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RESEARCH ARTICLE

Cutaneous Leishmaniasis caused by *Leishmania tropica* in Foum Jamâa (Azilal, Morocco)

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Abstract

Cutaneous leishmaniases (CL) are parasitic disease with a wide range of clinical symptoms and they are common in the human population in different localities such as Foum Jamâa in Azilal province, Morocco. The main objective of this study was to investigate the micro-environmental factors that may act as a factor of recrudescence for CL from risk January 2006 to December 2009 on 655 patients distributed in 3 sectors in Foum Jamâa. We also carried out an molecular detection of *Leishmania* and sand fly species responsible of CL in this focus. Free distribution tests were used to analyze the effect of each factor in the epidemiological assessments. Skin scrapings spotted on glass slides were collected and the ITS1 PCR-RFLP was used to identify the *Leishmania* parasite responsible for the recent cases of CL in FJ. Morphological identification was performed on 1072 sand flies (23% females and 77% males) collected by sticky paper traps during 6 months. Ours results showed that highest rate of positive lesions was found in the age group of 9 years old or under. However, there is no statistically significant correlation between gender and the rate of CL in presenting patients. Those results showed also that the distribution of positive cases was more significantly influenced by environmental factors common to each sector (altitude, sewerage, garbage...) than by individual specific. Ours results showed that the disease had caused by *Leishmania tropica* and 57% of the total collected flies were identified as *Phlebotomus (Paraphlebotomus) sergenti* (Parrot).

INTRODUCTION

Cutaneous leishmaniasis (CL) is endemic in many regions of the world such as Latin America, the Mediterranean basin, and western Asia from the Middle East to central Asia (**Alvar *et al.*, 2012**). In North Africa, the disease is highly prevalent in Morocco, posing a public health problem. The total CL cases reported in 2010 were 8849 against 5276 cases in 2008, showing a clear tendency for an increase during recent years (**OMS, 2010**).

In Morocco, three species of *Leishmania* are endemic, causing human CL. *L. major* is responsible of zoonotic CL and is localized in areas south of the Atlas Mountains (**Rioux *et al.*, 1986**). In the North of the country, some sporadic cases of CL due to *L. infantum* were observed (**Lemrani *et al.*, 1999 ; Rhajaoui, 2009**). *L. tropica* presents its largest geographic distribution and highest incidence rate in Morocco. It is found throughout the center of the country in a band stretching from the Atlantic Ocean along the length of the Atlas Mountains almost to the Mediterranean Sea (**Rhajaoui, 2009**). In recent years, several epidemics with hundreds of cases have been reported in several Moroccan cities (**Marty *et al.*, 1989**; **Rhajaoui *et al.*, 2004, 2007**; **Rhajaoui, 2009**). *L. tropica* is recognized as a very heterogeneous species and many authors readily demonstrate its intraspecific heterogeneity

However, its genetic and isoenzyme diversity varies according to the North Africa country concerned. In fact, in terms of isoenzyme profile variability, Morocco is characterized by the largest number of zymodemes ever described in *L. tropica*, with eight zymodemes (*L. tropica* MON-102, MON-107, MON-109, MON-112, MON-113, MON-122, MON-123, and MON-279) not all transmitted by *Phlebotomus sergenti* and not all isolated from human reservoir. These zymodemes, detected from human CL, dogs, and sandflies (**Dereure *et al,* 1991**; **Lemrani *et al.,* 2002**; **Pratlong *et al.,* 1991**).

On the other hand, microsatellite analysis revealed that two genetically very distinct populations of *L. tropica* co-exist within the same focus in Morocco: population ‘‘Morocco A’’ is related to population ‘‘Asia’’, whereas population ‘‘Morocco B’’ is genetically closer to the other African populations. This data suggests both anthroponotic and zoonotic transmission cycles of the parasites present in the same Moroccan CL foci (**Schwenkenbecher *et al.*, 2006**).

