Research Signpost
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

Recent Advances in Pharmaceutical Sciences VI, 2016: 189-211 ISBN: 978-81-308-0566-5
Editors: Diego Muñoz-Torrero, Àngela Domínguez and Àngels Manresa

11. Current status of Leishmaniosis in the Balearic Islands

M. Magdalena Alcover[1], Cristina Ballart[1,2], Teresa Serra[3], Montserrat Portús[1] and Montserrat Gállego[1,2]

[1]Department of Biology, Health and Environment. Section of Parasitology, Faculty of Pharmacy Universitat de Barcelona, 08028 Barcelona, Spain; [2]ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic-Universitat de Barcelona, Barcelona, Spain
[3]Institut Universitari d'Investigació en Ciències de la Salut (IUNICS) Universitat de les Illes Balears, Spain

Abstract. Data on leishmaniosis and its vectors (sand flies) in the Balearic Islands are scarce and restricted mainly to Majorca. According to the official data, the overall rate of human leishmaniosis (HL) is 0.7-3.5 cases per year/100,000 inhabitants (for the period 2001-2015), and the reported prevalence of canine leishmaniosis (CanL) varies between 0 and 45%, depending on the island and the dog population tested. In the present study, we investigated the sand fly fauna and current status of CanL in the Balearic Islands. Four sand fly species were captured: *Phlebotomus perniciosus*, a known vector in the Mediterranean area, *P. sergenti*, *P. papatasi* and *Sergentomyia minuta*. *P. perniciosus* was found throughout the island of Majorca, from sea level to the mountains, being detected in 70% of the capture sites and with a density of 6.7 specimens/m^2. The global density of *P. perniciosus* in Minorca was of 3.4 specimens/m^2, which constitutes a significant decrease

Correspondence/Reprint request: Dr. Montserrat Gállego Culleré, Department of Biology, Health and Environment, Section of Parasitology, Faculty of Pharmacy, Universitat de Barcelona, Av. Joan XXIII, 27-31 08028 Barcelona, Spain. E-mail: mgallego@ub.edu

compared to the results of a previous study performed 20 years ago. The influence of environmental factors on the presence or density of *P. perniciosus* differed according to the physiography of the area studied. A standard questionnaire sent to the local veterinarians in the Balearic Islands revealed that 73.8% of veterinarians had confirmed CanL cases in the previous 12 months and thought the disease was increasing in Minorca. The global seroprevalence of CanL in Minorca was 24%, being 31% among animals who had never left the island, which shows the existence of an autochthonous focus of CanL unrelated with an increasing vector density.

Introduction

Leishmaniosis is one of the world's most neglected diseases [1]. A total of 98 countries and 3 territories on 5 continents have reported endemic leishmaniosis transmission [2]. Caused by infection with a protozoan parasite of the genus *Leishmania*, this parasitic disease affects man and other mammals, and maintains its cycle by transmission through the bite of sand flies [1]. The presence of sand flies is critical for the development of the disease and one of the factors influencing its heterogeneous distribution [3].

In the Mediterranean region, leishmaniosis is caused by *L. infantum* and is considered a zoonotic disease with a secondary epidemiological cycle [4]; dogs act as the main host and parasite reservoir, and man as a secondary host [5 – 7]. The prevalence of leishmaniosis has recently increased in established endemic regions [8 – 10] and cases have emerged in areas previously considered non-endemic [11 – 14]. This increase may be due to various factors, including environmental changes and global warming [15 – 17], as indicated in predictive risk map models of leishmaniosis vectors and reservoir hosts [17 – 18].

In the Balearic Islands, previous studies have shown a heterogeneous distribution of both human and canine leishmaniosis [19 – 28]. Human leishmaniosis (HL) was designated as a notifiable disease ("Enfermedad de Declaración Obligatoria") in Spain from 1982 to 1996 [29]. Thereafter it was considered a regionally endemic disease, and each autonomous community with devolved powers over health could decide whether or not to keep it in its notifiable disease list. The Ministry of Health of the Govern de les Illes Balears decreed HL to be a "Malaltia de Declaració Obligatoria" (MDO) requiring individual numerical declaration [30]. In 2015, the disease was once again given mandatory notification status throughout the Spanish territory [31].

Leishmaniosis is included in the list of communicable diseases of importance for public health and the international trade of animals and animal products in the World Organisation for Animal Health (OIE). In Spain, canine leishmaniosis (CanL) was declared a notifiable disease by the Real Decreto 526/2014 [32], which regulates its notification.

Two main clinical forms of HL are found in Spain, as in other Mediterranean regions: cutaneous and visceral. Cases of mucosal involvement (mucocutaneous leishmaniosis) are rare [33]. Dogs usually show clinical signs of skin and visceral involvement concomitantly [5 – 6]. Entomological studies have demonstrated the presence of *Phlebotomus perniciosus*, a proven vector of leishmaniosis in Spain, in all the Balearic Islands [26 – 28, 34 – 36], but without considering the factors that could influence its distribution in the area.

The aim of the current work was to gain new epidemiological data on leishmaniosis in the Balearic Islands by studying the distribution of human and canine leishmaniosis and their vectors in the islands of Minorca and Majorca, where previous data indicate different degrees of endemicity [19 – 28].

1. Area of study

The study was carried out in the Balearic Islands (Spain), an archipelago in the western Mediterranean comprising four main islands (Majorca, Minorca, Ibiza and Formentera). Geologically, the Balearic Islands are a continuation of the Betica Mountains, with the exception of Minorca, which is a continuation of the Pyrenees [37]. The Balearic Islands have a Mediterranean climate, with a mean annual temperature of about 16-17 ºC, except in the high mountains of Majorca, the Serra de Tramuntana, where it drops to 13 ºC. Annual rainfall oscillates from a maximum in autumn (66.9mm) to a minimum in summer (8.6 mm), with considerable differences between the mountainous north and the arid south in Majorca, where altitudes range from sea level to 1,445 m.a.s.l. [38].

In 2011, Majorca had a population of 862,425 inhabitants, Ibiza 129,562, Minorca 92,434 and Formentera 9,147 [39]. The canine census in 2012, according to the data provided by the *Col.legi de Veterinaris de les Illes Balears*, was of 250,596 dogs, with 166,858 on the island of Majorca, 13,956 on Minorca, and the rest on Ibiza and Formentera.

2. Entomological survey in the Balearic Islands

Entomological studies of sand flies on the Balearic Islands are scarce. The first was conducted by Pittaluga and de Buen (1918) [40], who detected *Phlebotomus perniciosus*, *P. papatasi* and *Sergentomyia minuta* in Majorca. Later, Gil Collado (1977) [41] reported the occurrence of these three species in the Balearic Islands, but without distinguishing between the islands. In an update of sand fly species in Spain, *P. ariasi* was added to those present in Majorca [34]. More recent maps elaborated by different authors also show this distribution [35, 42 – 47] (Fig .1).

Our entomological study was focused on the islands of Majorca and Minorca. Prior entomological data were available for both islands [26, 47], but they were incomplete, especially in the case of Majorca, and failed to take into account the possible influence of environmental and climatic variables on the presence and distribution of leishmaniosis vectors.

