

A review of human and canine leishmaniosis in Catalonia, and associated vector distribution.

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Received: 21.01.06

Accepted: 03.07.06

...
perquè em sé perdurable
en les coses que em volten,
i sé que algú, en el temps,
servarà el meu record.

(Paraules al vent)
Miquel Martí i Pol

Al Profesor Ignacio Navarrete

Summary: This paper is a review of the distributions of human and canine leishmaniosis and their vectors in Catalonia. Official data (BEC) place the mean annual incidence rate of human leishmaniosis at 0.5 cases/100000 inhabitants, reaching higher than 1 case/100000 inhabitants in the southern farming regions of Priorat, Garrigues, Baix Ebre and Ribera d'Ebre, where the disease has been recognised as being endemic since the pioneering studies of the first half of the 20th century. Comparison between officially declared cases and those registered in the Laboratory of Parasitology at the UB, indicates a high rate of under-declaration and the true incidence may be three times higher than the declared one. The scarce, randomly selected seroepidemiological surveys available suggest a canine infection prevalence of between 2 and 10%, with a geographical distribution parallel to that of human leishmaniosis. Entomological surveys produce variable results, as quantitative analysis is hampered by a large number of factors. Nevertheless, they reveal the presence of *P. perniciosus* and *P. ariasi* throughout Catalonia, with a relative abundance related to bioclimatic conditions.

Key Words: Leishmaniosis, epidemiology, Catalonia.

Resumen: Se efectúa una revisión de la distribución de las leishmaniosis humana y canina y de sus vectores en Cataluña. Según los datos oficiales (BEC) la tasa de incidencia anual media de la leishmaniosis humana se sitúa en 0,5 casos/100000 habitantes, con valores superiores a un caso/100000 en las comarcas agrícolas del sur (Priorat, Garrigues, Baix Ebre, Ribera d'Ebre) que fueron ya consideradas las de mayor endemia en los trabajos pioneros de inicios del siglo XX. La comparación entre los casos declarados y los registrados en el Laboratorio de Parasitología de la UB, permite observar una importante subdeclaración y estimar la incidencia real en tres veces la declarada. Las escasas encuestas seroepidemiológicas en muestras no seleccionadas con que se cuenta sitúan la prevalencia de la infección canina entre un 2 y un 10%, con una distribución geográfica paralela a la de la leishmaniosis humana. Las encuestas entomológicas aportan resultados muy variables, condicionados por un gran número de factores que dificultan su análisis cuantitativo. No obstante permiten observar la presencia simultánea de *P. perniciosus* y *P. ariasi* en la mayor parte del territorio catalán, con abundancias relativas de ambas especies determinadas por el bioclima de la zona.

Palabras Clave: Leishmaniosis, epidemiología, Cataluña.

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1. Introducción

Human leishmaniosis has been known in Catalonia since 1912, when the first case of kala-azar in the Iberian Peninsula was diagnosed in a boy living near Tortosa (Pittaluga, 1912). During the first half of the 20th century leishmaniosis was recognized as a major health problem in Spain, particular-

ly along the Mediterranean coast, the Centre (Madrid and neighbouring areas) and Extremadura, as can be deduced from the numerous studies published during this period, which laid the foundations for epidemiological and clinical knowledge of this disease in Spain (reviewed in Botet and Portús, 1993).

The incidence of leishmaniosis seems to have reduced since the end of the forties, presumably as a result of decreasing vector density, caused by the introduction of insecticides in agriculture and the domestic environment, as well as during the malaria control programme (Gil Collado, 1977).

In 1982, leishmaniosis was included on a list of diseases for which the declaration of cases is obligatory which led to official records of the number of cases. Despite this obligatory declaration, there is little knowledge of the real incidence, relative proportions of the two main presentations (visceral and cutaneous), or geographical distribution of this disease in humans.

