal crop is yam, followed by manioc and corn. Agriculture is mostly an activity of the male population. As the plantations are rather distant from the houses, donkeys are necessary for transportation of land products; this leads to a close

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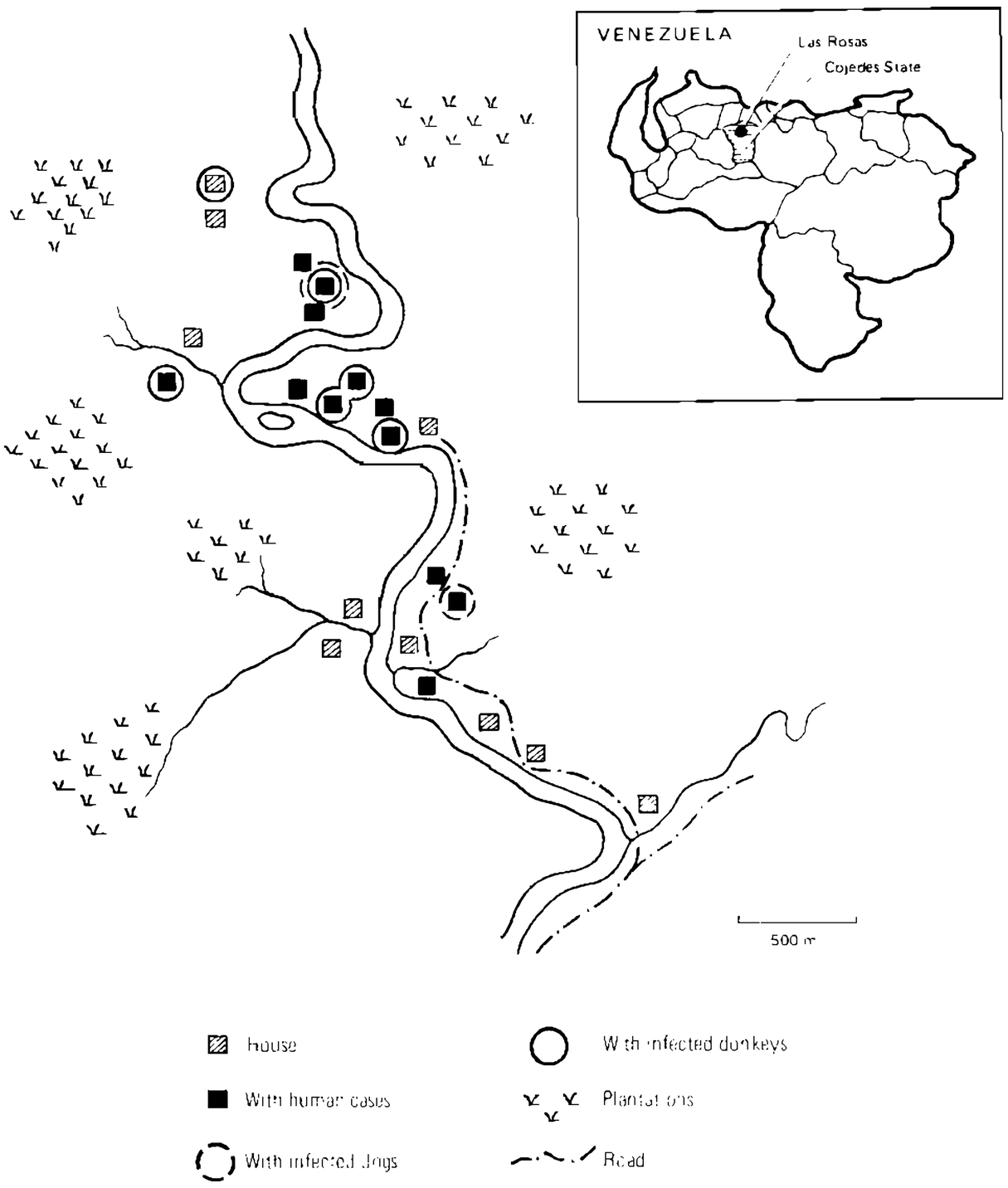

Map. 1. The study area: Las Rosas, Cojedes State, Venezuela.

Location of houses and of human, canine and equine cases of cutaneous leishmaniasis.

association of men with those animals, which are kept close to – and in a few instances inside – the dwellings, after the day's work. Dogs usually do not follow the peasants to the plantations, remaining around the houses most of the time.