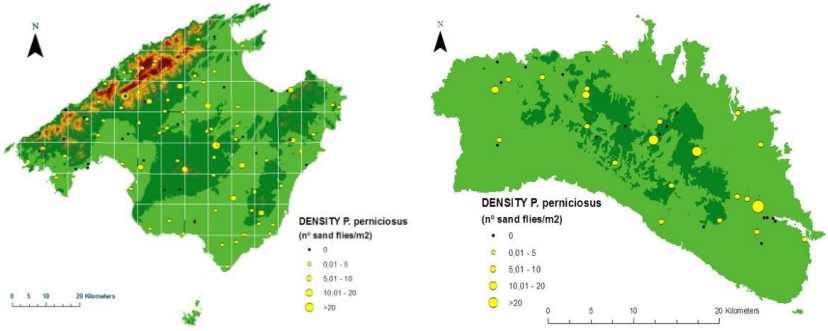
In July 2008, sand fly captures were carried out in Majorca with sticky castor oil traps (20x20 cm) according to a standardized methodology (Fig. 2) [15, 48, 49]. The sampling sites consisted of holes used to drain embankments or containment walls, and were distributed among 77 grids (5x5 km square) covering the entire island (Fig. 3a). Sand fly captures were carried out in Minorca between 2 and 4 July 2009, placing sticky traps in the

Figure 1. Distribution of sand flies in Spain (modified from Gállego et al., 1992) [35]. (⊙: Dubious appointment of *P. ariasi*, ⬬: *P. perniciosus*, ✱: *P. sergenti*, △: *P. papatasi*, ☒: *S. minuta*).

same 39 stations as in a previous study in 1988 [26], but now using the standardized methodology (Fig. 3b). The characteristics of the stations and sampling methodology (site location, type of trap, number of traps placed and collected, and meteorological data for the days of capture) and ecological and environmental factors were recorded in a PDA, using the Pendragon Forms v.5.0 software, associated with a GPS to record the coordinates of the sampling site. Sand flies were removed from the sticky traps with a brush and fixed in 96% ethanol to remove the castor oil, and then placed in 70% ethanol until identification. Males of all the species and *Sergentomyia* females were observed and identified under a stereomicroscope. Females of the genus *Phlebotomus* were mounted on glass slides in Hoyer medium and identified in an optical microscope using the keys of Gállego et al. [35].

(a) **(b)** **(c)**

Figure 2. Sticky traps used in the capture of sand flies (a); example of a sampling site (b), adhesive paper with captured sand flies (c).



(a) **(b)**

Figure 3. Sampling sites showing the density of *P. perniciosus*, in Majorca (2008) (a) and in Minorca (2009) (b).

2.1. Qualitative and quantitative analysis of the sand flies

On the island of Majorca, 88.2% of the traps were recovered, representing a surface of 135.68 m^2. A total of 14,412 specimens were captured, with 4 species identified (Table 1): *P. perniciosus*, *P. sergenti*, *P. papatasi* and *S. minuta*. *P. perniciosus* was captured throughout the island in 77 of the 111 stations prospected (69.36%), at 6 to 772 m.a.s.l. *P. sergenti* and *P. papatasi* were captured in only 14 and 1 of the stations, respectively, and always in a low number. The entomological surveys carried out in Majorca did not confirm the presence of *P. ariasi*, a proven vector of *L. infantum*, as this species was not found, despite sampling the whole island from 0 to 772 m.a.s.l and using a large number of traps.

On the island of Minorca, 92.38% of the traps were recovered, representing a surface of 54.32 m^2. The qualitative composition of sand flies in Minorca in 2009 showed no change had occurred over the 20 years since the previous study of 1988) [28], the same 3 species being identified on both occasions (Table 1): *P. perniciosus*, *P. sergenti* and *S. minuta*. On the contrary, changes in the density of the species were observed, with a significant decrease regarding *P. perniciosus*.

No environmental changes (newly urbanized areas, road construction, changes in land use) between 1988 and 2009 were observed. Meteorological factors were also analysed (mean daily temperature, average rainfall, wind speed, and humidity, all in different periods, and winter temperature) (Table 2), since climate change is another risk factor for the emergence or re-emergence of leishmaniosis foci [17, 52]. The meteorological data provided by the Spanish Meteorological Agency (AEMet) for the years of the two studies were compared using a linear regression model. This analysis revealed a decrease in average temperature of 0.9 °C in the month before the capture period in 2009 compared to 1988, thus showing a negative

Table 1. The sand fly fauna of Majorca (2008) and Minorca (1988, 2009)

		Sex ratio (M:F)	Number	Density (n/m^2)
Majorca (2008) [50] 135.56 m^2	*P. perniciosus*	4:1	921	6.72
	P. sergenti	24:1	25	0.18
	P. papatasi	3:0	3	0.02
	S. minuta	1.4:1	14,412	99.3
Minorca (1988) [28] 65.52 m^2	*P. perniciosus*	16.5:1	421	6.4
	P. sergenti	7.8:1	167	2.5
	S. minuta	1:1.4	937	14.3
Minorca (2009) [51] 54.32 m^2	*P. perniciosus*	8.5:1	179	3.4
	P. sergenti	9.5:1	21	0.4
	S. minuta	1.1:1	1,090	20.5

correlation with *P. perniciosus* density. These results disagree with Kuhn's model [53], which predicts that an increase in temperature would reduce the density of this species. Another difference observed between the two years was the average wind speed during capture, which was significantly lower (by 2.4 km/h) in 2009 than in 1988. Several authors have mentioned the negative influence of this meteorological variable on both the activity [54] and density [55] of sand flies. Thus, while less wind would not have led to an increase in the density of *P. perniciosus* in Minorca, it could have favoured sand flies leaving their resting places and biting animals.

Table 2. Results of the analysis of the meteorological factors associated with the decrease of *P. perniciosus* density in Minorca (Modified from Alcover et al., 2013) [51].

	Bivariate analysis		Linear model analysis	
Variable 2009	**IRR (CI)**	**p-Value**	**IRR (CI)**	**P-Value**
Mean daily temperature (ºC) (period2)	0.95	0.016	-0.91	0.002
Wind speed (Km/h) (period1)	0.91	0.058	-2.4	3.434×10^{-5}

Period 1: sampling Day 1 (traps set) to Day 4 (traps recovered)

Period 2: the month before sampling Day 1

Abbreviations: IRR, incidence risk ratio; p-Value, Pr(>|z|); CI, confidence interval; ºC, degree Celsius; Km/h, kilometres per hour

2.2. Factors influencing the presence or density of *Phlebotomus perniciosus*

To determine the predictors of the presence of *P. perniciosus* on the island of Majorca, a logistic regression analysis was performed using the presence/absence of the species as dependent variables. For independent variables, a panel of 57 ecological, epidemiological, environmental and meteorological characteristics was used. The independent variables of a preliminary bivariate analysis with a p-value ≤ 0.2 were used for a multivariate analysis. In the final multivariate model, only the independent variables with $p \leq 0.05$ were considered. SPSS v.20 was used (Table 2). The effect of meteorological and capture station variables on *P. perniciosus* density in Minorca was evaluated by generalized linear models based on negative binomial distribution [56] using the glm.nb function of the MASS statistical package, available on free software R [57]. After an initial bivariate analysis, the resulting independent variables with a value of

$p \leq 0.2$ were used in a multivariate analysis. In the multivariate model, independent variables with $p \leq 0.05$ were considered (Table 3).