The data on canine leishmaniosis are even more fragmentary, mainly due to the fact that the application of reliable diagnostic techniques, mainly serological, has not been introduced until recent years. Despite the opinion of veterinarian practitioners that the incidence of the disease has increased during recent decades, there also exists the assumption that this observation is only the result of the application of serological diagnostic techniques that have facilitated diagnosis.

The *Grup de Parasitologia Clínica* in the Laboratory of Parasitology at the *Universitat de Barcelona* has been working on leishmaniosis for more than 20 years. The diagnosis of human and canine leishmaniosis has been one of the principal research objectives of this group during these years, together with eco-epidemiological studies. As a consequence, the group has provided diagnostic support to hospitals in Catalonia, and acted as a reference centre for strain identification. In the present study, we summarize the data obtained on the distribution of human leishmaniosis (HL) cases, including the data published in the *Butlletí Epidemiològic de Catalunya*, and those obtained from samples analysed in our laboratory, together

with the results of different sero-epidemiological studies on canine leishmaniosis and data on sandfly distribution, in order to achieve an overall approximation to the real epidemiological situation of the disease in Catalonia.

2. Human leishmaniosis

2.1. Historical records

The first documented case of leishmaniosis in Spain was diagnosed in a boy from the Baix Ebre region, in southern Catalonia (Pittaluga, 1912). Nevertheless, before kala-azar was recognized, the clinical condition was known by practitioners in the area as “*mal de melsa*” (“spleen disease”) (Sala Ginabreda, 1947). Also the number of deaths in this region that was attributed to leukaemia, Hodking disease or splenic anaemia, diseases which could be clinically confused with leishmaniosis, was abnormally high (Pittaluga, 1912).

The recompilation of HL cases carried out by Pittaluga (1925), located most cases of leishmaniosis in Catalonia in the province of Tarragona, with 53 cases diagnosed between 1912 and 1925, and only 7 in the province of Barcelona during the same period (Fig. 1).



Fig. 1. Human leishmaniosis in Spain (Pittaluga, 1925).

The first cases in the province of Girona (north-east Catalonia) were recorded in 1930 (Pascual, 1930). A large historical recompilation of kala-azar cases in Catalonia was published in 1947

by Sala Ginabreda who recorded 52 kala-azar cases from the city of Barcelona in their personal archives from 1930 to 1947 and 99 cases previously published by others in Catalonia, with reference to their geographical origin. Another extensive study on leishmaniosis performed in the province of Tarragona (Pereperez, 1947) recorded 148 cases of human leishmaniosis- 43 of visceral leishmaniosis (VL) and 105 of cutaneous leishmaniosis (CL) - during a five years period (1942-1947). All these results indicated a high prevalence of leishmaniosis in southern regions of Catalonia (Baix Ebre, Ribera d'Ebre, Priorat, Baix Camp) and, to a lesser extent,

Barcelona and the Baix Llobregat area. An extensive historical review of leishmaniosis in Spain was published by Botet and Portús (1993).

2.2. Official records from 1982-2004

708 cases of leishmaniosis have been declared in Catalonia from 1982 to the end of 2003 and published in the *Butlletí Epidemiològic de Catalunya* (BEC), implying an average rate of 32 cases per year (a maximum of 62 in 1985 and minimum of 14 in 1989). In 2004, after the publication of the BEC, 32 cases were recorded (*Departament de Salut, Generalitat de Catalunya*, personal communica-

Table 1.- Human leishmaniosis cases in Catalonia. Butlletí Epidemiològic de Catalunya (BEC) (1983-2004).