Search for leishmaniasis in the human population. In house to house visits all inhabitants were examined for ulcers, other active skin lesions, skin scars and mucosal alterations. Smears obtained from skin lesions after local anesthesia with 1% lidocaine were stained with Giemsa's. Intradermal tests were performed with 0.1 ml leishmanial antigen, results being recorded 48 hours later; a papule of more than 5 millimeters or vesiculation with or without necrosis were regarded as positive. Strains of the parasite were isolated from the confirmed cases through subcutaneous inoculation of a triturate from the lesion in the hind feet of hamsters. Treatment was carried out with Glucantime (n-methylglucamine antimoniate, 50 mg/k, intramuscularly).

Search for canine and equine infections. A census of dogs was also made in all houses. The animals were examined and, if showing ulcers, skin thickenings or other skin lesions, they were weighed, anesthetized with nembutal (25 mg/k, intraperitoneally), and material from the lesions was used for prints stained with Giemsa. If leishmaniae were found, the parasites were isolated through hamster inoculation, as in the case of humans. As kala-azar has been previously reported in Cojedes State the dogs with cutaneous leishmaniae were also submitted to venipuncture for hemoculture in NNN and LIT media and for complement fixation tests. One of the sick dogs was taken to the laboratory and necropsied, spleen and liver macerates being injected intraperitoneally in hamsters.

All donkeys existing in Las Rosas were also examined. Ulcers and other skin lesions were examined as in the case of dogs. A skin fragment was kept for maceration and inoculation in hamsters. From three of the donkeys with leishmaniae in the skin lesions blood was collected for hemoculture in NNN and LIT media.

Search for wild animal reservoirs. With the help of a squad from the World Health Organization which was capturing local wild mammals to search for trypanosome infections, we set baited traps inside houses and outdoors, at 50 meters from the dwellings and in the plantations, far from the houses. The traps were baited with pieces of pineapple and corn. The animals captured were taken to the laboratory, anesthetized with chloroform and examined for skin lesions, which were biopsied for the preparation of Giemsa-stained smears and hamster inoculation. They were also submitted to cardiac puncture for blood examination (fresh preparations and Giemsa-stained thick and thin smears) and hemocultures, and, after necropsy, pieces of spleen and liver were obtained for stained smears and subcutaneous and intraperitoneal inoculation in hamsters.

Search for the phlebotomine vectors. During a whole year, from October 1978 through September 1979, the following types of captures of sandflies were performed weekly:

– indoors, on the walls and on the inhabitants, from 6 to 9 p.m.

– outdoors, near the houses and in the woods far from the houses, simultaneously on man, dog and donkey used as baits, from 6 to 9 p.m.

– in "natural" resting places (tree holes, around tree trunks, under leaves, in ground burrows), from 3 to 6 p.m.

The human baits were treated patients of leishmaniasis, all volunteers who were advised of risks, probably minimal, in view of the previous natural infection. Dogs were tied to some support and donkeys were kept as quiet as possible by their owners. The sandflies were brought alive to the laboratory, in plaster-bottomed net-covered cardboard cups, mounted and identified to species in phenol, some specimens being dissected for the search of flagellates in the intestinal tract, before immersion in phenol.

Identification of the parasite. To identify to species level the agent of the disease in man and animals we took into account the size of the amastigotes and their numbers in the lesions, growth in culture media, lesions produced in hamsters and the distribution of promastigotes in the intestinal tract of sandflies (Lainson & Shaw, 1972, 1979; Johnson & Hertig, 1970). Subspecies identification was done (with one human and two equine strains) through the isozyme patterns by means of agarose gel electrophoresis, using the following enzymes: glucose-6-phosphate dehydrogenase, phospho-glucoseisomerase, aspartate aminotransferase, malate dehydrogenase, alanine aminotransferase and phosphoglucomutase.

RESULTS

Leishmaniasis in the human population. As seen in Table 1, of 124 persons living in Las Rosas (the entire population), 21 (16.1%) had ulcers but only in 16 (12.9%) the leishmanial etiology was confirmed by examination of the lesion smears. The infection rates were 15% for females and 10.9% for males. The youngest confirmed case was 3 years and the oldest 62 years.