Among climatic variables, temperature is known to affect sand fly activity. In the Balearic Islands, the summer temperatures trigger the end of sand fly diapause, after which they leave their resting sites at dusk and night-time hours, when the females bite vertebrate hosts [44, 58]. According to the results [26, 49], the capture of *P. perniciosus* on the islands of Majorca and Minorca was not influenced by meteorological variables, in contrast with other studies [15, 48, 55, 59]. In Majorca, *P. perniciosus* was captured at average temperatures ranging between 19.6 ºC and 27.4 ºC, regardless of the altitude of the trap location, while in Minorca the temperature ranged from 25.04 ºC to 25.08 ºC. This species was observed in a wide range of temperatures at which sand flies are reported to be active (above 15.6 ºC) [58]. The short period of capture, only during the month of July in both Minorca (just four days) and Majorca (full month), together with the geographical homogeneity of the capture sites, prevented the finding of significant differences in density (Minorca) or presence/absence (Majorca) of vectors between different capture points. In Majorca, it was difficult to find places suitable for traps in mountainous areas above 700 m.a.s.l.

Locations away from inhabited areas were positively correlated with the presence or density of *P. perniciosus* in both islands, as in other studies [15, 48, 59]. Rural and remote locations away from urban centres provide more suitable conditions for the vector to develop its terrestrial cycle [11, 60 – 61]. In Majorca, the species increased in areas with adjacent garrigue shrub vegetation, and in Minorca vector density was higher in natural environments far from human populations than in cultivated areas, where the use of pesticides could decrease sand fly density. Altitude is a variable that is positively or negatively related to the presence of sand flies, depending on the species considered [59]. In our case, we analysed its influence only on the populations of *P. perniciosus* in the island of Majorca, where there is more altitudinal variation. A greater abundance of *P. perniciosus* was detected in remote capture sites over 50 m.a.s.l. Stations at 0-50 m.a.s.l. were located in humid and breezy coastal areas and sand flies are very sensitive to windy conditions [11, 58, 60].

The presence of a specific type of farm or animal species can also favour the capture of *P. perniciosus.* Thus, a nearby sheep farm favoured the presence of this species in Majorca, as did cats in Minorca. Sheep farms provide sand flies with feeding opportunities, since they contain a large number of animals that spend the night outside, the time when sand flies are active.

Table 3. Bivariate and multivariate analysis of the environmental factors associated with *P. perniciosus* presence/absence in Majorca (2008) and its density in Minorca (2009). (Modified from Alcover et al., 2013 and 2014) [50 – 51].

MAJORCA	Bivariate analysis			Multivariate analysis		
	IRR	**p-Value**	**C.I. (95%)**	**IRR**	**p-Value**	**C.I. (95%)**
Altitude (m.a.s.l.)						
0-50	ref.			ref.		
51-150	3.133	0.020	1.195-8.214	8.653	0.015	1.514-49.441
>150	1.625	0.402	0.522-5.055	0.805	0.816	0.131-4.964
Settlement						
Within settlement	ref.			ref.		
Edge of / between settlement	5.339	0.001	1.950-14.617	8.080	0.008	1.737-37.596
Site category						
Embankment drainage holes	ref.					
Wall drainage holes (not embankment)	2.111	0.031	0.204-0.843			
Other holes in walls (not embankment)	0.308	0.148	0.062-1.522			
Natural rock crevices	0.235	0.313	0.014-3.917			
Farm building (holes)	0.264	0.166	0.040-1.735			
Sewer/drainage openings	-	0.999	-			
Wall construction						
Stone without mortar	ref.					
Stone / mortar	0.338	0.079	0.101-1.133			
Brick/mortar	0.263	0.009	0.097-0.714			
Other	1.974	0.414	0.386-10.089			
General environment						
Rural village	ref.					
Rural agriculture and forestry	2.977	0.032	1.095-8.091			
Coastal village	0.548	0.435	0.122-2.475			
Other settlement (non rural or non coastal village)	0.366	0.158	0.090-1.478			
Adjacent flora						
Aleppo pine and evergreen oaks	ref.			ref.		
Garrigue shrubs	14.529	0.001	2.949-71.587	38.051	0.001	4.900-295.469
None	0.935	0.904	0.313-2.795	1.308	0.707	0.323-5.307
Land cover (Corine)						
Urban area	ref.					
Agricultural area	5.525	<0.001	2.113-14.448			
Forest area	1.594	0.461	0.462-5.497			
Humid area	-	1.000	-			
Arable						
Cereals	ref.					
Root crop	0.167	0.231	0.009-3.118			
Other (not cereal or root crop)	0.333	0.268	0.048-2.328			
None	0.269	0.016	0.093-0.781			
Sheep farm animals near						
No	ref.			ref.		
Yes	2.720	0.019	1.177-6.289	19.989	0.001	3.557-112.322
Pigeon farm near						
No	ref.					
Yes	0.155	0.031	0.028-0.842			
Orientation						
Other (all orientations except south-east- and west-facing)	ref.			ref.		
South-east-facing	2.990	0.171	0.623-14.350	34.975	0.018	1.817-673.425
West-facing	0.716	0.500	0.271-1.892	0.457	0.263	0.116-1.798
Drain hole construction						
Plastic pipe	ref.			ref.		
Other (unlined, cement pipe)	2.250	0.061	0.964-5.249	3.451	0.05	1.002-11-880

Table 3. Continued

MINORCA	Bivariate analysis			Multivariate analysis		
	IRR	**p-Value**	**C.I. (95%)**	**IRR**	**p-Value**	**C.I. (95%)**
Site category						
Urban	ref.					
Rural (includes edge of and between locations)	8.7	0.015	1.371-52.959			
Orientation						
North-facing	ref.			ref.		
West-facing	0.45	0.405	0.064 – 3.168	0.08	0.017	0.009-0.771
South-facing	0.43	0.244	0.100- 1.869	1.02	0.955	0.431-2.427
East-facing	2.11×10^{-9}	0.996	0-Inf	0	1	0-Inf
Other (not applicable)						
Water course nearby						
No	ref.					
Yes	0.07	0.0252	0.004-0.751			
Wall construction						
Stone without mortar/Dry stone wall	ref.					
Stone/mortar	5.61	0.006	1.793-22.746	4.13	0.002	1.549-11.024
Stone/mortar (plaster/white)	0.18	0.207	0.007-2.773	0	1	0-Inf
Adjacent flora (within 100m)						
Natural vegetation	ref.			ref.		
Arable	0.24	0.0319	0.061-0.927	0.29	0.035	0.081-1.024
Cats						
Not seen	ref.			ref.		
Seen	9.59	0.011	2.242-91.704	16.39	0.001	2.616-102.669
Capture *S. minuta*						
No	ref.					
Yes	1.02	0.0001	1.006-1.039			

In Minorca, this variable was not taken into account, since livestock on the island is mainly cattle. However, an increase in the density of *P. perniciosus* was related with the presence of cats, which has epidemiological importance, since cats can act as reservoirs of *L. infantum* [62 – 63].

The type of wall was only an influencing factor in Minorca, where a positive correlation with sand fly density was observed when sticky traps were placed on stone mortar walls. Dry stone walls, very common in the Balearic Islands, have numerous interconnected holes, which facilitate air currents and consequently disturb sheltering sand flies and hamper sampling [11, 58, 60].

