

KALA-AZAR IN PORTUGAL. VII. EPIDEMIOLOGICAL SURVEY IN ALIJÓ (ENDEMIC REGION OF ALTO-DOURO)

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ABSTRACT: The municipality of Alijó is situated in an endemic area of kala-azar with 8,3 cases/100.000 inhabitants/year. This study is based on the Indirect Immunofluorescent Assay. The survey concerning dogs as reservoirs showed a 10 % prevalence rate in 1986/87 and 12,4 % in 1988/89, which was 20,2 % and 18,2 % respectively in the south of that area. The study of human groups at risk showed 11,9 % positive serological reactions with low titres. *Leishmania infantum* zymodeme MON-1 was isolated from 2 dogs as well as from one *Phlebotomus ariasi*. In another *P. ariasi*, *L. infantum* zymodeme MON-24 was isolated. This study showed a typical Mediterranean endemic focus of kala-azar with the dog as reservoir, *P. ariasi* as the principal vector and *L. infantum* zymodeme MON-1 as the responsible strain.

KEY WORDS: Kala-azar, *Leishmania infantum*, zymodemes, dog reservoir, *Phlebotomus*, Portugal.

INTRODUCTION

Kala-azar is endemic in Portugal where, in 1988, 45 proven cases were officially reported. Nearly 80 % of the patients came from the Region of Alto-Douro in the north of the country. The annual incidence of visceral leishmaniasis (V.L.) in this region is 8,3 cases/100.000 inhabitants, the disease being almost exclusively rural.

The region of Alto-Douro presents an estimated population of 433100 inhabitants (FRAZÃO, 1981), with altitudes ranging from 200 to 1000 m. The climate and vegetation are mediterranean (FRANCO, 1973). The mean annual temperature is 14° C (6° to 24° C). The mean annual rainfall is 800 mm³ (GASPAR, 1979).

In this region, the municipality of Alijó (18846 inhabitants and 286,12 km²) has shown the highest prevalence (86 cases from 1970 to 1984).

An epidemiological survey was performed from 1986 to 1989, and its results are presented in this paper.

MATERIAL AND METHODS

Ecological data

This study was carried out in a rural region called Alijó. It is situated in the north of the country and bordered by the Pinhão river (to the west), the Tua river (to the east) and the Douro river (to the south). The vegetation is mostly formed by oak type trees, juniper and chestnut trees, wild pine and some shrub species. There are also orchards and olive groves (ROBERT, 1984-85; Universidade de Trás-os-Montes e Alto Douro, 1988). The municipality may be partitioned in 3 distinct areas (Fig 1). «Terra Quente» (Hot land), in the south,

is an area of deep sheltered valleys, schistous ground and an altitude ranging from 200 to 600 m. The maximum average temperature is 24° C and the minimum is 8° C. The rainfall is under 600 mm³. Agricultural activity is port wine-related. «Terra Fria» (Cold land) is situated in the north. It is an area of granitic plateau with an altitude between 700 and 1000 m, characterized by a severe prolonged winter and a short hot summer. The average annual temperature is 12° C. Farming and goat herding are the most important activities. «Terra de Transição» (Transition land) is the widest of the 3 and is made up mainly of slopes with broad platforms. The annual average temperature is 13,6° C and the rainfall is 700 mm³. The main agricultural activity concerns farming and vineyards.

Technical data

From 1986 to 1987 canine blood samples were collected in the little town of Alijó and in 19 villages of the municipality, located in the three above-mentioned areas (Fig. 1). The sampling method and the blood collecting technique have been described elsewhere (ABRANCHES *et al.*, 1983, 1987). The prevalence of canine leishmaniasis was evaluated by Indirect Immunofluorescent Assay (IFA) with a significant titre $\geq 1:128$, established according to preliminary results (ABRANCHES, 1984; ABRANCHES *et al.*, 1987). In some suspected clinical cases parasitological examination by popliteal lymph node puncture was immediately carried out, according to a technique already described (ABRANCHES, 1984). In 1988 and 1989, sample collection was repeated in the same region. During this period a seroimmunological assay on humans at risk (people in permanent contact with ill dogs) was carried out. Vector studies were carried out only in «Vale de Mendiz» (Terra Quente) where the prevalence of canine infection was 37,8 %. CDR miniature light traps were used in the capture of the phlebotomine sandflies, following the method of KILLICK-KENDRICK (1987). The dissection of the females and the growth of promastigote forms in NNN medium were done according to methods recommended by KILLICK-KENDRICK & LEGER (pers. comm.). Identification of *Phlebotomus* was performed by the method of LEGER *et al.* (1983).