All lesions were ulcers, except one which was framboezoid and another, verrucose; 33% of the patients had multiple lesions. The sites of the lesions were: upper 47.6%, lower limbs 38.1%, head and neck 9.5%, abdomen 4.8%. Hamsters injected with triturate from ulcers became infected, showing benign lesions. Alterations on the nasal mucosa

TABLE I

Persons examined and cases of cutaneous leishmaniasis, by age and sex, in Las Rosas, Cojedes State, Venezuela, April 1978

Age groups, in years	Females			Males			Total				
	Examined	Clinical	Parasitologically confirmed	Examined	Clinical	Parasitologically confirmed	Examined	Clinical		Parasitologically confirmed	
								No.	%	No.	%
0 – 1	2	–	–	4	–	–	6	–	–	–	–
2 – 4	14	2	1	5	1	1	19	3	15.8	2	10.5
5 – 9	5	–	.	11	1	1	16	1	6.3	1	6.3
10 – 29	26	7	7	23	5	3	49	12	24.5	10	20.5
30 or more	13	1	1	21	4	2	34	5	14.7	3	8.8
Total	60	10	9	64	11	7	124	21	16.9	16	12.9

were later observed in four patients with cutaneous lesions with parasites (25%); biopsy with a Hartmann forceps was performed and the Giemsa stained mucosal smears were negative, but triturates injected subcutaneously in the hind paws of previously immunosuppressed hamsters confirmed the leishmanial nature of the mucosal lesion of three of the patients.

The intradermal test performed in 115 inhabitants was positive in 26, or 22.6%, being 16.9% among males, 28.6% among females and 5.1% in children less than 10 years of age.

Clinical cure was achieved in all patients with 1-4 series of Glucantime, except in two, who received additional treatment with the anti-leprosy drug Lamprene.

Leishmaniasis in dogs and donkeys. All dogs and donkeys present in the locality were examined. As seen in Table II, of 43 dogs 6 showed active cutaneous lesions, but only in 3 (7%) the leishmanial origin was confirmed parasitologically both in skin smears and by hamster inoculation. The location of the lesions was on muzzle, ear and nipple; two dogs had ulcers, the third showed scaly infiltration on the ears (Figs. 2 to 4). The confirmed infections were all found in females.

Of the 29 donkeys, all males, 7 had active cutaneous lesions and in 6 (21.9%), leishmaniae were detected. The lesions were located in glabrous or less hairy areas, the majority being ulcers of variable size (from about 5 millimeters to 5 centimeters in diameter), on scrotum, penis, tail or ear (Figs. 5 to 9).

All dogs and donkeys had multiple lesions, some with the same appearance as in humans and with few parasites in the smears. In the infected dogs no leishmaniae were detected in the viscera smears and the inoculation of viscera triturates in hamsters, hemocultures and complement fixation tests for kala-azar were negative. So were the hemocultures of the infected donkeys.

Search for wild reservoirs. Of the 118 synanthropic or wild animals examined, all mammals, none was found infected. Some had skin lesions but these were negative, both by direct examination of stained smears and hamster inoculation, and so were the hemocultures and hamsters injected with viscera triturate. The animals were: 22 *Rattus rattus* (rats), 51 *Proechimys semispinosus* (spiny-rats), 1 *Heteromys anomalus*, 1 *Oryzomys* sp. (long-tailed mouse), 1 *Echimys semivellosus* (spiny-rat), 4 *Dasyprocta agouti* (agouties), 1 *Agouti paca* (paca), 26 *Didelphis marsupialis* (opposums), 2 *Tamandua tetradactyla* (ant-eaters) and 9 Phyllostomidae (leaf-nosed bats). Trypanosomes were found in opossums, spiny-rats and ant-eater.

Search for the vectors. * As seen in Table III, in 622 hours of systematic captures performed during a complete year, 2,110 sandflies of 17 species were collected. Few species occurred in large numbers, such as *Lutzomyia panamensis*, *Lu. atroclavata*, *Lu.*

* *Lu.* is used as abbreviation for *Lutzomyia* to avoid confusion with *L.* for *Leishmania.*