3. Canine leishmaniosis (CanL) in the Balearic Islands

In 2015 leishmaniosis was included among the diseases of zoonotic origin in the list of diseases of official notification by the Government of the Balearic Islands [64].

The first study on CanL in the Balearic Islands dates from 1989 [19], when a prevalence of 14% was reported in Majorca. Subsequently, several studies have been published on the different islands [19 – 21, 25 – 28, 65 – 66], from which it was inferred that CanL was endemic in Majorca, Ibiza and Formentera, with a seroprevalence ranging between 6 and 45%

[19, 21, 25 – 28, 43]. The prevalence rose to 67% when molecular diagnostic techniques were used [66]. With respect to Minorca, Seguí [26 – 28] could not demonstrate the presence of an autochthonous focus of CanL in the island. The few positive cases detected (8/813, 0.98%) were regarded as imported, since they involved dogs introduced to the island from well-established foci. The characterization of the strains isolated from dogs in Majorca by Multilocus Isoenzyme Analysis (MLEE), led to the identification exclusively of zymodeme MON-1 of *L. infantum* [67].

In order to know the trends of CanL in the area, a standard questionnaire [12, 18] was sent to local veterinarians in 2009. Of the 111 questionnaires sent out to veterinary clinics, 42 were returned completed (a reply rate of 38%). Over 80% of surveyed veterinary clinics attended more than 20 dogs a week, and of these, 73.8% had confirmed CanL in more than 10 dogs in the previous 12 months. While 50% of the veterinarians thought that the incidence of CanL had not changed over time (Fig. 4), 26.2% perceived an increasing trend, mainly those from Minorca (3/6). Most veterinarians considered the new diagnosed cases as autochthonous (88.1%), including all those from Minorca (6/6).

In order to investigate the possibility of an autochthonous focus of CanL in Minorca, a cross-sectional study on CanL was performed in the island in April-June 2010 with the help of the practitioners of three veterinary clinics located in different areas of the island (Mercadal, Ciutadella and Sant Lluís) (Fig. 5). Veterinarians were asked to randomly select animals born on the island and without any history of travelling abroad, regardless of the presence of clinical signs.

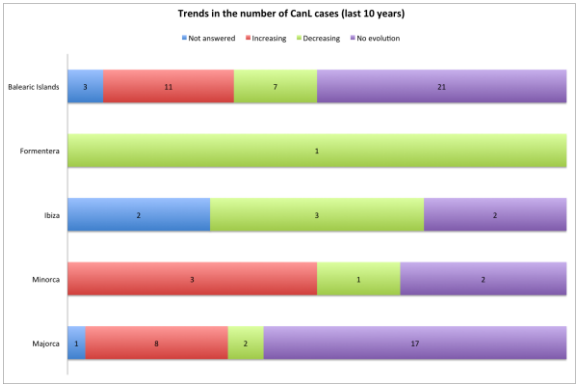


Figure 4. Veterinary questionnaire on CanL trends in the Balearic Islands in the last 10 years (number of replies).

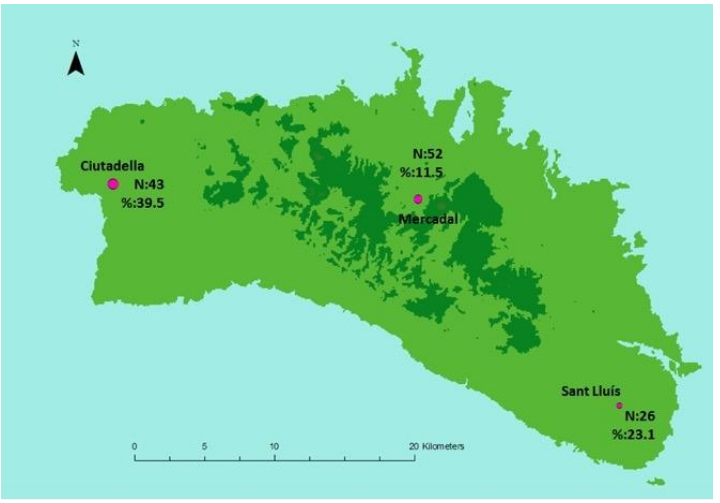


Figure 5. Prospective study of CanL in Minorca. N: number of dogs, %: seroprevalence.

In total, 121 dogs were selected with permission from the owners. The prospective study of CanL was carried out by serological analysis. Blood samples were obtained by cephalic vein puncture and sera were frozen and conserved at -40ºC until use. Serology was performed, with minor modifications, according to the protocols of "in-house" ELISA and Western Blot techniques [68], IFI [69] and a commercial ICF technique (Speed®Leish K, BVTGroup, Virbac) (Fig. 6). Dogs that tested clearly positive with at least two immunological methods were considered seropositive and probably infected [12]. Dogs that tested positive with at least two techniques but were borderline were considered doubtful.

The global seroprevalence was of 23.96%, and 31% among animals of known local origin and with no history of movements to endemic areas. The high seroprevalence in different localities in Minorca (Fig. 5) was similar to that found in known endemic areas in Spain [8, 70 – 72], indicating that dogs with suspected *Leishmania* infection had been included in the sample. Our results point to the emergence of an autochthonous focus of CanL in Minorca, which could be related with the continuous introduction of infected animals from endemic areas to the island while the vector is present, as reported by Seguí [27 – 28], rather than an increase in *P. perniciosus* density.

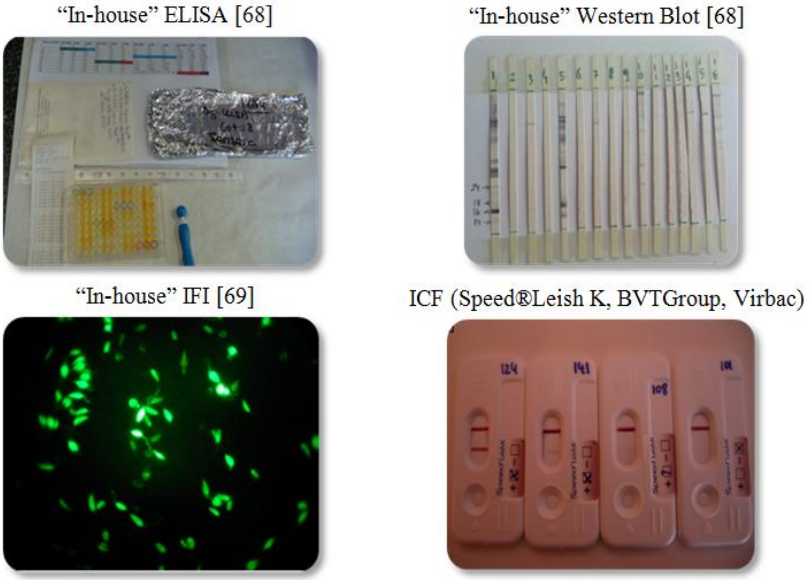


Figure 6. Leishmaniosis diagnostic techniques used to study CanL seroprevalence

4. Human leishmaniosis (HL)

The source of HL data in the Balearic Islands is a literature review of published cases, together with officially reported cases. The first description of a HL case in the area was in 1925, namely visceral leishmaniosis on the island of Majorca [73]. In 1926, a child was diagnosed with visceral leishmaniosis (VL) by Drs. Cervera and Darder, also in Majorca [26], and in 1935, a report on VL and cutaneous leishmaniosis (CL) in Spain by Dr. Najera Angulo [74] includes three cases in Majorca between 1925 and 1934. Finally, Gil Collado [41] reviewed the data from dermatological services in the islands from 1961 to 1973 and found only one case of cutaneous leishmaniosis during this period.