Much debate concerns the transmission cycle of *L. tropica* in North Africa. In Morocco, the disease is often described as being anthroponotic (**Guessous-Idrissi *et al.,* 1997**; **Rhajaoui, 2009**). However, the epidemiology of *L. tropica* in this country is by no means fully understood. In particular, sporadic cases occur in rural areas and small towns, which cannot readily be explained by local anthroponotic transmission. Either these latter are dependent foci in which the parasite requires repeated introduction, or there may be unidentified zoonotic sources (**Ashford, 2000**). This idea is supported by the greater diversity of *L. tropica* strains in the proven vector *P. sergenti* compared with the diversity observed in humans (**Ajaoud *et al.,* 2013; Guilvard *et al.,* 1991**). Thus, the precise role of humans, domestic dogs, and other animals as reservoir hosts is not well-established (**Guessous-Idrissi *et al.,* 1997**). Regarding the clinical aspect, CL with *L. tropica* is described as a single lesion starting as a nodule at the site of inoculation. A crust develops centrally which may fall away exposing an ulcer which heals gradually (**Rhajaoui, 2009**). In the region of Azilal, the first cases of CL that have been recorded by the provincial delegation of health goes back to 1996 and 1997 in Tanant, at 16 km from Foum Jamâa. Early in 2000, several provincial centers declared some scattered cases in this region. Several years later (2005-2009), the region saw a higher predominance of CL with a rate of impact of 0.06-0.45 % in 2007.

In addition to the high economic cost of the disease treatment, CL causes many social problems for patients (**Yiougo *et al.,* 2007**) and sometimes even after recovery, when it leaves undesirable scars on the face. In the present study, we questioned on the actual environmental factors that may incubate the inoculums and/or enhance the spreading of newly introduced cases into the FJ area (Azilal). We selected the ITS1-PCR method for detecting *Leishmania* in skin scrapings spotted on glass slides; the ITS1 PCR-RFLP was used to identify the *Leishmania* parasite responsible for the recent cases of CL in FJ. Considering the importance of the vector in the transmission of the CL cycle, an entomological survey was carried out in this focus to study the sand fly fauna, species composition, and the monthly prevalence of sand flies.

MATERIALS AND METHODS

Study sites

This study was conducted in Foum Jamâa region, the province of Azilal, Atlas of Morocco (figure 1). With a total area of about 63000 ha, the area was subdivided into 3 adjacent sectors: Beni Hssan (BH), Foum Jamâa (FJ) and Tabia (T). This region is located near the Western High Atlas National Park (32° 08’ N, -6° 60’ W) at different altitude from 523 to 1086 m above sea level and has an arid to semi-arid climate, with a mean annual rainfall of 300 mm and monthly mean temperatures that range from 45°C in August to 10°C in December. The agriculture remains the primary source of income and is mainly based on the production of wheat, almond, olive and lentils. Most households in the region have stables nearby and poor sanitation.

Epidemiologic features of the CL and the molecular diagnosis of the parasite

For each patient we filled a questionnaire with information about the patient (name, age, gender, and address) and micro-ecological data (habitat type, sewerage, breeding, dogs…). Data were analyzed using non-parametric khi² tests. For khi² test of independence between two variables and for khi² test between observed and expected values of each variable, the null hypothesis was respectively the independence between variables and the equal distribution of positive cases between the classes of each variable. For all test, the significance level was 0.05. Tissue samples were taken for some patients with suspected CL, who had consulted the health centers in Foum Jamaa. From the edge of lesions, smears were prepared, fixed with absolute methanol, and then stained with Giemsa. The whole slide was analyzed with a 100-immersion objective. 81 slides were subjected to DNA extraction and molecular identification of the causative *Leishmania* species. All microscopically positive and negative slides were then chequed for *Leishmania* kDNA by molecular assays. The smear scrapings were added to 250 ul lysis buffer (50 mM NaCl, 50 mM Tris, 10 mM EDTA, pH 7.4, 1% v/v, Triton X-100 and 100 mg of proteinase K per ml). Cell lysis was

accomplished after incubation overnight at 60 °C. Lysates were then subjected to phenol–chloroform extraction, as described by **Van Eys et *al.*, 1992**. DNA was purified by Qiagen kit according to the instructions of the manufacturer. A final volume of 30 ul obtained were kept at −20 ◦C until used. A PCR-restriction fragment length polymorphism (RFLP) approach was applied for the detection and identification of the *Leishmania* parasites. The ribosomal internal transcribed spacer 1 (ITS1) was amplified using the primer pair L5.8S and LITSR **(Schonian *et al.*, 2003**). Amplicons were analyzed on 1.5 % agarose gels by electrophoresis and visualized by UV light. A reaction was considered positive when a band of the correct sizes (300 to 350-bp) was observed. A negative and positive control containing distilled water and DNA of *L. infantum*, respectively, were included during PCR to ensure reliability, validity and to check for possible contaminations of the amplification reactions. PCR product was digested with the restriction endonuclease HaeIII (Invitrogen).

The resultant fragments were separated by electrophoresis on 3 % agarose gels. In order to comparison and confirmation of identified *Leishmania* species, three strains with those of WHO reference of *L. major* (MHOM / SU / 73 / 5ASKH), *L. tropica* (MHOM / SU / 74 / K27) and *L. infantum* (MHOM / TN / 1980 / IPT1) were used as the standard strains.