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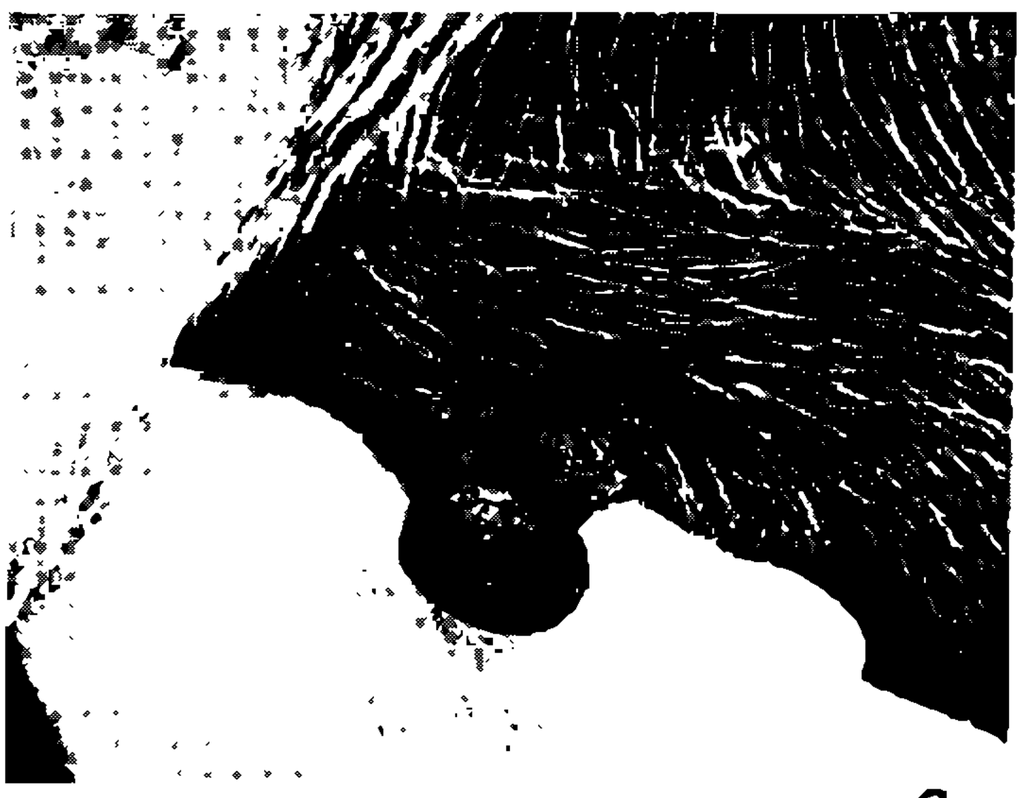

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Cutaneous leishmaniasis in Las Rosas, Cojedes State, Venezuela.

Fig. 1 – Dog, ulcer on nose. *Fig. 2* Dog, crusty lesion on ear. *Fig. 3* Dog, ulcer on nipple. *Fig. 4* – Donkey, proliferative lesion on ear. *Fig. 5* – Donkey, ulcers (a) and scars from previous ulcers (b) on scrotum. *Fig. 6* – Donkey, ulcers on scrotum.

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Cutaneous leishmaniasis in Las Rosas, Cojedes State, Venezuela.

Fig. 7 – Donkey, a large ulcer (natural size) on scrotum. *Fig. 8* – Donkey, ulcer on tail. *Fig. 9* – At the end of day's work, donkeys remain close to human dwellings.

TABLE II

Results of search for Leishmaniae in cutaneous lesions of dogs and donkeys (*Equus asinus*), in Las Rosas, Cojedes State, Venezuela, 1978-1979

Animals	Sex	Examined	With lesions		Parasitologically confirmed	
			No.	%	No.	%
Dogs	Females	27	5	18.5	3	11.1
	Males	16	1	6.3	–	–
	Both sexes	43	6	14	3	7
Donkeys	All males	28	7	25	6	21.4

trinidadensis and *Lu. gomezi,* which accounted for almost 90% of the total. The prevalence of each species according to the type of capture is presented in Table IV, where the number collected in houses, outdoors on human and animal baits and in "natural" resting places, as well as the time spent in each type of capture, are recorded. The only species collected inside houses were *Lu. panamensis* and *Lu. gomezi,* which were also almost the only ones obtained outdoors, on human and animal baits. Other species such as *Lu. lichyi, Lu. ovallesi* and *Lu. punctigeniculata* were only rarely seen on one or more of the baits, while the remaining species were found only in the natural resting places. Of the sandflies obtained inside houses only two females of *Lu. gomezi* were collected while feeding on the inhabitants, all other specimens being caught on the walls.

TABLE III

Species of sandflies collected in Las Rosas, Cojedes State, Venezuela, October 1978 through September 1979

Total number and percent of specimens.

Species of sandfly	No.	Percent
Lutzomyia panamensis (Shannon, 1926)	910	43.1
Lutzomyia atroclavata (Knab, 1913)	430	20.4
Lutzomyia trinidadensis (Newstead, 1922)	356	17
Lutzomyia gomezi (Nitzulescu, 1930)	179	8.5
Lutzomyia ovallesi (Ortíz, 1952)	77	3.7
Lutzomyia punctigeniculata (Floch & Abonnenc, 1944)	67	3.2
Lutzomyia shannoni (Dyar, 1929)	40	1.9
Lutzomyia migonei (França, 1920)	20	1
Lutzomyia lichyi (Floch & Abonnenc, 1950)	15	0.7
Lutzomyia olmeca bicolor (Fairchild & Theodor, 1971)	5	0.2
Lutzomyia antunesi (Coutinho, 1939)	3	0.1
Lutzomyia rangeliana (Ortíz, 1953)	3	0.1
Lutzomyia cayennensis (Floch & Abonnenc, 1941)	1	0.0
Lutzomyia evansi (Nuñez-Tovar, 1924)	1	0.0
Lutzomyia marajoensis (Damasceno & Causey, 1944)	1	0.0
Lutzomyia sp.	1	0.0
Brumptomyia sp.	1	0.0
Total	2,110	100