The first officially reported case of HL we could find dates from 5-11 September, 1982 [75], the year that HL became a notifiable disease in Spain. From 1982 to 1996, the Health Department of the Spanish Government collected data on HL in the Balearic Islands but without differentiating between islands (Table 4). In 1981 began a decentralization

Table 4. Distribution of human leishmaniosis cases reported in the Balearic Islands. CL: Cutaneous Leishmaniosis. VL: Visceral Leishmaniosis.

	Year	CL	VL	Majorca	Minorca	Ibiza-Formentera	Cases not assigned to Islands	Total	Report (year)
Notifiable disease, nationwide Boletines Epidemiológicos Semanales del Ministerio de Sanidad y Consumo.	**1982**	--	--	--	--	--	1	1	BES 1741 (1986)
	1983	--	--	--	--	--	1	1	BES 1741 (1986)
	1984	--	--	--	--	--	2	2	BES 1741 (1986)
	1985	--	--	--	--	--	3	3	BES 1741 (1986)
	1986	--	--	--	--	--	4	4	BES 1880 (1991)
	1987	--	--	--	--	--	0	0	BES 1880 (1991)
	1988	--	--	--	--	--	9	9	BES 1880 (1991)
	1989	--	--	--	--	--	8	8	BES 1880 (1991)
	1990	--	--	--	--	--	11	11	BES 1880 (1991)
	1991	--	--	--	--	--	8	8	SNE
	1992	--	--	--	--	--	22	22	SNE
	1993	--	--	--	--	--	5	5	SNE
	1994	--	--	--	--	--	0	0	SNE
	1995	--	--	--	--	--	0	0	SNE
	1996	--	--	--	--	--	0	0	SNE
Notifiable disease, regional Xarxa de Vigilancia Epidemiológica de les Illes Balears	**1997**	--	--	--	--	--	12	12	Full Set. 39/2005*
	1998	--	--	--	--	--	14	14	Full Set. 39/2005* +
	1999	4	8	--	--	--	12	12	Full Set. 39/2005*
	2000	0	2	--	--	--	2	2	Full Set. 39/2005*
	2001	0	6	5	0	1	0	6	Full Set. 8/2002*
	2002	2	2	3	0	1	0	4	Full Set. 20/2003*
	2003	2	8	10	0	0	0	10	Full Set. 37/2004*
	2004	14	7	17	0	3	1	21	Full Set. 22/2005*
	2005	25	11	36	0	0	0	36	Full Set. 49/2006*
	2006	25	10	33	0	1	1	35	Informe 2006*
	2007	9	13	19	0	2	0	22	Informe 2007*
	2008	6	5	11	0	0	0	11	Informe 2008*
	2009	6	8	11	0	3	0	14	Informe 2009*
	2010	7	1	7	0	1	0	8	Informe 2010*
	2011	17	3	17	1	2	0	20	Informe 2011*
	2012	14	6	20	0	2	0	20	Informe 2012*
	2013	12	4	14	0	2	0	16	Informe 2013*
	2014	17	7	20	0	4	0	24	Informe 2014*
	2015	--	--	26	0	2	0	28	Full Set. 14/2016*
	TOTAL National (1982 – 1996)						74	74	
	TOTAL Regional (1997 – 2015)	160	101	247	1	24	43	315	

* Accessible at http://www.caib.es/sacmicrofront/noticias.do?idsite=337&tipo=12245&mcont=84507

SNE: Data provided by the SNE (Sistema Nacional de Epidemiología)

BES: Boletin Epidemiológico Semanal (weekly report from Spanish Government)

Full Set.: Full Setmanal (Weekly report from Balearic Government)

\+ Data modified from the Full Set. and provided by the SNE Informe (Report from Balearic Government)

in Social Security healthcare, and consequently, in 1996 the responsibility for the declaration of HL cases was transferred to the Autonomous Communities [76]. Each region was made responsible for its own list of notifiable diseases, and the *Conselleria de Salut del Govern de les Illes Balears* included leishmaniosis in the list [30].

Since 1982 it have been notified 377 cases of HL in the Balearic Islands being the majority reported in Majorca (78.2% of these cases from 1997 to 2015) (obtained from data in Table 4).

From 1997 to 2015, 315 cases were notified at a regional level (Table 4). Nevertheless, the disease is underreported in the Balearic Islands. As an example, from 1999 to 2014, 261 cases were notified 85 of which were detected through active searches, which represents 32.6% of underreporting (Fig. 7). In the years 2003, 2007 and 2013, the percentage of underreporting reached levels of 70, 54.5 and 62.5%, respectively.

An active search for HL cases in Minorca by Seguí [26 – 28] revealed two cases in the Hospital Virgen de Monte Toro (Mahon), but both were considered imported. One was a case of VL diagnosed in 1983 in a 23-year-old woman of Minorcan origin, but living in Almeria. The second case was of CL, diagnosed in a man who had spent some years living in Valencia. In 1994, Portús et al. [36] published a case of VL involving a

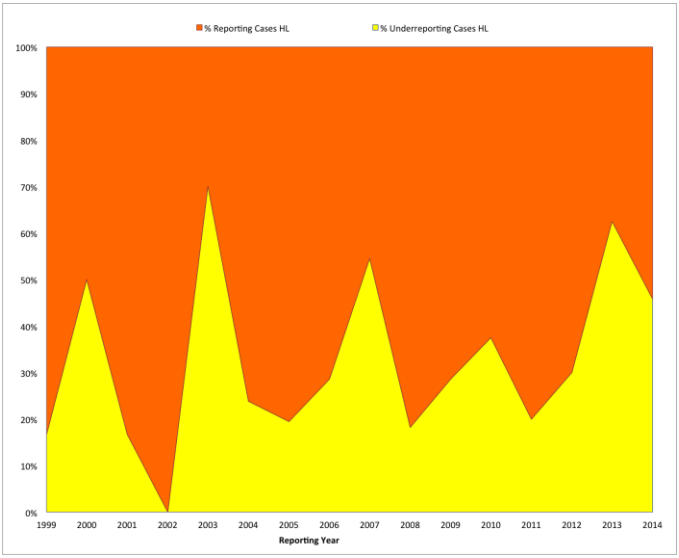


Figure 7. Percentage of reported and underreported HL cases in "*Xarxa de Vigilància Epidemiològica*" [24].

44-year-old woman who had not left the island for 13 years, her last trip outside being in 1978 to the Costa Brava. The possibility of a cryptic form of leishmaniosis acquired 13 years earlier was suggested. And finally, one case of CL was notified in 2011 [24] in an 11-month-old baby who had previously travelled to Ibiza, suggesting that it was an introduced case (Table 4).

The characterization of the strains isolated from humans in Majorca showed the presence of four zymodemes of *L. infantum* in the island (MON-1, MON-24, MON-34 and MON-108) [67].

5. General considerations

Entomological studies are often used as indicators of leishmaniosis vectors and therefore of the disease risk. Such studies are being standardized to determine which factors influence the incidence of the disease [14, 15, 48, 50, 51, 60, 61,77]. Most of these studies are performed using sticky traps, which allow sand flies to be caught in a relatively large area over a short period of time, but other traps, such as light traps (CDC traps), are often used in Europe. Sticky traps do not use any attraction device [78 – 79] and are placed mainly in crevices of sheltered walls used by sand flies as resting sites [80].