Comarca	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	Total
1 Alt Camp				1											3								4
2 Alt Empordà					3	2	3	0	1	2	1		2		1			1				1	17
3 Alt Penedès				1				2															3
4 Alta Ribagorça																							0
5 Alt Urgell																							0
6 Anoia	1				1										1				1		1		5
7 Bages					1				2	1	3	1					3						11
8 Baix Camp	1		1	1					1	1	1	1		2				1				1	11
9 Baix Ebre	1	1	5	2	2	4	1			2				1	2	1			1				24
10 Baix Empordà		1	4	1		1	1								1		1		1				11
11 Baix Llobregat	4	3		2	2	4		1	2	9	5	4			1	4	3	2	3	6	6	5	66
12 Baix Penedès	1		2		1											2	1						7
13 Barcelonès	20	11	21	31	17	30	16	8	24	10	12	15	12	32	11	7	10	7	13	11	18	9	345
14 Berguedà																	1				1		2
15 Cerdanya																							0
16 Conca de Barberà				2																			2
17 Garraf					1	1	1		2	2	1				1		1	1		1			12
18 Garrigues	1	2	5				1	1														1	11
19 Garrotxa							1																3
20 Gironès		1	3	5	2	1				1		1			1			1	1		2		18
21 Maresme	1		1	1		1	1		3	2	2					1					1		14
22 Montsià																			2				2
23 Noguera		1			2						2												5
24 Osona	1	1			1				1			1						1	1	1			8
25 Pallars Jussà	1	1													1								3
26 Pallars Sobirà					1																		1
27 Pla de l'Estany																							0
28 Pla d'Urgell										1													1
29 Priorat		1	1	5		3		1							1				1			1	14
30 Ribera d'Ebre	2		1	2							1					1					1		8
31 Ripollès																1							1
32 Segarra																1			1				2
33 Segrià	3	1		1		1			1		2												9
34 Selva			4								1	1		1				1					8
35 Solsonès																							0
36 Tarragonès		1		2		1				3	5	3			1	1	2		1	1	1	1	23
37 Terra Alta																							0
38 Urgell					1															1			2
39 Val d'Aran																							0
40 Vallès Occidental	2		1	2	2		2	3	3	1	1		3				2		5	5	4	4	40
41 Vallès Oriental					1				2	1	0	5			2		2			1		1	15
Catalonia	39	25	51	62	35	50	26	14	42	36	37	32	17	36	27	19	26	15	33	27	34	25	708

tion). As these cases have yet to be published, and we lack information on their distribution, they are not included in this review. From 1982 to 1996, only numerical information was recorded -the number of cases in each region -“comarca”. From 1997, cases were recorded individually, providing information about the age and gender of the patient, but not on the pathology, visceral or cutaneous. Most cases were declared by hospitals situated in Barcelona and Baix Llobregat, as well as other regions along the Mediterranean coast (Table 1).

The disease is distributed throughout Catalonia, but predominantly along the Mediterranean coast. As one might expect, the highest number of cases occur in places with higher human density such as Barcelona and the surrounding industrial towns and, to a lesser extent, the cities of Tarragona and Girona (Fig. 2).

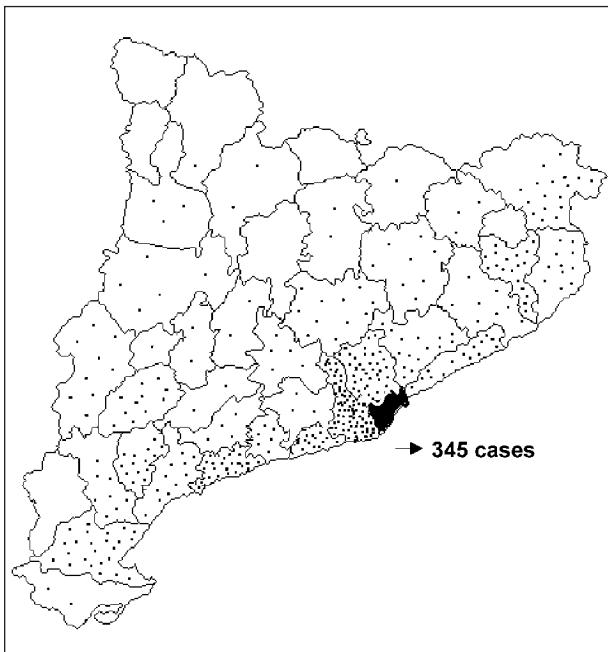


Fig. 2. Human leishmaniosis in Catalonia during the period 1982-2003. Geographical distribution of cases. Each dot represents one case. Data from *Butlletí Epidemiològic de Catalunya* (1983-2004).