The monthly frequency of sandflies in Las Rosas, based on the systematic captures of all types is presented in Table V. It shows that the density of sandflies and, in particular, that of *Lu. panamensis*, was low between December and April, rising sharply to peaks in May and August and disappearing in February and March. *Lu. gomezi*, present in smaller numbers, kept its low density throughout the year, with risings less pronounced than in *Lu. panamensis*) in November and May.

In the search for natural infections of sandflies with *Leishmania* we dissected and examined the intestinal tract of 819 females: 424 *Lu. panamensis*, 130 *Lu. atroclavata*, 108 *Lu. trinidadensis*, 93 *Lu. gomezi* and 69 of less frequent species. We found none with promastigotes. However, in several specimes of *Lu. atroclavata*, *Lu. ovallesi*, *Lu. punctigeniculata*, *Lu. shannoni* and *Lu. trinidadensis* the intestinal tract harboured epimastigotes and trypomastigotes, which are developmental stages of trypanosomes.

The parasites. In the lesions of the naturally infected hosts (man, dog and donkey) the amastigotes were usually scarce, relatively small and morphologically similar. Cultures of lesions were obtained, in most cases, after several attempts, in NNN and LIT media kept at 22°C, but growth of promastigotes was poor. Hamsters inoculated with strains from man and animals developed moderate lesions, which were usually nodular with a tendency to ulcerate, sometimes curing spontaneously. Specimens of *Lu. panamensis* and *Lu. gomezi* which fed on nodules on the foot of a hamster injected with a donkey strain became infected, showing promastigotes in the midgut and pyloric triangle (Aguilar, 1981). These characters led to the diagnosis of *Leishmania braziliensis.* Two puppies injected subcutaneously in the nose with material of an ulcer from a donkey developed nodules (with parasites), which became ulcerated for a very short period, apparently healing spontaneously. Though smears from the site of the cured lesions showed no leishmaniae, the persistence of viable parasites was demonstrated by hamster inoculation up

to one year later. The strains submitted to biochemical characterization at the Oswaldo Cruz Institute through isozyme pattern (one human and two equine) were shown to belong to the subspecies *Leishmania braziliensis braziliensis* (Aguilar et al., 1982).

DISCUSSION

Human infections. The relatively high number of persons with active lesions associated with the rather low prevalence of skin test positivity and the finding of incipient mucosal involvement only in persons who had cutaneous lesions acquired during the present outbreak, are consistent with the information that cutaneous leishmaniasis was not endemic in the area before. Although the first cases were adult males who possibly acquired their infection in the plantations far from the houses, the presence of the disease in women and very young children suggests that transmission also occurred inside houses or in their vicinity.

Canine and equine infections. Wild mammals are usually regarded as the sources of infections in American cutaneous leishmaniasis, while domestic animals and man would merely be victims, or accidental hosts of the parasites. Since the first infected dogs were discovered by Pedroso (1913) in Brasil, this animal has been found with the disease in many of the endemic areas. While studying a focus of "uta" in the province of Huaruchiri in the Peruvian Andes, Herrer (1951) was the first to find a large proportion of infected dogs (40 out of 513, or 9%). This led to the assumption, generally accepted until recently, that "uta" is the only form of New World cutaneous leishmaniasis in which dogs are a reservoir host (Lainson & Shaw, 1972; Bray, 1974). However, recent studies have shown not only that in dogs the infection is the rule in foci of several countries (TableVI), including in places where no parasitized wild animals were detected (Dias et al., 1977), but also that in some areas the infection rate among dogs is equal or higher than that recorded by Herrer in Peru: 9% in Panama (Herrer & Christensen, 1976); 10.5% in Ilha Grande, Rio de Janeiro State (Araujo Fº, 1978) and 16.9% in Viana, Espirito Santo State (Falqueto, 1983), both in Brazil.

