

LEISHMANIASIS IN THE PROVINCE OF SALAMANCA, SPAIN. PREVALENCE IN DOGS AND SEASONAL DYNAMICS OF VECTORS

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SUMMARY. Of 433 dogs examined in 4 natural zones of Salamanca Province, Spain, the prevalence of canine leishmaniasis (IFAT titres $\geq 1/80$) was 0-3 % in most mountain villages and from the Castillian plateau (> 600 m. a. s. l.) and 10-15 % in villages situated on hillsides (« flanc du coteau ») with elevations < 600 m. a. s. l. An exception was the high prevalence found in the area around the city of Salamanca owing to its height (800 m. a. s. l.) and flood-plain situation.

Of 5,105 sandflies captured with sticky traps, *P. perniciosus* and *P. ariasi* were well represented at the sites with high prevalences of canine leishmaniasis but not elsewhere. Their abundance in the periurban area of the city of Salamanca is attributed mainly to human transformation of the environment. Of the two species, only with *P. ariasi* can a linear relationship be observed between density and the prevalence of canine leishmaniasis, which seems to show that in Salamanca *P. ariasi* is its main vector. The probable lower observed vectorial capacity of *P. perniciosus* could be due to the fact that no progressive physiological ageing towards the end of the summer was observed.

Patients with leishmaniasis in the Province come from sites with the highest prevalence of canine leishmaniasis.

Key-words: Leishmaniasis. Prevalence. Vectors.

La leishmaniose dans la province de Salamanque, Espagne. Prévalence chez les chiens et dynamique saisonnière des vecteurs.

RÉSUMÉ. De 433 chiens analysés, dans les quatre zones naturelles de la province de Salamanca, les pourcentages de Leishmaniose canine (titres IFAT = 1/80) ont été de 0-3 % dans la plupart des villages des montagnes — à climat alpin — et dans les villages de la plaine de la province (700-900 m au-dessus du niveau de la mer) et de 10-15 % dans les villages situés à mi-versant et à des altitudes inférieures aux 600 m (au-dessus du niveau de la mer). Une exception parmi les hautes prévalences a été la zone périphérique de Salamanca capital, par son altitude (800 m) et sa situation dans une « vega ».

Des 5 105 phlébotomes capturés au moyen de trappes adhésives, *P. perniciosus* et *P. ariasi* ont été bien représentés seulement dans les lieux ayant de hautes prévalences de Leishmaniose canine. Leur abondance dans la zone périphérique de Salamanca capital est attribuée, fondamentalement, aux transformations anthropogéniques de l'environnement. Des deux espèces, uniquement chez *P. ariasi* peut être observé un rapport linéaire entre sa densité et la prévalence de la Leishmaniose, ce qu'indiquerait qu'à Salamanca *P. ariasi* est son vecteur principal. Probable-

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ment, le plus faible pouvoir vectoriel de *P. perniciosus* soit dû au fait qu'on n'ait pas observé un vieillissement progressif dans l'âge physiologique des femelles vers la fin de la période estivale.

Les Leishmanioses humaines provinciales d'origine connue proviennent des lieux qui ont donné de plus fortes prévalences de Leishmaniose canine dans cette enquête.

Mots-clés : Leishmaniose canine. Prévalence. Vecteurs.

Introduction

In the first half on this century Hernandez Martin (1930) and Iglesias (1934) reported leishmaniasis in dogs and humans in villages of Salamanca Province (Spain). Today the prevalence of infections has not varied much; occasional cases continue to be recorded in both hosts (*Bol. Epidemiol.*, 1986; Simon Vicente, pers. comm.).

In spite of the persistence of leishmaniasis in this province, until now no studies have been carried out on the epidemiology of the disease, except for those of the above authors. Their studies were limited to foci discovered at La Fregeneda (zone III, *Fig. 1*). There are other reports or citings of *Phlebotomus* species (see Cordero *et al.*, 1980).