Nevertheless, the inclusion of population density data reveals that the annual rates of declared incidence of this disease remain highest in the southern regions, particularly Priorat, Garrigues, Ribera d’Ebre and Baix Ebre (Fig. 3).

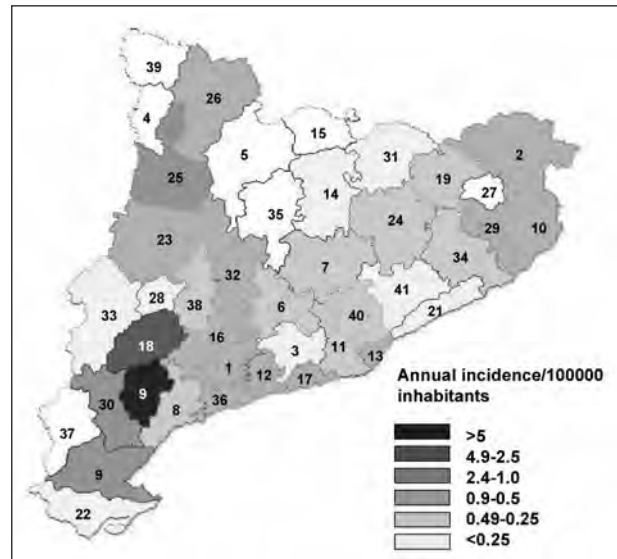


Fig. 3. Human leishmaniosis in Catalonia during the period 1982-2003. Data from *Butlletí Epidemiològic de Catalunya* (1983-2004). (Annual incidence rate was calculated from the number of cases declared during the period 1982-2003 and the census of 1996).

From 1997 onwards, children represent only 15.5% of patients, while 69.3% of cases were in males (Table 2).

Table 2. Cases of leishmaniosis registered in the Laboratory of Parasitology at the UB and the *Butlletí Epidemiològic de Catalunya* (BEC) during years 1998-2004.

	Lab. Parasitol.	BEC
Number of cases (1998-2004)	152	192
Children < 10 years old*	17 (11)	26 (15)
Males*	114 (75)	124 (69)
Cutaneous leishmaniosis	8**	
HIV co-infection	120	
Other immunosuppressive pathologies (transplant, esplenectomy, lymphoma)	7	

* Number (%)

** Three imported cases, one from Morocco and two from South America

2.3. Records from our archives

During the period 1998-2004 we registered 152 new cases of autochthonous leishmaniosis from the samples sent to our laboratory for diagnosis or strains isolated in hospitals and sent to our cryobank for iden-

tification. The hospitals that, by-and-large, sent samples to us regularly, represent 15% of the hospital beds in Catalonia (data from *Servei d'Informació i Estudis. Direcció General de Recursos Sanitaris. Departament de Salut, Generalitat de Catalunya*) and included 27% of AIDS cases registered (CEESCAT, 2005). Consequently, if we assume that we were able to register all the cases of leishmaniosis found in those hospitals and we extrapolate the percentage of AIDS patients to the percentage of leishmaniosis patients, it could be estimated that the real number of cases of leishmaniosis in Catalonia during the period 1998-2004 was near 560, what leads to an annual incidence of 1.3 cases in 100000 inhabitants. This assumption is only an approximation as some hospitals only send us cases with diagnostic problems and/or the remittance of samples is restricted to some hospital services. Actually, in some years (1999, 2001 and 2003), the number of cases we registered was numerically higher than that registered in the BEC. This under-declaration of leishmaniosis cases observed in Catalonia is comparable to that observed in the whole of Spain, where the number of officially declared cases is approximately 120 per year (0.3 cases by 100000 inhabitants), whilst the true figure is estimated to be about 300 cases (200 of VL and 100 of CL) (Alvar, 2001).