Concerning the infection of equines, the first reference is due to Mazza (1926), who found leishmaniae in an ulcer from a horse (*Equus caballus*) in Argentina. Years later, Alencar (1959) in Brazil detected these parasites in a donkey in Ceará State. However, it is in Venezuela that the high frequency of infection among donkeys has been observed in several rural communities during outbreaks of cutaneous leishmaniasis in the human population. This was first verified by Pons & Londres (1968) in Zulia State, then by Bonfante et al. (1979, 1981) in Lara State and by ourselves (Aguilar et al., 1979) in Cojedes State, where the proportion of parasitologically confirmed cases was higher in donkeys than in humans and dogs.

It seems to us that in numerous foci of American cutaneous leishmaniasis the domestic animals have not been examined to a desirable extent. For example, in the Amazon Region of Brazil, which is one of the most important endemic areas in the country (and where many wild mammals have been found parasitized), the infection has been reported only in one cat (Mello, 1940) and two dogs (Mayrink et al., 1981) and this may not reflect the real situation, as there is a tendency of the local workers to search for the infection mostly in wild animals. The same may be said concerning donkeys; it is possible that the equine infection be present in areas where donkeys are numerous and closely linked with the human activities, as is some of Venezuelan foci. More attention should be paid to donkeys as possible hosts in other countries.

Probable vectors. The aim of the sandfly captures was to determine the species feeding on man, dog and donkey, the known domestic hosts of cutaneous leishmaniasis in the area. For this reason the collecting was chiefly oriented to this purpose rather than to obtain a broader knowledge of the local phlebotomine fauna, including species that feed on rodents and wild mammals of other orders that are known to be sources of leishmanial infections in other areas.

Of the species of sandflies collected in the various types of captures, *Lutzomyia panamensis* has been the most numerous (Table III). In the captures performed inside houses (Table IV) the number of sandflies was relatively low, perhaps due to the fact that in Las Rosas, since 1962, the houses are periodically sprayed with insecticides, as the area is endemic for Chagas' disease. The fact that *Lu. panamensis* and *Lu. gomezi* were the only species seen in houses, coincides with the observation of Ramirez-Perez et al. (1981) in Aragua State. In Las Rosas, inside human dwellings the proportion of *Lu. panamensis* and *Lu. gomezi* was 3 to 1. The two species account for nearly all sandfly

TABLE IV

Sandflies collected in systematic captures performed in houses and outdoors on humans, dogs and donkeys (*Equus asinus*) used as baits and in natural resting places in Las Rosas, Cojedes State, Venezuela, from October 1978 through September, 1979

Species of sandfly	In houses		Outdoors							
			Humans		Dogs		Donkeys		Natural resting places	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
Lu. antunesi	–	–	–	–	–	–	–		1	2
Lu. atroclavata	–	–	–	–	–	–	–	–	171	259
Lu. evansi	–	–	–	–	–	–	1	–		–
Lu. olmeca bicolor	–	–	–	–	–	–	–	–	5	–
Lu. gomezi	13	1	77	8	1	10	26	38	–	5
Lu. lichyi	–	–	10	–	1	–	2	–	2	–
Lu. marajoensis	–	–	–	–	1	–	–	–	–	–
Lu. migonei	–	–	–	–	–	–	–	–	5	15
Lu. ovallesi	–	–	5	–	2	4	–	–	17	49
Lu. panamensis	40	2	293	–	18	–	527	12	18	–
Lu. punctigeniculata	–	–	1	–	2	1	–		5	58
Lu. rangeliana	–	–	–	–	–	–	–	–	2	1
Lu. shannoni	–	–	–	–		–	–	–	8	32
Lu. trinidadensis	–	–	–	–	–	–	–	–	133	223
Lu. sp.	–	–	–	–	1	–	–	–	–	–
Brumptomyia sp.	–	–	–	–	–	–	–	–	1	–
Total, each sex	53	3	386	8	26	15	556	50	369	644
Total, both sexes	56		394		41		606		1,013	
Hours spent	135.5		156		41.5		61.5		227.5	

specimens feeding on man, dog and donkey in the outdoor captures. Of the sandflies biting man 75.9% were *Lu. panamensis*, 19.9% *Lu. gomezi* and only 4.2% of the remaining species. But the bait attracting the largest number of *Lu. panamensis* was the donkey, followed by man and, lastly, dog, the average numbers per 10-hours of captures being 85, 18 and 6 respectively. *Lu. gomezi*, in much smaller numbers than *Lu. panamensis*, preferred to bite man than donkey. The other species collected while feeding on the baits were *Lu. lichyi* on all three hosts, *Lu. ovallesi* and *Lu. punctigeniculata* on man and dog and *Lu. evansi* on donkey, but the number of specimens of each of these four species was too small for including them among the possible vectors (Table IV). *Lu. atroclavata* and *Lu. trinidadensis*, although abundant in the locality, as one can see by their high density in natural shelters, have not been collected biting man, dog or donkey. Ortiz (1968) associates them to small rodents and reptiles and in our survey nearly all sandflies found infected with epimastigotes and trypomastigotes were obtained in tree holes inhabited by lizards and/or small rodents, and at least *Lu. trinidadensis* is regarded as saurophilic, being an incriminated vector of lizard trypanosomes (McConnell & Correa, 1964; Christensen & Telford, 1972).