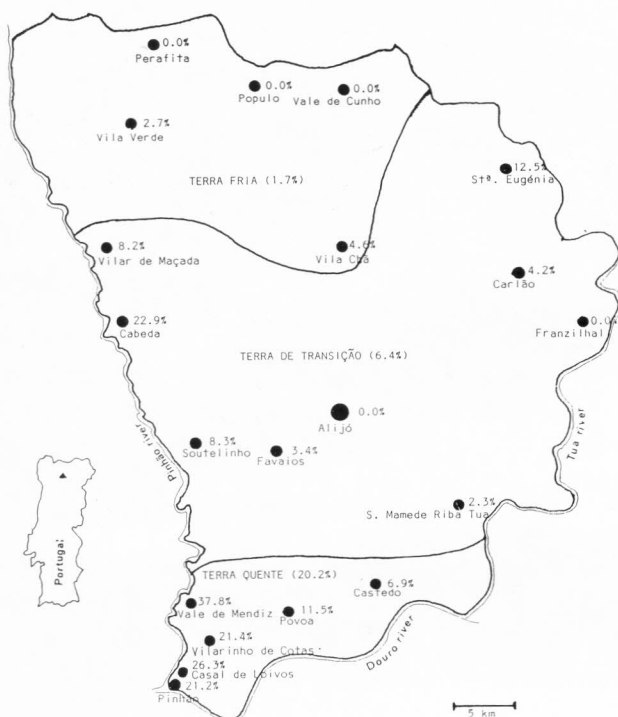


Fig. 1.— Map of municipality of Alijó with the local prevalences of canine leishmaniasis.

RESULTS

The number of officially registered dogs in the municipality was 1638 in 1986. A total of 712 were studied in the first survey and 437 in the second (Table 1).

The prevalence rate obtained in the first survey (1986-87) was 10 %, being 20,2 % in «Terra Quente», 1,7 % in «Terra Fria» and 6,4 % in «Terra de Transição». These differences between «Terra Quente» and «Terra Fria» ($p \leq 105$) and between «Terra de Transição» and «Terra Fria» ($p \leq 0,028$) are significant. In the second survey (1988-1989) the total and partial prevalences were similar (12,4 %) with, respectively, 18,2, 0, and 8,5 % in the three sub-zones considered.

In the serological survey performed in 1988, 253 human sera (64 from children) were analyzed. We observed 30 positive reactions (11,9 %), with no significant titres (1:8 to 1:32), 5 of these from children. The results showed that 77,7 % of people with positive reactions lived in «Terra Quente».

The vector study of 179 female phlebotomes (161 *P. ariasi* and 9 *P. perniciosus*) showed two specimens of the first species with promastigotes (1,2 %).

Three strains of *Leishmania* were obtained in NNN culture medium growth. Two of them, isolated from a dog and from a sandfly, were indentified as *L. infantum* zymodeme MON-1. The other strain, also isolated from

Places	1st SURVEY (1986/1987)				2nd SURVEY (1988/1989)			
	No. of sera	IFA $\geq 1/128$	%	Conf.lim. 5%	No. of sera	IFA $\geq 1/128$	%	Conf.lim. 5%
Pinhão	33	7	21,2	8,9-38,9	43	10	23,3	11,8-38,6
Casal Loivos	19	5	26,3	9,2-51,2	22	5	22,7	7,8-45,4
Vilar. Cotas	42	9	21,4	10,3-36,8	25	7	28,0	12,1-49,4
Vale de Mendiz	45	17	37,8	23,8-53,5	35	10	28,6	14,6-46,3
Povoa	26	3	11,5	2,5-30,2	34	0	0,0	0,0-10,3
Castedo	58	4	6,9	1,9-16,7	55	7	12,7	5,3-24,5
SUB-TOTAL	223	45	20,2	15,1-26,1	214	39	18,2	13,3-24,1
Soutelinho	12	1	8,3	0,2-38,5	4	0	0,0	0,0-60,2
Cabeda	35	8	22,9	10,4-40,1	33	8	24,2	11,1-42,2
Vilar. Maçada	49	4	8,2	2,3-19,6	30	6	20,0	7,7-38,6
Favaio	59	2	3,4	0,4-11,7	37	2	5,4	0,7-18,2
Alijó	47	0	0,0	0,0- 7,6	ND			
S.M. Riba Tua	43	1	2,3	0,1-12,3	23	0	0,0	0,0-14,8
Franzilhal	17	0	0,0	0,0-19,5	30	0	0,0	0,0-11,6
Carlão	71	3	4,2	0,9-11,9	48	0	0,0	0,0- 7,4
St. Eugénia	40	5	12,5	4,2-26,8	31	4	12,9	3,6-29,8
SUB-TOTAL	373	24	6,4	4,2- 9,4	236	20	8,5	5,2-12,8
Vila Verde	37	1	2,7	0,1-14,2	15	0	0,0	0,0-21,8
Perafita	36	0	0,0	0,0- 9,7	ND			
Pópulo	4	0	0,0	0,0-60,2	ND			
Vila Chã	22	1	4,6	0,1-22,8	12	0	0,0	0,0-26,5
Vale Cunho	17	0	0,0	0,0-19,5	ND			
SUB-TOTAL	116	2	1,7	0,2- 6,2	27	0	0,0	0,0-12,8
TOTAL	712	71	10,0	7,9-12,4	477	59	12,4	9,5-15,7