Collection and identification of sand flies

Sandflies were collected in the summers (May–October) of 2010 mainly on sticky traps (paper coated with castor oil). The collections were carried bi-weekly on 8 stations, representing 3 main different biotopes in the region of FJ indoor (dwelling, ruined house, cave); outdoor (bridge, windows) and animal shelters (hen-house, stable, stable henhouse) and regularly distributed to cover all supervised area. The traps installed after sunset were collected before sunrise in the catching sites. Collected sand flies were placed in glass vials containing 70 % ethanol. After sex determination, all collected sand flies were mounted on glass slides, using Canada balsam, and were identified by morphological taxonomic keys according to **Boussaa. 2008**.

RESULTS

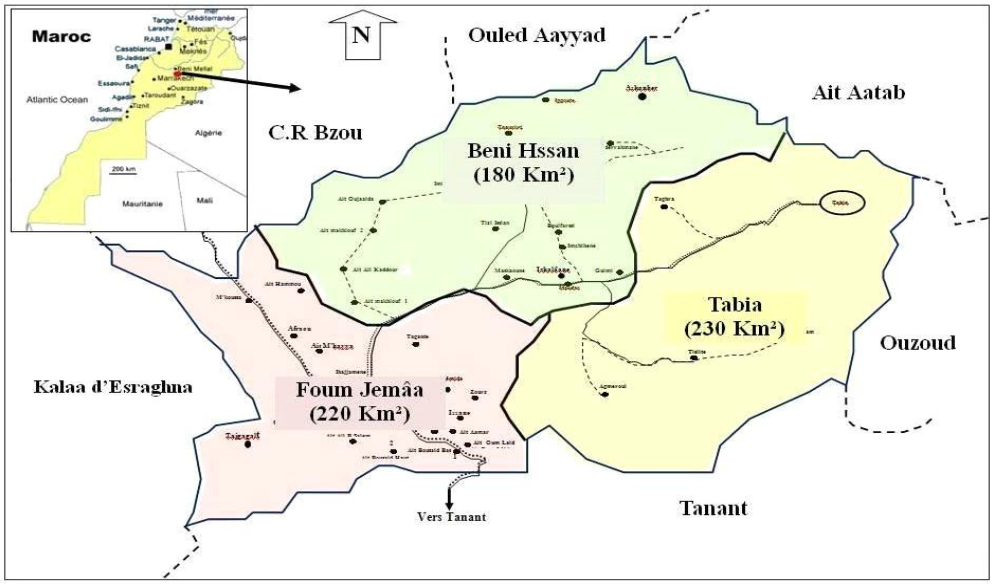


Fig 1. Position of the Foum Jamâa region (province of Azilal) indicated by red Spot in Moroccan map

Epidemiological survey

In this study, 655 patients of both sexes and different age groups were included. Figures 2, 3 and 4 summarize the result of repartition of the 479 positive cases according to studied parameters. Results of non-parametric khi² test was reported on graphs, letters a and b indicate, respectively that the observed value is significantly lower or greater than that expected according to the null hypothesis of equal distribution of the 479 positives cases between all modalities of each parameter, all values were reported in terms of percentage of the total positive cases.

The repartition of positives cases according to the sex (213 **[**44%**]** male; 266 **[**56%**]** female; $p = 0.004$) was not significant. The disease has been found significantly prevalent in the age group of 9 years or under (67%; $p \leq 0.0001$) (figure 2).