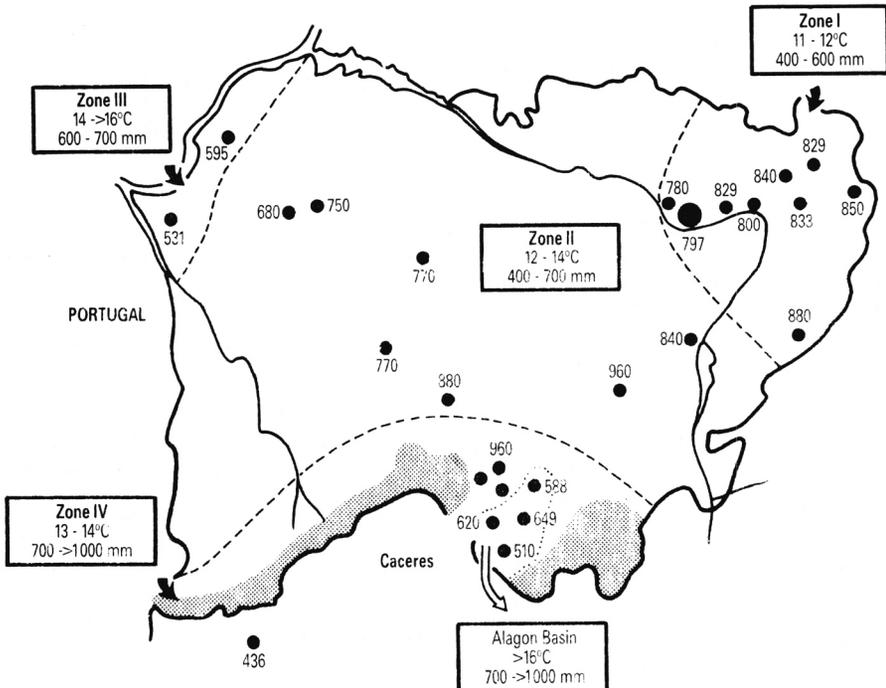


Fig. 1. — Province of Salamanca. Natural zones with their mean annual rainfall and temperature and situation of sampling sites. Next to each sampling sites figure the heights above sea level. The shaded zones represent the mountainous areas.

The aim of the present work was to determine the prevalence of canine leishmaniasis (CANL) and to study the seasonal distribution and physiological ages of the sandfly species present at each site.

Material and methods

For the study of the prevalence of canine leishmaniasis four ecologically homogeneous areas, depending on their climate and altitude characteristics, were chosen according to the methods of Lanotte *et al.* (1978) (*Fig. 1*). The study also included Villasbuenas de Gata (Caceres Province) which is ecologically similar to the Alagon basin (*Fig. 1*) since one case of infantile leishmaniasis was recorded there during the study period.

The villages sampled were chosen in such a way that all the different geographic entities were represented. The dogs not from the sampling areas and those who had not lived over a summer season were excluded.

The same criteria were applied to the animals sampled from the area around the City of Salamanca (periurban area: PA) which was analyzed separately from its own zone (Zone I).

Sera were studied by the indirect fluorescence antibody test (IFAT) according to the standard protocol (Bray, 1985) using promastigotes of *L. donovani infantum* strain MCNES 1841 CNMB as antigen and a fluorescent conjugate (ICN immunobiological) at a dilution of 1/40.

To isolate the parasites, where possible the dogs showing a positive IFAT reaction were sacrificed. Samples of spleen from these animals were examined microscopically. NNN culture media and hamsters were also inoculated; these were examined according to the usual procedures.

Specimens of *Phlebotomus* and their relative frequency were determined between May-October, 1986. Capture was made with sticky traps (20 × 20 cm) of known efficacy (Rioux *et al.*, 1982). These were placed at fixed sites (stables, chicken-coops, caves, fissures in rocks, walls, etc.) in villages where canine blood samples had been taken and in neighbouring villages. The traps were replaced every 7-15 days.

Live specimens of *Phlebotomus* were also captured by aspiration in a cave located in PA. The physiological age of the females was studied according to the method of Polovodova (*in* Detinova, 1963). We also studied whether the females had spermatozooids in their spermathecae and observed the contents of their accessory glands. In fed and/or parous specimens the mid-gut was examined for the presence of *Leishmania* promastigotes.