Table 1.— Local prevalence of canine leishmaniasis. ND=not done.

the vector, belonged to zymodeme MON-24 (PIRES *et al.*, 1991).

DISCUSSION

The municipality of Alijó is an endemic area of Mediterranean V.L. with some specific characteristics. Thus, for the first time in our studies in Portugal, it was possible to define bioclimatic areas with significantly different prevalence rates. The area with the highest prevalence (20,2 %) is «Terra Quente», characterized by sheltered slopes, schistous ground and with altitudes below 500 m. In areas with higher hills the prevalence is clearly inferior (6,4 %). The disease almost disappears (1,7 %) in the northern part of this zone, characterized by granitic ground with cold and prolonged winters. In the survey done a year and a half later, a similar difference between the three zones was seen.

However, nine cases of kala-azar were reported in the municipality of Alijó, from 1984 to 1989. Most of these were in the «Terra Quente» area, 8 in children. In our study, the number of positive IFA reactions with low titres in the human population at risk was high (11,9 %). This is possibly due to residual antibodies being maintained in the human population in endemic areas, a fact whose epidemiological significance was demonstrated by KELLINA (1981). This was not found in our previous studies in the Lisbon metropolitan endemic area, where the incidence of this disease is much lower, 0,2 cases/100.000 inhabitants/year (ABRANCHES *et al.*, 1987), such difference being significant ($p < 6 \times 10^{-9}$). These data seem to point to the existence of symptomless infections, as already was suggested by several authors in the Mediterranean sub-region, Asia and Brazil (PAMPIGLIONE *et al.*, 1974; SUKKAR *et al.*, 1984; BADARO *et al.*, 1986). Nevertheless, in a former study concerning dogs in Alcácer do Sal, the area of a previous focus that is now considered extinguished, we found several positive reactions (IFA) with low titres. In this study, however, a significant statistical correlation was also verified with canine Hepatozoonosis (ABRANCHES, 1984) and this observation suggests that careful interpretation of data is required.

The preliminary results on insect vectors gave good evidence that *P. ariasi* is involved.

We also consider that *Leishmania infantum* Nicolle, 1908, zymodeme MON-1 is probably the strain responsible for leishmaniasis in the region. This zymodeme was isolated from a dog and from a *P. ariasi* female in the area studied and is, up to now, the only variant identified in Portugal, independently of its human, canine or vulpine origin (ABRANCHES, CONCEIÇÃO-SILVA & SILVA-PEREIRA, 1986; ABRANCHES *et al.*, 1987). Zymodeme MON-24 was also isolated, recently, in the area, from a vector (*P. ariasi*), but it is unlikely to be also responsible for human and canine visceral leishmaniasis cases. This zymodeme was observed in cutaneous lesions in Algeria (BELLAZZOUG *et al.*, 1985), in Italy (GRAMICCIA,

GRADONI & POZIO, 1986) and in Tunisia (PRATLONG *et al.*, 1989). Only once was it related with visceral lesions in an AIDS patient (GRADONI, GRAMICCIA & BETTI, 1990).

In conclusion, the municipality of Alijó is a stable endemic focus of leishmaniasis. This focus is very active for human and canine populations and its characteristics are typically mediterranean. The probable vector is *P. ariasi*. Confirmation of the capacity of *P. perniciosus* to transmit the parasite requires further studies. The infection is caused by *L. infantum* zymodeme MON-1.

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