Two basic types of methodology have been used for the placement of these traps: transects, which aim to capture sand flies in the maximum number of places in the chosen routes [48, 59, 61, 79], or in at least one spot within each grid over a given area [18].

Herein, we report entomological studies carried out on the islands of Majorca and Minorca. The use of different methodologies, type of traps, and criteria for choosing capture sites makes it difficult to compare results from different studies.

Since the study objectives in the two islands were not identical, the methodology used also differed. In Minorca, the aim was to observe any changes in the density of *P. perniciosus* that might explain the emergence of CanL in the island, and therefore the traps were set along the same transects as in a previous study in 1988 [28]. However, while the traps in 1988 were left for a variable number of consecutive nights (from 2 to 8), in 2009 traps were placed on four nights, as stipulated in the EDEN (Emerging Diseases in a changing European Environment) methodology, and as performed in recent studies in Spain and other countries [15, 48, 60 – 61, 77], as well as in our study in Majorca.

With respect to the island of Majorca, and in contrast with Minorca, data are available on an autochthonous focus of HL and CanL, but information on the sand fly fauna, in particular *P. perniciosus*, is very fragmented and incomplete. Thus, the aim of the current work was to study the distribution of the leishmaniosis vector in the island, and the factors influencing its occurrence.

The variables analyzed also differed between islands, with fewer analyzed in Minorca (28) than Majorca (57). In Majorca, the study was more extensive, covering the entire island, and was focused on the distribution of *P. perniciosus* and the factors that influence it. Additionally, since Majorca is larger and more geographically diverse than Minorca, it was possible to study the effect of a higher number of variables on the occurrence of *P. perniciosus*.

Another difference between the two studies was the statistical approach. Thus, in Minorca we used the density of *P. perniciosus* as the dependent variable, since we wanted data that was comparable with those of a previous study [28], while for Majorca, the variable was the absence/presence of the vector, which has also been used in other studies carried out in Spain [15, 48].

Among the variables finally included in the bivariate analysis of data collected on both islands (14), three were found to have a statistically significant positive and/or negative influence on *P. perniciosus*: the location of the sampling site (defined in relation to the nearest settlement: inside, at the edge or between settlements); the type of wall construction (stone without mortar, stone/mortar, brick/mortar, other); and the adjacent flora (nearest vegetation <100 m to the sampling site).

A location away from settlements was positively correlated with *P. perniciosus* presence and/or density, as in other studies [18, 48, 60]. Rural locations away from urban areas would provide the right conditions for the vector to develop its terrestrial cycle [11, 60 – 61]. Moreover, the use of insecticides in urbanized or cultivated areas would reduce the population of sand flies [1, 63].

We also detected a positive correlation in sampling sites where sticky traps were placed on walls constructed of bricks or stone with mortar. Dry stone walls are also very widespread in the two islands, but their numerous holes impede a comprehensive sampling and therefore the provision of representative data. Furthermore, the holes in dry stone walls are often interconnected, facilitating air currents that would be unfavorable for sand flies [11, 60 – 61].

The "adjacent flora" variable had a positive influence on the vector presence in both islands, but an accurate comparison of results was hindered by different categorization of the data. Thus, in Majorca the presence of the vector was linked to scrub vegetation, whereas in Minorca it can only be concluded that a natural, uncultivated habitat had a positive influence. Natural environments are located away from human populations, while in cultivated areas pesticide use may decrease the presence and/or density of *P. perniciosus*.

In our studies, the possible presence of animals, including dogs, was not generally related with *P. perniciosus* detection, coinciding with other studies [15, 48, 59], which may be explained by the opportunistic feeding behaviour of this species [81 – 82]. Also, the type of trap influenced what sampling sites could be used and therefore could have affected the results, as sticky traps are usually placed far from kennels or livestock.

As mentioned above, vector studies are essential to understand the epidemiology of leishmaniosis outbreaks in different areas and apply control measures. The complexity of the vector-parasite-host-environment-climate relationships in the biological ecosystem requires the development of prevention programs that integrate the surveillance and control of the parasite, vector and reservoir of leishmaniosis [1, 11, 65].

Wide-ranging strategies for controlling leishmaniosis have been proposed, and traditional approaches are discussed by various authors [83 – 84]. Currently, these strategies focus primarily on the early diagnosis of the disease [85], treatment of infected humans and animals [1, 83 – 84], finding suitable molecules for the treatment and control of the disease [86], vaccine development [7, 85] and the application of different methods of vector control [87].

Regarding HL, the number of cases being reported in the Balearic Islands is likely to increase [45], given the current level of underreporting. This would provide more reliable data on its incidence and distribution, of particular importance in the Balearic Islands. Despite the diagnosis of some cases of HL in Minorca, the displacements of such patients to endemic areas makes it difficult to establish the geographical origin of the infection. The islands of Ibiza, Formentera and Majorca, which are very close and well connected to Minorca, are highly endemic areas of both CanL [19 – 21] and HL [22 – 24].

The aforementioned emergence of CanL on the island of Minorca is probably due to a lack of measures to prevent infected dogs entering the island from endemic areas. The recent inclusion of leishmaniosis among

diseases to be notified by veterinarians in the Balearic Islands will provide more accurate data on current distribution and prevalence of the disease.

6. Conclusion

P. perniciosus is the only vector of *L. infantum* species found in the islands of Majorca and Minorca. This sand fly species was captured throughout both islands, which points to a risk of leishmaniosis transmission. In Majorca, *P. perniciosus* is most likely to be found at altitudes of 51-150 m.s.a.l., in garrigue vegetation, between and at the edge of settlements, and in the proximity of sheep farms. The global density of *P. perniciosus* in Minorca has decreased significantly since the study performed 20 years ago, specifically in rural areas. The emergence and establishment of an autochthonous focus of CanL in Minorca is apparently related with the continuous introduction of *Leishmania*-infected dogs rather than an increase in *P. perniciosus* density. Although HL is a notifiable disease, it is clearly underreported in the Balearic Islands.

Acknowledgements

This work was supported by grants of the Ministerio de Educación y Ciencia of Spain (CGL2007-66943-C02-01/BOS), and Departament d'Universitats, Recerca i Societat de la Informació de la Generalitat de Catalunya (Spain) (2009SGR385). The Spanish Meteorological Agency (AEMet) provided the meteorological data for the study. The *Sistema Nacional de Epidemiología* confirmed the HL cases. Grateful acknowledgement is given to M.G. Seguí for helping in the location of the capture stations and A. Lanau for their active participation in the sand fly captures. We thank the veterinarians R. Aranda, M. Huguet and E. Sintes, for their active participation in collecting blood samples of dogs, and the farm owners that allowed us to collect blood samples from their dogs and to capture the sand flies. We are also grateful for the help of the Col.legi de Veterinaris of Balearic Islands, particularly R. García and A. Figueroa, as well as the veterinarians who completed the questionnaires. Thanks are due to J. Abellán, from the Centre Insular Sanitat Menorca, who provided data on the human case of leishmaniosis declared in Minorca. Thanks are due to J. Martín-Sánchez, X. Castells and A. Picado for helping in the statistical study.