2.4. Clinical presentation

The lack of official statistics on the clinical presentation of leishmaniosis hampers statements regarding the real morbidity rate of CL, which is assumed to go undeclared or even undiagnosed in many cases. Actually, the five cases of autochthonous CL recorded by us during a seven-year period (Table 2) did not represent the real number of CL cases treated in the hospitals that send samples to our laboratory for analysis. From most of them, we received no samples from the dermatology department throughout this time. Nevertheless, CL which had high morbidity rates during the first half of the past century (Perepérez, 1947), seems to be now much less frequent than before.

2.5. Age and gender distribution

The main reports on leishmaniosis in Catalonia (Perepérez, 1947, Sala Ginabreda, 1947),

as in other areas in Spain (Pittaluga, 1925, Nájera, 1933), during the first half of the past century characterized leishmaniosis, mainly in its visceral form, as a paediatric disease, affecting both males and females to a similar extent. Although the results published may be biased as most statistics were obtained from paediatric hospitals and surgeries, there is no doubt that leishmaniosis was a disease mainly affecting children, in both its forms, VL and CL (Botet and Portús, 1993).

The present epidemiological situation associates leishmaniosis with immunosuppressive conditions (Desjeux *et al.*, 2000), particularly co-infection with HIV, but also with other immunosuppressive pathologies, which has altered the spectrum of the population at risk. Only 11% (7-17) of our cases and 15% (11-22) of those published in the BEC correspond to children less than 10 years old.

There is no agreement on the influence of gender in the distribution of human leishmaniosis (both VL and CL) around the world that may be attributed to biases in sampling methods in some studies, as a consequence of different socio-cultural situations. In Colombia, for example, the higher infection rate reported in man than in women has been attributed to gender-linked differences in access to health care (Vélez *et al.*, 2001). Early works on leishmaniosis in Catalonia judged that there were no differences in male and female distribution of kala-azar (reviewed in Gil Collado, 1977, Botet and Portús, 1993), as well as of oriental sore (Perepérez, 1947). The uneven distribution of leishmaniosis cases declared in Catalonia during the period 1998-2004, with 69 (62-75)% cases found in males may be attributed to the gender bias in AIDS cases (75% in males) declared during this time period (CEESCAT, 2005). It is worth emphasizing that 79% of the leishmaniosis cases we recorded corresponded to HIV co-infected patients.

3. Canine leishmaniosis

Unfortunately, there are no official statistics on canine leishmaniosis. The only existing records on canine leishmaniosis in Catalonia we have are those reviewed by Gil Collado (1977), who brought together data collected by Vilà and Torredemè (1913) in

Tarragona (which recorded a 6% prevalence rate in dogs), Cartañá (1933) in Barcelona (1.7% prevalence rate) and Covalada *et al.* (1951), also in Barcelona (2% prevalence rate). Sero-epidemiological surveys in random dog populations carried out by our team during recent years (Table 3) have revealed similar results to those published in the first half of the 20th century, despite their use of serological diagnostic techniques that are more sensitive than parasitological ones.

Table 3.- Seroprevalence of anti-*Leishmania* antibodies in dogs in different regions in Catalonia in random sampling studies.

	Number of dogs studied	Seroprevalence rate in dogs (%)	HL incidence
Baix Camp*	959	1.9	0.36
Barcelonès*	146	4.8	0.74
Priorat**	1196	10.2	6.91

* Portús *et al.*, 1987;

** Fisa *et al.*, 1999.

HL (human leishmaniosis) incidence refers to the mean number of annual cases per 100000 inhabitants (BEC, 1983-2004).