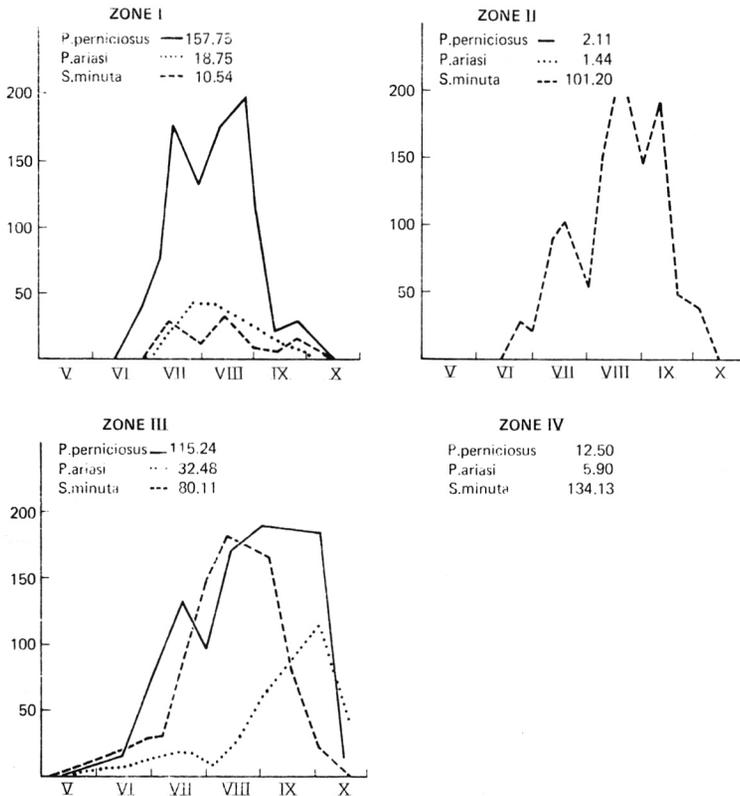


FIG. 2. — Seasonal dynamics and mean density of *P. perniciosus*, *P. ariasi* and *P. minuta* in zones I (periurban area of city of Salamanca), II and III. In zone IV the densities of one sampling are given. The abscissas reflect densities per m² of sticky trap, and ordinates the months of capture. The number following each species represents the mean density over the season.

Results

CANINE LEISHMANIASIS: PREVALENCE

The total number of dogs analyzed was 433, most of which (422) came from sites shown in *figure 1*. The other 11 were provided by a veterinary clinic in Salamanca City. The prevalence of leishmaniasis in the latter 11 dogs was considered separate from the others, as shown in *Table I* which also shows IFAT-positive animals from each zone (at titres equal to or greater than 1/80).

In randomly-selected dogs, the highest percentages of positivity were found in Villasbuenas de Gata (15 %) and, in Salamanca Province, in zone III (13.2 %) followed by zone IV (4.4 %), zone I (3.3 %) and zone II (2.9 %). Zone I can be subdivided into PA and the rest of the zone with prevalence rates of 9.09 % and 2.02 %, respectively. Likewise, in zone IV the dogs taken from the villages in the

TABLE I. — Canine leishmaniasis.
IFAT-positive dogs at titres equal to or greater than 1/80.

Origin	Sera analyzed	1/80 to 1/160	1/320 to 1/640	$\geq 1/1,280$	Total positive cases and prevalence
Zone I	121	2	2	—	4 (3.3 %)
Zone II	105	2	1	—	3 (2.9 %)
Zone III	68	6	2	1	9 (13.2 %)
Zone IV	90	2	2	—	4 (4.4 %)
Veterinary clinic	11	1	3	1	5 (45.45 %)
Villasbuernas de Gata	38	2	2	2	6 (15 %)

lower part of the Alagon basin, at heights below 600 m. a. s. l., showed a prevalence of 10.5 % whereas no leishmaniasis was observed in dogs taken from the villages in zone IV situated above this altitude.

The percentages of positives as a function of the age of the animals were as follows: 6-10 years, 8 %; 1-5 years, 5.7 %, and ≤ 1 year, 0 %.