References

1. World Health Organization. 2012, WHO Technical Report Series, 975, pp.216.
2. Alvar, J., Vélez, I.D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J., den Boer, M., the WHO Leishmaniasis Control Team. 2012, *PLoS ONE*, 7(5) e35671.
3. Rioux, J.A., Golvan, Y.J., Houin, R., Croset, H., Tour, S. 1970, *Revue de Médecine*, 18: 1039-1052.
4. Garnham, P.C.L. 1965, *Am. Zool.*, 5: 141-151.
5. Dereure, J. 1999, *In: Les leishmanioses. Collection Médecine tropicale de l'AUPELF-UREF*, 109-127.
6. Gállego, M. 2004, *Rev Sci Tech.*, 23: 661-676.
7. Gramiccia, M., Gradoni, L. 2005, *Int. J. Parasitol.*, 35(11-12):1169-1180.
8. Gálvez, R., Miró, G., Descalzo, M.A., Nieto, J., Dado, D., Martín, O., Cubero, E., Molina, R. 2010, *Vet. Parasitol.*, 169: 327–334.
9. Martín-Sánchez, J., Morales-Yuste, M., Acedo-Sánchez, C., Barón, S., Díaz, V., Morillas-Márquez, F. 2009, *Emerg. Infect. Dis*., 15(5):795-798.
10. Molina, R., Jiménez, M.I., Cruz, I., Iriso, A., Martín-Martín, I., Sevillano, O., Melero, S., Bernal, J. 2012, *Vet. Parasitol.*, 190: 268-271.
11. Maroli, M., Feliciangeli, M.D., Bichaud, L., Charrel, R.N., Gradoni, L. 2013, *Med. Vet. Entomol.*, 27: 123-147.
12. Ballart, C., Alcover, M.M., Picado, A., Nieto, J., Castillejo, S., Portús, M., Gállego, M. 2013, *Prev. Vet. Med.*, 109: 116–127.
13. Miró, G., Checa, R., Montoya, A., Hernández, L., Dado, D., Gálvez, R. 2012, *Parasit Vectors*, 5: 60.
14. Morosetti, G., Bongiorno, G., Beran, B., Scalone, A., Moser, J., Gramiccia, M., Gradoni, L., Maroli, M. 2009, *Geospat. Health*, 4:115-127.
15. Gálvez, R., Descalzo, M.A., Miró, G., Jiménez, M.I., Martín, O., Dos Santos-Brandao, F., Guerrero, I., Cubero, E., Molina, R. 2010, *Acta Trop.*, 115: 95-102.
16. Githeko, A.K., Lindsay, S.W., Confalonieri, U.E., Patz, J.A. 2000, *Bull. World Health Organ*, 78(9): 1136-1147..
17. Peterson, A.T., Shaw, J. 2003, *Int. J. Parasitol*., 33: 919–931.
18. Gálvez, R., Miró, G., Descalzo, M.A., Molina, R. 2011, *Prev. Vet. Med.*, 102: 59–65.
19. Matas-Mir, B., Rovira-Alos, J. 1989, *Conselleria de Sanitat i Seguretat Social del Govern Balear*, Palma de Mallorca, pp.110.
20. http://www.scalibor.es/leishmaniosis/prevalencia.asp (last access 20/04/2016).
21. Chicharro, C., Nieto, J., García, E., Cruz, I., Cañavate, C., Flores, M., Cuadrado, J., Alvar, J. 2004, Proceedings of the Ninth EMOP, Valencia (Spain), pp.161.
22. Riera, C., Fisa, R., Udina, M., Gállego, M., Portús, M. 2004, *Trans. R. Soc. Trop. Med. Hyg.*, 98: 102-110.
23. Riera, C., Fisa, R., López-Chejade, P., Serra, T., Girona, E., Jiménez, M.T., Muncunill, J., Sedeño, M., Mascaró, M., Udina, M., Gállego, M., Carrió, J., Forteza, A., Portús M. 2008, *Transfusion*, 48: 1383-1389.

24. Xarxa de Vigilància Epidemiològica de les Illes Balears. Informes anuales 2006-2015. *Govern de les Illes Balears* http://www.caib.es/sacmicrofront/noticias.do?idsite=337&tipo=12245&mcont=84507
25. Pujol, A., Cortés, E., Ranz, A., Vela, C., Aguiló, C., Martí, B. 2007, *Revista del Col·legi Oficial de Veterinaris de les Illes Balears Veterinària*, 32: 9-12.
26. Seguí, M.G. 1991, Tesis Doctoral, Facultad de Farmacia, Universitat de Barcelona, pp.324.
27. Seguí, M.G. 1991, *Rev. Men.*, 191: 153-178.
28. Seguí, M.G. 1991, *Rev. Cien.*, 9: 91-101.
29. BOE núm. 13, de 15 de enero de 1982, pp.907.
30. BOCAIB Núm. 17 08-02-1997: 1706-1708. https://www.caib.es/sacmicrofront/noticia.do?idsite=274&cont=59988&lang=CA
31. BOE núm. 65, de 17 de marzo de 2015, pp.25012.
32. BOE núm. 167, de 10 de julio de 2014, pp. 54170-54178.
33. Alvar, J., Cañavate, C., Gutiérrez-Solar, B., Jiménez, M., Laguna, F., López-Vélez, R., Molina, R., Moreno, J. 1997, *Clin. Microbiol. Rev.*, 10, 2: 298-319.
34. Gil-Collado, J., Morillas-Márquez, F., Sanchís-Marin, M.C. 1989, *Rev. San. Hig. Públ.*, 63: 15-34.
35. Gállego, J., Botet, J., Gállego, M., Portús, M. 1992, *In: "In memoriam" al profesor Doctor D. E. de P. Martínez Gómez. Publicaciones de la Universidad de Córdoba*, 581-600.
36. Portús, M., Gállego, M., Seguí, M.G., Sole, J. 1994, *Parasite*, 1: 87-88.
37. Martín-Algarra, A., Vera, J.A. 2004, *SGE-IGME*, 352-354.
38. Rivas Martínez, 1983, *Lazaroa*, 5: 33-43.
39. http://ibestat.caib.es/ibestat/inici (last access 20/04/2016).
40. Pittaluga, G., de Buen, S. 1917, *Boletín del Instituto Nacional de Higiene de Alfonso XIII*, 50: 137- 145.
41. Gil Collado, J. 1977, *In*: *Colloques Internationaux du Centre National de la Recherche Scientifique*, 239: 177-189.
42. Molina, R., Aransay, A., Nieto, J., Cañavate, C., Chicharro, C., Sans, A., Flores, M., Cruz, I., García, E., Cuadrado, J., Alvar, J. 2005, *Proceedings of ISOPS V*, 82 (1): OP-10.
43. Alvar, J. 2001, Laboratorios Intervet S.A. Salamanca, pp.200.
44. Lucientes, J., Castillo, J.A., Gracia, M.J., Peribáñez, M.A. 2005, *Rev. Elec. Vet. REDVET*, 6: 1-8.
45. Amela, C., Suarez, B., Isidoro, B., Sierra, M.J., Santos, S., Simón, F. 2012, *Centro de Coordinación de Alertas y Emergencias sanitarias (CCAES), Ministerio de Sanidad, Servicios Sociales e Igualdad*, Madrid, pp.26.
46. Suárez-Rodríguez, B., Isidoro-Fernández, B., Santos-Sanz, S., Sierra-Moros, M.J., Molina-Moreno, R., Astray-Mochales, J., Amela-Heras, C. 2012, *Rev. Esp. Sal. Públ.*, 86: 555-564.
47. Lladó, M.T., Rotger, M.J. 1990, *Conselleria de Sanitat i Seguretat Social del Govern Balear*. Palma de Mallorca, pp.109.