Although without direct evidence, and in spite of not finding any specimen infected with promastigotes, our observations point to *Lu. panamensis* as the probable vector of cutaneous leishmaniasis in Las Rosas. It has been found in the majority of the endemic areas of Venezuela and was proven to be a vector in another part of the country (Pifano et al., 1959). Another species which might also be a vector is *Lu. gomezi*, already found naturally infected in Panama.

TABLE V

Monthly frequency of sandflies - all species, *Lu. panamensis* and *Lu. gomezi* – in Las Rosas, Cojedes State, Venezuela, October 1978 through September 1979

Number of specimens and mean per 10 hours of capture.

Year and month		Hours spent	Number			Mean per 10 hours of capture		
			All species	*Lu. panamensis*	*Lu. gomezi*	All species	*Lu. panamensis*	*Lu. gomezi*
1978	October	43.5	93	27	2	21.4	6.2	0.7
	November	53	227	121	45	42.8	22.8	8.5
	December	35	56	27	3	16	7.7	0.9
1979	January	66	109	27	7	16.5	4.1	1.1
	February	51	71	–	–	13.9	–	–
	March	67	61	–	28	9.1	–	4.2
	April	69	84	3	19	12.2	0.4	2.8
	May	64	541	301	49	84.5	47.0	7.7
	June	21	220	3	7	104.8	1.4	3.3
	July	58	222	79	7	38.3	13.6	1.2
	August	53	351	285	5	66.2	54.8	0.9
	September	41.5	75	37	7	18.1	8.9	1.7
Total		622	2,110	910	179	33.9	14.6	29

The parasite. Morphological and biological characters of all strains from humans, dogs and donkeys identified them to *Leishmania braziliensis* (Aguilar et al., 1979) and the peripylarian development in sandflies, of a donkey strain confirmed this diagnosis. The same species of parasite was later reported by Bonfante et al. (1981) in equine leishmaniasis in another Venezuelan State, Lara. The biochemical identification of the subspecies *Lu. braziliensis braziliensis* in human and donkey strains is corroborated by the presence of nasal mucosa alterations of leishmanial origin in one-fourth of the human patients. So, we believe the agent of the disease in all three hosts to be the same.

Are humans and domestic animals sources of infection? The number of wild or synanthropic animals examined in Las Rosas has been too small to exclude them as primary sources of infection in the leishmaniasis outbreak. However, the fact that in the locality the disease was not known and that apparently no alterations occurred in the environment which could facilitate the proximity of wild animals and humans and, in addition, the existance of dogs and specially donkeys infected, called our attention for the necessity of investigating the eventual role of the domestic animals and man in the transmission of the disease.

In Brazil, in several ocasions it has been verified that the agent of cutaneous leishmaniasis of man and dog is the same. *Leishmania braziliensis braziliensis* has been found in these two hosts in Bahia (Barreto et al., 1982), Espirito Santo (Falqueto, 1983), Rio de Janeiro (Marzochi et al., 1982) and São Paulo (Machado et al., 1982). In Venezuela, during the Las Rosas outbreak, the species of parasite in humans, donkeys and dogs was *L. braziliensis*, the subspecies *braziliensis* being confirmed at least for the two first hosts. As there are species of sandflies which are susceptible and eclectic so as to feed frequently on man, dog and donkey, the possibility that they can become infected on those hosts and transmit their parasites should be tested. In Las Rosas, *Lu. panamensis*, the most suspected species, was collected in larger numbers on donkey, then on man and lastly dog and this coincided with the rate of natural infection among the three hosts (Table VII).

Map 1 shows the coincident infection of humans and dogs or donkeys in several houses. In one house with four human cases, the six dwellers lived in close association with two infected animals – one dog and one donkey, which were often under the same roof during our weekly visits.