No amastigotes were observed in spleen smears of any of the 4 dogs sacrificed with IFAT titres between 1/160 and 1/1,280, although 3 were positive by the assays in NNN media and hamsters. The unsuccessful culture was from an animal showing patent signs of disease at the time of sacrifice and several myases in the subocular region. This dog had been treated with unknown antibiotics and other drugs.

PHLEBOTOMUS AND ITS POPULATION DYNAMICS

Sandflies captured on 710 sticky traps were 1,953 males and 239 females of *Phlebotomus perniciosus*; 539 males and 25 females of *P. ariasi*; 774 males and 1,572 females of *Sergentomya minuta*, and 3 males of *P. sergenti*.

The first three species were present in all the four study zones. *P. sergenti* was only found at La Fregeneda (zone III) (450 m. a. s. l.), at the site most distant from the Duero and Agueda Rivers of all the 15 sites set up within this municipality along a stretch rising from 140 to 531 m. a. s. l. over 10 km.

Figure 2 shows the seasonal abundance of each species and their mean densities (total number of specimens/total surface area of sticky traps in m²) for each of the zones. The data relating to zone I in fact correspond to PA since outside this zone either no captures were made or, like in zone II, the species captured were mainly *S. minuta*.

In zone IV, the data shown refer to a single sampling made between 8th and 15th of August. In this sampling period, the density of *P. perniciosus* was considerably lower than that observed for the same dates in PA and zone III; by contrast, the density of *P. ariasi* showed no differences.

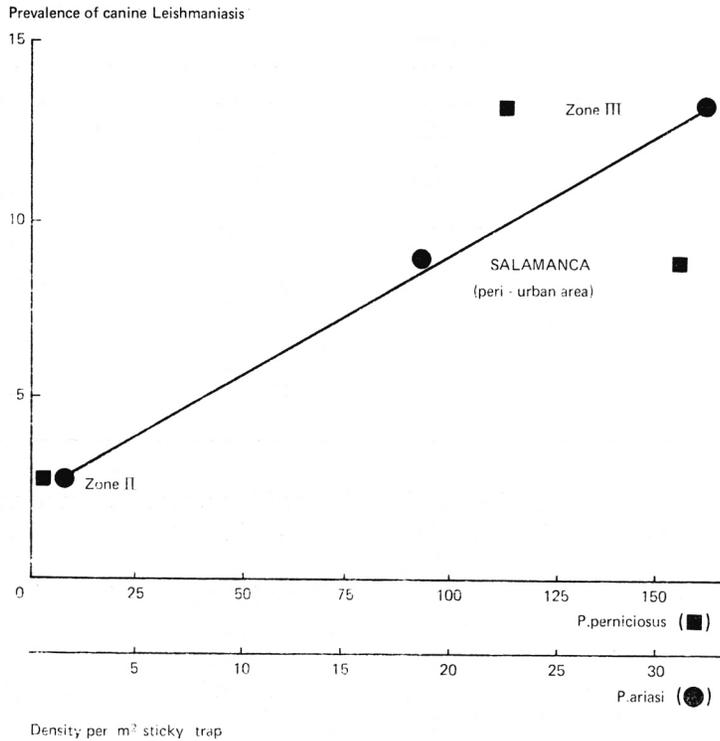


FIG. 3. — Relationship between the mean density of *P. ariasi* per m² of sticky trap and prevalence of canine leishmaniasis. Zone IV has been omitted owing to the lack of representative data of its *Phlebotomus* fauna and in zone I only the enclave comprised by the city of Salamanca is considered (see text).

All the species showed greatest densities at altitudes below 500 m. a. s. l. (Table II); in Salamanca Province, these altitudes are only found in small enclaves of the broad Duero basin (zone III) or on the descending limbs of the Castillian plateau in the Alagon basin (Fig. 1).

Finally, it was interesting that of the 128 females of *P. perniciosus* captured

TABLE II. — Number of specimens captured per m² of sticky trap according to elevation a. s. l. of sampling sites.