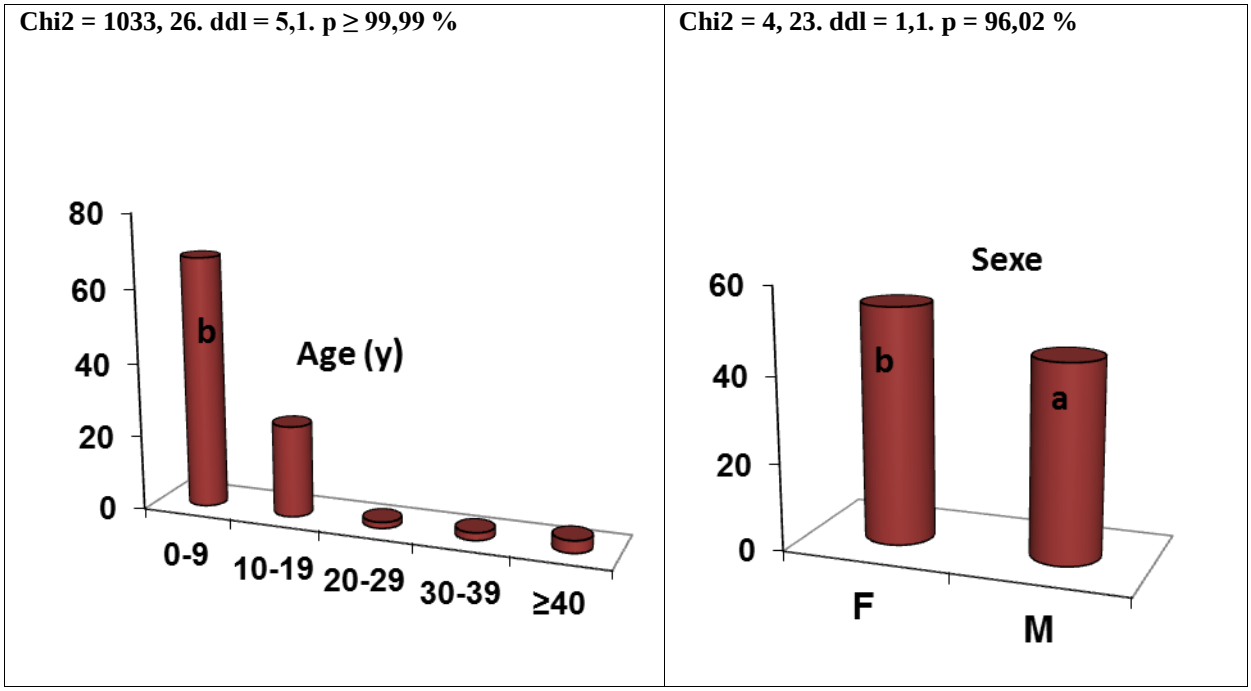


Fig 2. The prevalence of cutaneous leishmaniasis by age and sex in Foum Jamâa region (Azilal, Morocco) recorded from 2006 to 2009

From 2006 to 2008, temporal evolution of the number of new CL positive cases was relatively stable, but a significant increase was detected in 2009 (36.5%, p≤10-4) (figure 3. 1). Most patients (97.5%) are originally from Foum Jamâa, and all of them are Moroccans (figure 3. 2). Among the three sectors of FJ region, Tabia shows significantly the lowest number of positive cases (6.3%, p≤10-4), and there is almost no difference between the two other sectors (BH & FJ) with respectively 44.7% and 49.1% (figure 3. 3). The repartition of the positive cases according to altitude was very significant (p≤10-4), the highest number of positive cases (88.3%) was recorded at intermediate elevation (between 700 to 900 m) (figure 3. 4).

Micro-environmental factors around patients' houses seem to have direct impact on CL infection. Among the three modes of household refuse evacuation, namely: private focus (pf), public garbage collection (rp) and throw away in the river (sg), 83.7% of the patients declared that their family had a private focus of garbage (figure 4. 1), this percent was significantly higher (p≤10-4).

The habitat type was represented by two main types of home construction with equal frequency as concluded from our field observations.
The traditional method uses local materials (mixture of soil & straw) and the recent construction using concrete, so the house was qualified as permanent structure. The percent of positive cases was significantly (24.6%, p≤10-4) lower in the first type while it was significantly important (75.4%, p≤10-4) in permanent structure (figure 4. 2).

According to animal breeding, the lowest statistically significant percentage of positive cases (20.3%, p≤10-4) was found among farmers with livestock outside the house (figure 4. 3). While the highest number of positive cases was recorded with state of animal breeding inside habitat (37.6%).
The factor of the number of dogs, both domestic and stray dogs, was apparently more linked to positivity. The percent of positive cases was significantly higher [74.1%, p≤10-4], where 2 or more dogs were present (figure 4. 4).