TABLE VI

Some records of American Cutaneous Leishmaniasis in domestic animals

Host	Country and (in parentheses) State	References
Domestic dog (*Canis familiaris*)	Costa Rica (Heredia)	Soto et al., 1976
	Panama	Herrer & Christensen, 1976
	Venezuela (Yaracuy)	Pifano, 1940
	Venezuela (Yaracuy)	Medina & Romero, 1960
	Venezuela (Zulia)	Homez-Chacin, 1958
	Venezuela (Zulia)	Pons & Londres, 1968
	Venezuela (Lara)	Bonfante et al., 1972
	Venezuela (Cojedes)	Aguilar et al., 1979
	Brazil (Amazonas)	Mayrink et al., 1981
	Brazil (Ceará)	Deane & Deane, 1955
	Brazil (Bahia)	Barreto et al., 1982
	Brazil (Espirito Santo)	Falqueto, 1983
	Brazil (Rio de Janeiro)	Souza et al., 1980
	Brazil (Rio de Janeiro)	Araújo F9, 1978
	Brazil (Rio de Janeiro)	Marzochi et al., 1982
	Brazil (São Paulo)	Pedroso, 1913
	Brazil (São Paulo)	Brumpt & Pedroso, 1913
	Brazil (São Paulo)	Forattini & Santos, 1955
	Brazil (São Paulo)	Machado et al., 1982
	Brazil (Minas Gerais)	Dias, 1982
	Brazil (Minas Gerais)	Dias et al., 1977
	Brazil (Minas Gerais)	Mayrink et al., 1979
	Peru (Huarochiri)	Herrer, 1951
	Argentina (Salta)	Mazza, 1926, 1927
	Argentina (Tucuman)	Romaña et al., 1949
Domestic cat (*Felis cattus*)	Brazil (Pará)	Mello, 1940
Horse (*Equus caballus*)	Argentina (Salta)	Mazza, 1927
Donkey (*Equus asinus*)	Venezuela (Zulia)	Pons & Londres, 1968
	Venezuela (Lara)	Bonfante et al., 1979, 1981
	Venezuela (Cojedes)	Aguilar et al., 1979
	Brazil (Ceará)	Alencar, 1959

TABLE VII

Relation between prevalence of cutaneous leishmaniasis and the number of *Lu. panamensis* caught while feeding on the known domestic hosts – donkey, man and dog –, in Las Rosas, Cojedes State, Venezuela, 1978-1979

Host	Prevalence of cutaneous leishmaniasis	Mean number of *Lu. panamensis* per 10 hours capture
Donkey	21.4	85.7
Man	12.9	18.8
Dog	7	4.4

In Las Rosas, informations from most of the older inhabitants suggest that cutaneous leishmaniasis might have been imported through infected donkeys from endemic areas. The first human case, which occurred in 1974, belonged to a family that had imported a donkey presenting multiple ulcers on penis and scrotum. Ulcers appeared in two human cases and some of the dogs and donkeys prior to the intensification of the outbreak, in 1978.

The presence of parasites in the blood of wild animals has been frequently revealed through hemocultures used as a routine method of diagnosis. In man and domestic animals hemocultures have not been carried out on the same scale as in wild animals, and those performed have usually been negative. In spite of this, the mucosal metastasis in man suggests the possible passage of the parasites through the blood, and cultures of bone marrow or blood have sporadically been positive (Deane et al., 1966; Bowdre et al., 1981; Ramos et al., 1982). So, the possibility of man and domestic animals being sources of infection should be investigated. We suggest that systematic hemocultures and, even better, xenodiagnosis with the adequate species of sandflies be performed in human, canine and equine cases. This should help to clarify their role in the epidemiological chain of American cutaneous leishmaniasis.

RESUMO

Durante um surto de leishmaniose tegumentar em Las Rosas, localidade previamente não endêmica do Estado Cojedes, Venezuela, 12,9% dos habitantes, 7% dos cães e 21,4% dos asnos (*Equus asinus*) apresentavam lesões com parasitos. O agente etiológico nos três hospedeiros foi identificado como *Leishmania braziliensis*, sendo da subespécie *braziliensis* pelo menos em pessoas e asnos. O transmissor provável foi *Lutzomyia panamensis*.

Não comprovamos a infecção numa pequena amostra de mamíferos silvestres examinados. O surto esteve aparentemente relacionado com a importação de asnos com úlceras de áreas endêmicas.

Chamamos a atenção para o fato de que não é apenas nos focos de "uta", mas também nos de outras formas de leishmaniose tegumentar americana que os cães estão freqüentemente parasitados. Enfatizamos a necessidade de se procurar a infecção em asnos e de se proceder a hemoculturas e a xenodiagnósticos com flebótomos em casos humanos, caninos e equinos para investigar seu possível papel como fontes de infecção e não o de meros "fins de linha" na cadeia epidemiológica da doença.

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