Height in meters a. s. l.	<i>P. perniciosus</i>	<i>P. ariasi</i>	<i>S. minuta</i>
700-800	120	16	105
400-500	992	313	966
100-300	661	235	361

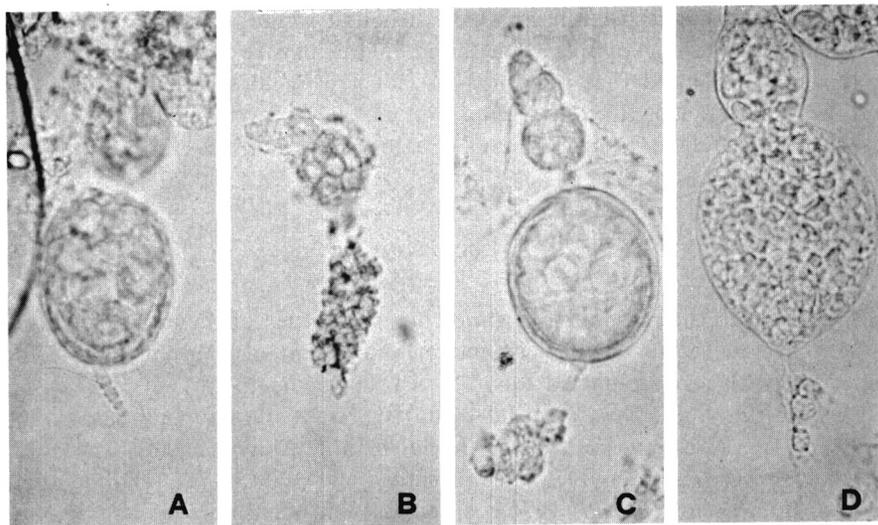


FIG. 4. — *P. perniciosus*. Ovarioles. A, nulliparous female ; B, C and D, parous females, showing pedicel at different degrees of retraction.

in zone III on sticky traps, only 6 had blood/eggs in their abdomens whereas none of the 109 captured (alive or on sticky traps) in zone I showed this characteristic. In contrast, 25 females of *P. ariasi* found in zone I, 3 were fed/gravid.

PHYSIOLOGICAL AGE

A total of 109 females were captured live in a cave in PA, 99 of which were *P. perniciosus*. Eighty-one were clearly nulliparous (Fig. 4 a) and 18 parous. Of the latter, some females had oviposited 1 or 2 times (Fig. 4 c, d). In the others, it was difficult to ascertain parity since the pedicel of the ovarioles was completely distended (Fig. 4, b). Accordingly, table III only shows the percentage of nulliparous and parous females in the different captures and does not specify the number of ovipositions. Of the 18 parous females, 3 had a few spermatozooids in their spermathecae and 2 did not show granules in the accessory glands. None of the

TABLE III. — *P. perniciosus*. Percentage of nulliparous and/or parous females in a population from the periurban area of the city of Salamanca.

Physiological state	Date				
	23-07	1-08	15-08	1-09	15-09
Nulliparous (%)	80	50	80	60	80
Parous (%)	20	50	20	40	20

nulliparous females had been inseminated although 15 had granules in their accessory glands. No promastigotes were found in the parous females.

It should be noted that none of these 99 live females of *P. perniciosus* had blood in their stomachs, like those captured on the sticky traps in the same zone, none showed a follicular development greater than stage I-II of Christopher's scale.

Discussion

According to the reasoning of Abranches *et al.* (1983), it is not possible to evaluate the prevalence of canine leishmaniosis in Salamanca Province by a direct comparison with those reported for other European foci.

Despite this limitation, we believe that CANL is unimportant in Salamanca except for the 3 enclaves (PA, La Fregeneda and Alagón basin); the fact that titres of 1/80 were considered significant instead of 1/160 (a more reliable indicator of epizooty) (Lanotte *et al.*, 1974), supports such a notion.

In the 3 foci studied, the prevalence of CANL were clearly higher than those of the surrounding areas and are quite similar to the foci in the Mediterranean and Portugal (see Bettini and Gradoni, 1986). However, caution should be used in this kind of comparison (Abranches *et al.*, 1983). Of the 3 foci, two (PA and La Fregeneda) must be limited in size since CANL was not found outside the municipal limits. In contrast, the third probably comprises many of the villages of the low mountain area situated between Salamanca and Cáceres. Such an idea is based on the fact that of the 4 villages sampled in an area below 600 m. s. a. l. (3 in Salamanca and 1 in Cáceres) all showed a high prevalence. It seems reasonable to assume that the same would hold for other ecologically similar villages in the same zone. However a more detailed analysis of CANL in this zone was outside the scope of this work.