The comparison of the seroprevalence rate of anti-*Leishmania* antibodies in dogs and the incidence of human leishmaniosis reveals a notable geographical correspondence between the two parameters.

Other sero-epidemiological surveys, in allegedly random dog populations, have been performed on dogs from kennels run by animal protection societies. In this case, sampling was carried out by kennel personnel and blood was sent to our or other reference laboratories for analysis. Seroprevalence rates in these cases were higher than in the sero-epidemiological studies on the more random dog populations we sampled (Table 4).

In our opinion, this lack of agreement between the two sets of results may be caused by the biased sampling method used in the kennels. The possibility that these differences in sero-prevalence rates were a consequence of employing different analytical tests can be discounted, as serodiagnosis performed in different laboratories (our laboratory and that of the *Diputació Provincial de Barcelona*) and using different diagnostic kits produced very similar results for each kennel. We report this circumstance as an exam-

Table 4. Seroprevalence of anti-*Leishmania* antibodies in dogs in different regions in Catalonia based on studies performed in kennels handled by animal protection societies.

	Number of dogs studied	Seroprevalence rate (%)
Anoia*	95	11.6
Bages	112	10.7
Barcelonès	67	8.9
Barcelonès*	754	9.2
Berguedà	54	7.4
Berguedà*	51	11.8
Garraf*	28	21.4
Osona	29	6.8

* Grau *et al.*, 2000

ple of the introduction of bias in sampling during epidemiological studies that, in this case, leads to significant errors in the quantification of the true rate of prevalence of canine infection.

4. Sandfly vectors

The distribution of sandflies in Spain has been the object of various reviews (Gil Collado, 1977; Gil Collado *et al.*, 1989, Gállego *et al.*, 1992) that have outlined the distribution map of different species around the country. In Catalonia, six sandfly species are known: *Phlebotomus ariasi*, *P. mascittii*, *P. papatasi*, *P. perniciosus*, *P. sergenti* and *Sergentomyia minuta*. However, only *P. ariasi* and *P. perniciosus* are believed to be responsible for leishmaniosis transmission (Rioux *et al.*, 1986, Guilvard *et al.*, 1996). The density and abundance of both species is very variable and the data obtained in different surveys varies according to capture site, weather, the kind of traps used and other circumstances, which has hindered the comparison of results from different studies. Notable differences in the density and abundance of sandfly species have been attributed to different trapping methods (Gallego *et al.*, 1992). In general, light traps catch more species and specimens than sticky traps or aspirators (Gemetchu *et al.*, 1977). However, even use of the same methodology, in studies performed by the same research team results may be very variable, explaining the inaccuracy of attempts to establish a direct relationship between leishmaniosis prevalence

and quantitative entomological data, particularly when researchers have attempted to establish relationships based on scant data. For example, the densities of *P. perniciosus* obtained sampling identical locations in the town of Torroja del Priorat, using sticky paper traps, laid for two weeks in the middle of June, were 11 specimens/m² in 1991 and 106 in 1992. The results obtained from sandfly captures performed in transects carried out during the month of July during the years 1987-1989 in different regions of Catalonia with different bioclimatic compositions are summarised in table 5.

Table 5. *Phlebotomus perniciosus* and *P. ariasi* densities in different regions in Catalonia.

	Altitude m a.s.l.	It	Bioclimate	<i>P. perniciosus</i> density	<i>P. ariasi</i> density
Barcelonès	60-230	351-410	Low meso- mediterranean-upper thermomediterranean	2.2	0.2
Bages	100-800	160-260	Low supra- mediterranean-upper mesomediterranean	4.5	1.3
Baix Llobregat	40-500	301-350	Low meso- mediterranean	4.9	0.6
Lleida*	300-1700	111-260	Upper supra- temperate-low supra- mediterranean-upper meso- mediterranean	11.3	7.7
Priorat	100-800	261-350	Upper-low mesomediterranean	11.9	2.1

* Gállego *et al.*, 1990. Transect ran through the regions of Noguera, Pallars Jussà and Pallars Sobirà. Density results in the table are calculated taking into account the surfaces of both sides of the sticky paper (0,08 m²), rather than just one (0,04 m²), as was done in the original publication of these results.