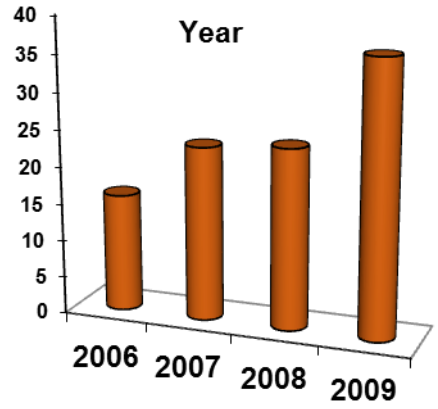
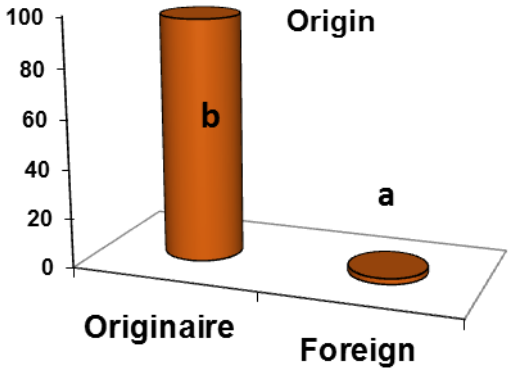
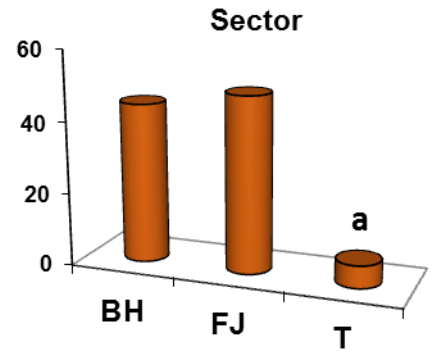
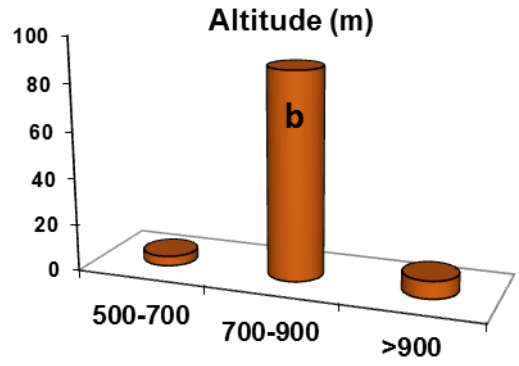
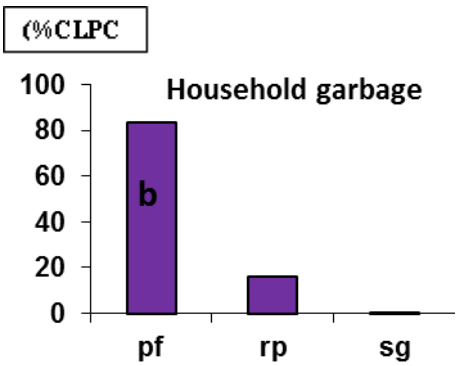
1 **Chi2 = 42,09. Dd l= 3,1. P ≥ 99,99 %**	**2** **Chi2 = 436,01. Dd l= 1,1. P ≥ 99,99 %**
	
3 **Chi2=159,34, ddl=2,1-p≥99,99%**	**4** **Chi2=675,47, ddl=6,1-p≥99,99%**
	

Fig 3. Positive cutaneous leishmaniasis by years of study, origin, sector and altitude patient in Foum Jamâa region (Azilal, Morocco) recorded from 2006 to 2009

1

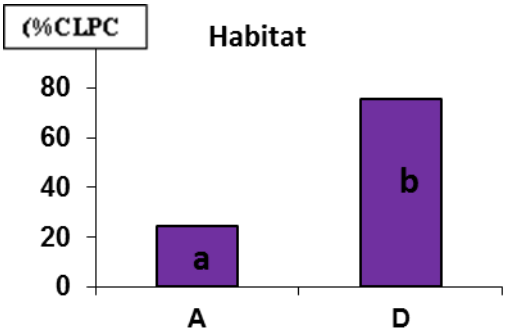
Chi2 = 546,30. Ddl = 2,1. p ≥ 99,99 %



1: household garbage (pf: private focus, rp: public garbage collection, sg: river) (p≤10-4).

2

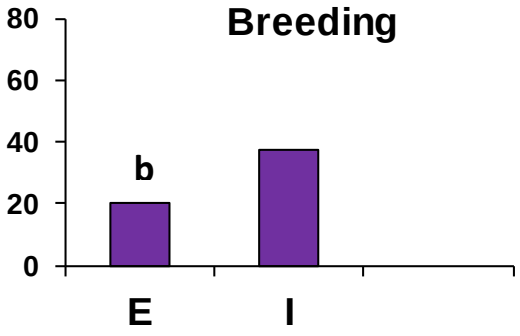
Chi2 = 123,28. Ddl = 1,1. P ≥ 99,99 %



2: Habitat type (A: traditional, D: modern) (p≤10-4)

3

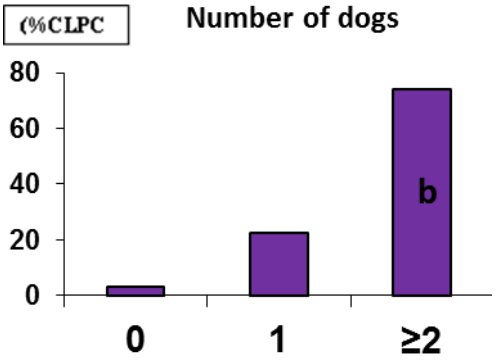
Chi2 = 38,41. Ddl = 2,1. p ≥ 99,99 %



3: Breeding (E: extern livestock, I: intern livestock) (p≤10-4).