48. Barón, S.D., Morillas-Márquez, F., Morales-Yuste, M., Díaz-Sáez, V., Irigaray, C., Martín-Sánchez, J. 2011, *Parasitology*, 138: 1234-1244.
49. Ballart, C., Barón, S., Alcover, M.M., Portús, M., Gállego, M. 2012, *Acta Trop.*, 122:155– 159.
50. Alcover, M.M., Ballart, C., Martín-Sánchez, J., Serra, T., Castillejo, S., Portús, M., Gállego, M. 2014, *Parasit Vectors*,4;7:421.
51. Alcover, M.M., Ballart, C., Serra, T., Castells, X., Scalone, A., Castillejo, S., Riera, C., Tebar, S., Gramiccia, M., Portús, M., Gállego, M. 2013, *Acta Trop.*, 128:642-651.
52. Aspöck, H., Gerersdorfer, T., Formayer, H., Walochnik, J. 2008, *Wier. kiln. Wochenschr.*, 120 (Suppl 4): 24-29.
53. Kuhn, K.G. 1999, *Trop. Med. Int. Health*, 7: 1-2.
54. Killick-Kendrick, R., Rioux, J.A., Bailly, M., Guy, F.M., Davidson, I., Knechtu, R., Ward, R.D., Guilvar, E., Perieres, J., Dubois, H., 1984, *Ann. Parasitol. Hum. Comp.*, 59 (6): 555-572.
55. Branco, S., Alves-Pires, C., Maia, C., Cortes, S., Cristovão, J.M.S., Gonçalves, L., Campino, L., Afonso, M.O. 2013, *Acta Trop.*, 125: 339-348.
56. Hilbe, J.M. 2007, *Cambridge University Press*, NY, pp.264.
57. R Development Core Team. R. 2012, *R Foundation for Statistical Computing*, ISBN 3-900051-07-0. http://www.R-project.org/ (last access 20.04.16).
58. Killick-Kendrick, R. 1999, *Clin. Dermatol.*, 17: 279-289.
59. Ballart, C., Guerrero, I., Castells, X., Barón, S., Castillejo, S., Alcover, M.M., Portús, M., Gállego, M. 2014, *Geospath. Health*, 8: 367-381.
60. Rioux, J.A., Golvan, Y.J. 1969, *In: Monographies de l'Institut National de la Santé et de la Recherche Médicale*, pp.224
61. Rioux, J.A., Carron, S., Dereure, J., Périères, J., Zeraia, L., Franquet, E., Babinot, M., Gállego, M., Prudhomme, J. 2013, *Parasite*, 20: 34.
62. Gramiccia, M. 2011, *Vet. Parasitol.*, 181(1):23-30.
63. Martín-Sánchez, J., Acedo, C., Muñoz-Pérez, M., Pesson, B., Marchal, O., Morillas-Márquez, F. 2007, *Vet. Parasitol.*, 145(3-4):267-73.
64. Decret 21/2015. BOIB, 2015, N1 56: 18679-18686.
65. Alvar, J., Cañavate, C., Molina, R., Moreno, J., Nieto, J. 2004, *Adv. Parasitol.*, 57:1-88.
66. Solano-Gallego, L., Morell, P., Arboix, M., Alberola, J., Ferrer, Ll. 2001, *J. Clin. Microbiol.*, 39(2): 560-563.
67. Chicharro, C., Morales, M.A., Serra, T., Ares, M., Salas, A., Alvar, J. 2002, *Trans. R. Soc. Trop. Med. Hyg.*, 96 Suppl 1:S93-9.
68. Riera, C., Valladares, J.E., Gállego, M., Aisa, M.J., Castillejo, S., Fisa, R., Ribas, N., Carrió, J., Alberola, J., Arboix, M. 1999, *Vet. Parasitol.*, 84: 33-47.
69. Gradoni, L., Gramiccia, M. 2008, In*: OIE Manual of Diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). 6th edition. Paris: Office International des Epizooties*,240-250.
70. Morillas, F., Sanchez Rabasco, F., Ocaña, J., Martin-Sanchez, J., Ocaña-Wihelmi, J., Acedo, C., Sanchiz-Marin, MC. 1996, *Parasitol. Res*., 82:569-570.

71. Aisa, M.J., castillejo, S., Gállego, M., Fisa, R., Riera, C., de Colmenares, M., Torras, S., Roura, X., Sentis, J., Portús, M. 1998, Am. J. *Trop. Med. Hyg*., 58(2):154-159.
72. Morales-Yuste, M., Morillas-Márquez, F., Díaz-Sáez, V., Barón-López, S., Acedo-Sánchez, C., Martín-Sánchez, J. 2012, *Parasitol. Res.*, 111:155-164.
73. Pittaluga, G. 1925, *Societé des Nations*, pp.28.
74. Nájera Angulo, L. 1935, *Revista Médica de Barcelona*, pp. 34- 51.
75. Boletines Epidemiológicos Semanales del Ministerio de Sanidad y Consumo. (1982–1996).
76. BOE núm. 21 de 24 enero 1996, 2153-2158.
77. Farkas, R., Tánczos, B., Bongiorno, G., Maroli, M., Dereure, J., Ready, P.D. 2011, *Vector-Borne and Zoonot. Dis*., 11(7): 823-834.
78. Alexander, B., Maroli, M. 2003, *Med. Vet. Entomol.*, 17: 1-8.
79. Rioux, J.A., Golvan, Y.J., Croset, H., Houin, R., Juminer, B., Bain, O., Tour, S. 1967, *Ann. Parasitol. Hum. Comp.*, 42 (6): 561-603.
80. Léger, N., Depaquit, J. 1999, *Collection Médecine Tropicale de l'AUPELF-UREF*, 90-108.
81. de Colmenares, M., Portús, M., Botet, J., Dobaño, C., Gállego, M., Wolff, M., Seguí, M.G. 1995, *J. Med. Entomol.*, 32: 229-233.
82. Jiménez, M., González, O., Iriso, A., Marco, E., Alegret, A., Fúster, F., Molina, R. 2013, *Parasitol. Res.*, 112: 2453-2459.
83. Solano-Gallego, L., Koutinas, A., Miró, G., Cardoso, L., Pennisi, M.G., Ferrer, L., Bourdeaug, P., Oliva, G., Banethi, G. 2009, *Vet. Parasitol.*, 165: 1-18.
84. Solano-Gallego, L., Miró, G., Koutinas, A., Cardos, L., Pennisi, M.G., Ferrer, L., Bourdeau, P., Oliva, G., Baneth, G. 2011, *Parasit. Vectors*, 4: 86.
85. Ready, P.D. 2014, *Clin. Epidemiol*., 6: 147–154.
86. Ready, P.D. 2008, *Rev. sci. tech. Off. int.*, 2008, 27 (2), 399-412.
87. Sharma, U., Singh, S. 2008, *J. Vector Borne Dis.*, 45, 255-272.