The focus at La Fregeneda and the one in the Alagón basin can be explained by their low altitude and by their topographic characteristics (flanc de coteau), factors that strongly favour the transmission of leishmaniasis (Rioux *et al.*, 1980). Undoubtedly, the most atypical focus is the one in PA owing to its altitude and flood plain situation. The area is reduced to the outskirts of the city in which rural and urban activities overlap, a situation that probably contributes to the transmission of several diseases by arthropods (Gilot and Pautou, 1982). In Salamanca Province, as in France (Rioux *et al.*, 1967), a close correlation exists between the prevalence of CANL and the density of *Phlebotomus*. It was observed that *P. ariasi* and *P. perniciosus* are well represented in all the provincial foci, even the one in PA which reaches similar densities to those observed in areas in which human leishmaniasis is relatively common. The distribution/abundance of these two species in PA is probably more related to human transformation of the environment than to natural factors. At least this seems to be the case since outside PA, throughout zones I and II, only the ubiquitous species *S. minuta* is abundant.

In the three foci of CANL reported in this study, both species—*P. perniciosus* and *P. ariasi*—were taken together, unlike other foci on the Mediterranean coast

where there is usually a predominance of one species over the other, such as *P. perniciosus* in Marseilles (Gilot *et al.*, 1983) and in Italy (Maroli *et al.*, 1987) or *P. ariasi* in the Cévennes (Rioux *et al.*, 1967) or where both species are well represented in a given region, they are usually separated altitudinally (e. g. in Almeria, Spain; Martínez Ortega, 1986). As in Salamanca both species have been reported occurring together in Portugal (Ferreirares, 1956, *in* Rioux *et al.*, 1967).

Since both species have been cited as vectors of CANL (Rioux *et al.*, 1979; Pires, 1984), either species, or both (as in Portugal, Pires, 1984), may serve as vectors in Salamanca but as may be seen in *figure 3* the prevalence of CANL is linear and corresponds with the density of *P. ariasi* but not with *P. perniciosus*. This suggests that in Salamanca, as in the Cévennes (see Dye, 1986), the former species could be the main vector of the disease.

In any case, more information is needed to confirm the above hypothesis. This is specially relevant, mainly taking into account that *P. perniciosus* was considerably more abundant than *P. ariasi*. Biological differences between both species might contribute to *P. ariasi* being of greater epizootiological importance.

According to our findings no progressive ageing of the populations of *P. perniciosus* towards the end of the summer was observed (see *Table III*), unlike *P. ariasi* in France (Guilvard *et al.*, 1980). However, the ageing observed for our population from the cave in PA might be influenced by the capture method employed (Wilkes and Rioux, 1980) and by the reduced sample size which may reflect why no fed/gravid females were observed in the samples. The latter aspect might also be influenced by the fact that the females were always captured at the same site (in the cave in PA) where for unknown reasons fed/gravid females might not rest or else only enter to oviposit after which they land on the walls of the cave, where they are easily captured. It is perhaps why many of the females studied had completely distended pedicels. Apart from this apparent anomaly, the rest of the characters studied in the dissections coincide with what has been observed in other species (Magnarelli *et al.*, 1984).

As in Portugal (Abranches *et al.*, 1983) and Italy (Pozio *et al.*, 1981), it may be seen that in Salamanca Province, clinical cases of the disease in humans are infrequent. However, it seems clear that the zones of greatest prevalence are those offering the greatest risk for the population since it was there that six known cases of visceral leishmaniasis and the six cutaneous cases reported from 1973 to the present (pers. comm. Dept. of Dermatology, University Clinical Hospital, Salamanca) were discovered. The 24 cutaneous cases of leishmaniasis located in the Province of Salamanca between 1968 and 1973 (Gill Collado, 1977) could support such a correlation but unfortunately we are unaware of their provincial origin.

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