4

Chi2 = 384,96. Ddl = 2,1. P ≥ 99,99 %



4: Number of dogs around both domestic and stray dogs (p≤10-4).

Fig 4. Influence of micro-ecological factors on the repartition of cutaneous leishmaniasis positive cases (%CLPC) between 2006 and 2009 in the Foum Jamâa region (province of Azilal, Morocco).
***a and b indicate a significant statistical difference between classes, respectively lower and greater, and p-value was indicated in between bracket for each factor.**

Micro-environmental factors around patients' houses seem to have direct impact on CL

In this study, *Leishmania* DNA was detected in 78 of 81 samples examined by ITS1-PCR (96.20%). The *Leishmania* parasites in 78 clinical samples amplified by PCR were identified as *L. tropica* by their typical restriction profiles (figure 5). ITS1-PCR produced one single band, which was 300-bp in size. The RFLP analysis revealed two fragments (185- and 57-bp) (figure 6), specific for *L. tropica*.

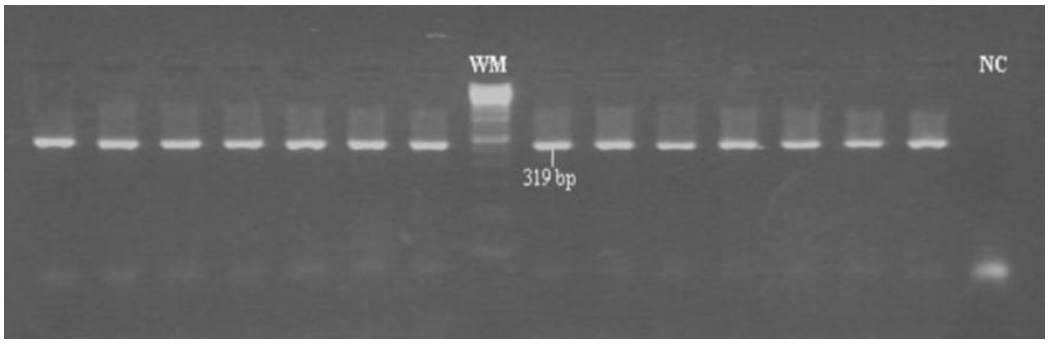


Fig 5. Results of PCR-based amplication of DNA extracted from Giemsa-stained lesion smears from Foum Jamaà. The bands shown on 1.5% agarose gel stained with ethidium bromide. WM = size marker, NC = negative control. All other lanes correspond to clinical materials from patients.

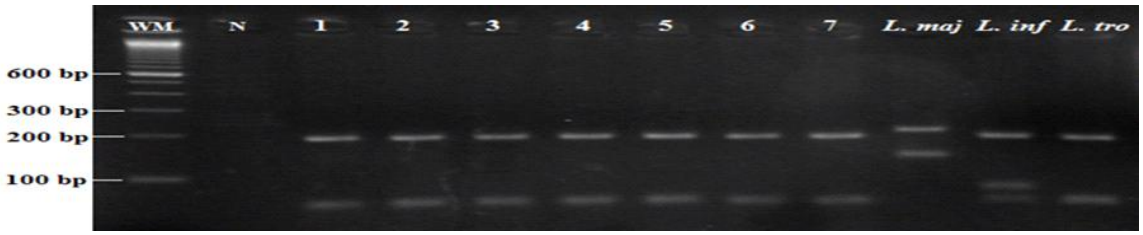


Fig 6. Molecular identification of causative CL species. Restriction fragment length polymorphism (RFLP) analysis of the amplified internal transcribed spacer 1 region (ITS1) digested with restriction enzyme HaeIII and analyzed by electrophoresis on 2.5% agarose gel.