It (thermicity index), calculated as ten times the sum of the yearly average temperature, the average minimum temperature of the coldest month in the year and the average maximum temperature of the coldest month in the year (Rivas Martínez, 1987).

In all cases, captures were carried out using sticky paper traps that were laid in place for 4-7 days.

The sandfly density results obtained in entomological prospections performed using the transect methodology and castor oil sticky paper traps, can only provide a rough estimation of sandfly distribution over the region and quantitative values are only comparable between those obtained within the same transect. The mean sandfly density in transects carried out in Catalonia was very variable, but always higher for *P. perniciosus* than for *P. ariasi*, the latter attaining higher densities in colder regions. Studies performed in the South of France (Rioux *et al.*, 1977) estimated the mean density of *P. ariasi* to be 5 specimens/m², at which level the probability of *Leishmania* transmission is very slight. A higher density of 20 specimens/m² was found in areas with higher transmission rates. These data are consistent with results obtained in Catalonia, where the highest sandfly densities were found in rural regions with high human leishmaniasis incidence.

Density also varies with altitude (Table 6), as was observed in transects carried out in the province of Lleida and the Priorat region. *P. perniciosus* density decreased with altitude in both transects while that of *P. ariasi* was only high at high altitudes in the province of Lleida (the Pyrenees) with a supratemperate subhumid bioclimate (Rivas Martínez, 1987).

Table 6.- Sandfly density at different altitudes in the province of Lleida and the Priorat region.

Altitude (m a.s.l.)	Density (specimens/m ²) from sticky traps			
	<i>P. ariasi</i>		<i>P. perniciosus</i>	
	Lleida*	Priorat	Lleida*	Priorat
<200	-	5.9	-	21.3
200-400	1.5	1.5	31.7	14.7
401-600	4.6	2.6	24.3	8.4
601-800	3.8	0.7	12.1	0.5
801-1000	1.8	-	1.6	-
>1000	19.7	-	1.9	-

- No captures were made at this altitude in this area;

* Gállego *et al.*, 1990 (density results were published having taken into account the surface of only one side of the sticky traps).

5. Concluding remarks

The most significant event in the history of the epidemiology of leishmaniosis over the last 50 years, in Catalonia as in other areas of the west Mediterranean region, has been the emergence of AIDS. As Gil Collado (1977) has indicated, the number of infant cases has decreased from the middle of the past century, probably due to the reduction in the density of vector population but also due to an increase in socio-economic conditions that has led to better infant nutrition. Most old studies on leishmaniosis in Spain associated the disease with areas with low economic and hygienic conditions (Botet and Portús, 1997). The incidence of infantile and cutaneous leishmaniosis has probably stabilized since the 70s, with the lowest number of cases occurring now. If we take into account the number of cases of infantile leishmaniosis recorded in BEC and the number of cases recorded in our laboratory, we estimate that the real number of infantile visceral leishmaniosis cases in Catalonia may be around 5 per year.

The areas of highest endemicity, in other words those with the highest incidence rates, are still those in southern Catalonia. Nevertheless, human concentration in big cities and industrial areas has led to a concentration of human leishmaniosis cases around these areas. The bias introduced to the population at risk, predominantly adult males co-infected with HIV, has led to a similar bias in the population affected by leishmaniosis.

Although we lack complete distribution maps for canine leishmaniosis, the few seroepidemiological surveys performed on a representative randomly selected canine population suggest that distributions of seroprevalence rates correlate with human leishmaniosis incidence.

P. perniciosus and *P. ariasi* are present throughout Catalonia, with geographical differences in relative abundance that can be attributed to bioclimatic variations.

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