Three reference strains were used for comparison: *L. inf = L. infantum* (MHOM/TN/1980/IPT1); *L. maj = L. major* (MHOM/SU/73/5ASKH); *L. trop = L. tropica* (MHOM/SU/74/K27). WM = molecular size marker; NC = negative control. All other lanes show digested PCR product from clinical materials; lanes 1–7 = *L. tropica.*

Entomological survey

Taxonomic and relative abundance

In this study, 1072 sand flies were collected using 1259 sticky traps from FJ region throughout 6 months: between May to October 2010 (table 1). The sex-ratio indicated that more males
(837 [78.07%]) were collected than females (235 [21.92%]); the male/female ratio was 3.3:1. Overall, six species were identified, consisting of five *Phlebotomus spp*. and one *Sergentomyia.*

The most dominant species was *P (Paraphlebotomus) sergenti* (Parrot) (56%), followed by *P. (Larroussius) longicuspis* (Nitzulescu) (24%). These two abundant species constituted 80% of the total collected flies.

Table 1: The numbers and species of sandfly collected, in Foum Jamâa region (Azilal)

Espèces	Mâle	Femelle	Total	%
P. sergenti	**450**	**147**	**597**	**56**
P. longicuspis	**225**	**30**	**255**	**24**
P. papatasi	**46**	**23**	**69**	**7**
P. perniciosus	**45**	**1**	**46**	**4**
S. minuta	**70**	**34**	**104**	**9**
P. chabaudi	**1**	**0**	**1**	**0**
Total	**837**	**235**	**1072**	**100**

Temporal distribution of sand flies

Monthly monitoring of sand flies vector activity during 6 months-study is shown in table 2 and figure 7. Sand flies flight activity of total population was strongly marked during two periods: the most important one, started in the end of May and reached a peak in June for all sand flies species except *S. minuta* which peaked during July, while the activity of the others species decreased to a minimum during this month. The second period of intense sand flies activity corresponds to August- September-October depending on sand flies species and fall to low levels in the end of October.

Table 2: Temporal distribution of sandflies species in Foum Jamâa region

	Number of trap	*P. papatasi*	*P. sergenti*	*P. longicuspis*	*P. perniciosus*	*S. minuta*	*P. chabaudi*
May	**206**	**1**	**96**	**47**	**7**	**0**	**0**
June	**206**	**47**	**280**	**69**	**22**	**14**	**0**
July	**213**	**6**	**21**	**35**	**1**	**17**	**0**
August	**216**	**8**	**58**	**31**	**2**	**55**	**0**
September	**220**	**1**	**71**	**52**	**4**	**14**	**1**
October	**198**	**6**	**71**	**21**	**10**	**4**	**0**
Total	**1259**	**69**	**597**	**255**	**46**	**104**	**1**

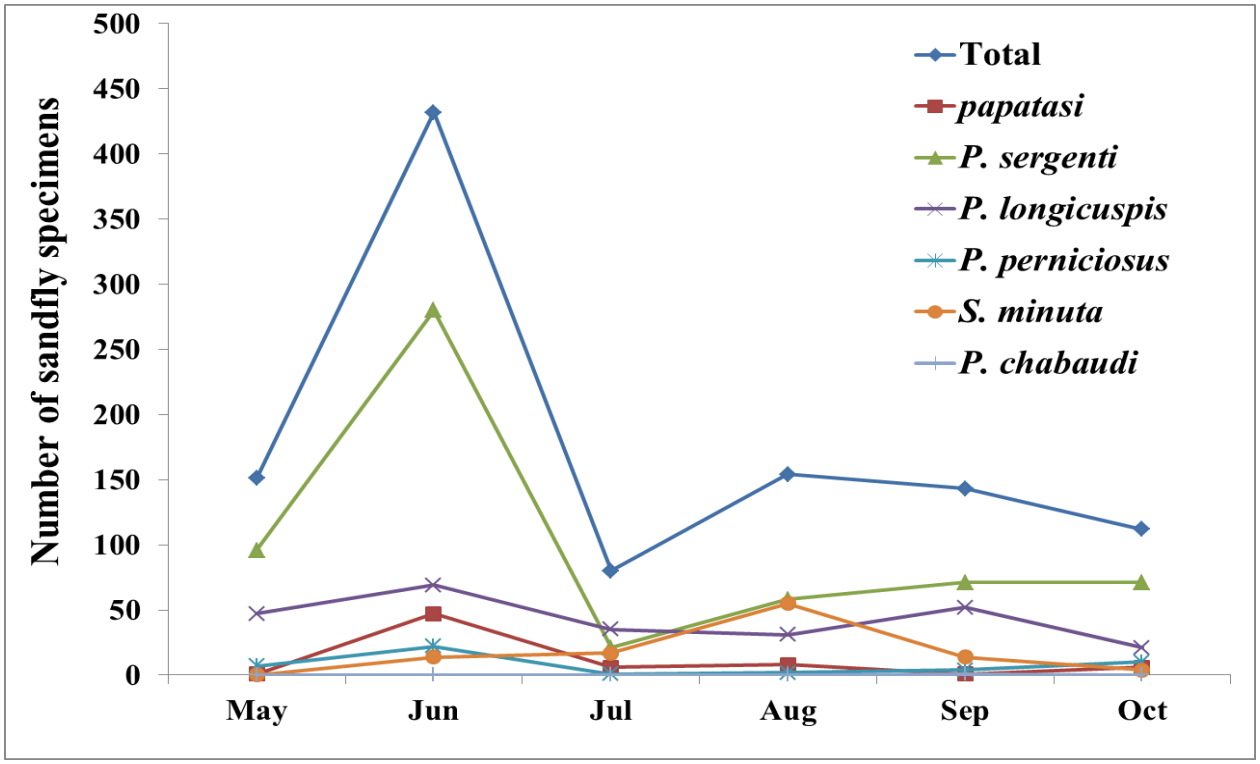


Fig 7. Monthly monitoring of sand flies vector activity in Foum Jamâa region.

Sand flies occurrence according to biotope

The distribution of the collected total number of sand flies according to biotope was shown in figure 8. The highest number of sand flies captured was observed in the animal shelters with a value of 2.33 sandflies/trap, followed by indoor with about 1.52 sandflies/trap. The lower number of captured sandflies was registered in the outdoor (0.69 sandflies/trap).

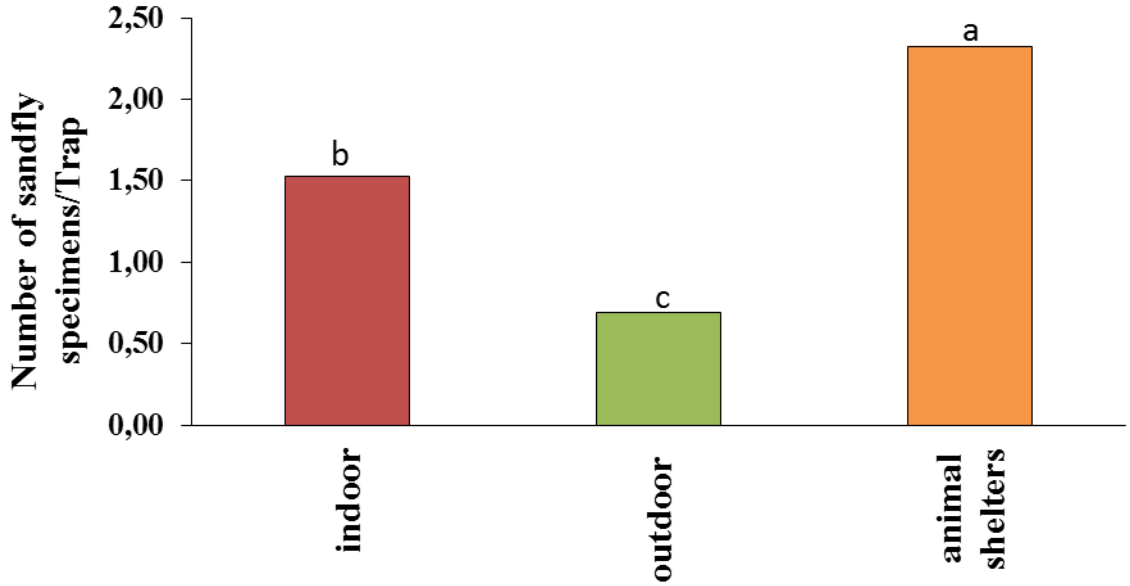


Fig 8. Relative abundances of sandfly species in each category of biotope sampled in Foum Jamâa region (Azilal, Atlas of Morocco)

Discussion

Fieldwork observation highlights a strong heterogeneity in the ecological conditions in FJ area and this affects the spatial distribution of the CL disease. In addition to differences between sectors owing to population density, altitude may contribute to the persistence of CL. Distribution of positives cases according to altitude suggests a transmission by one species of *Phlebotomus* in the region, which has preference of altitude between 700 to 900 m above sea level. Different species vectors will transmit with specificity different parasites species, and consequently each case must get specific treatment. According to Dedet (**Dedet, 2001**), the geographical distribution of leishmaniasis was related to environmental conditions that can influence the distribution and density of both the sandfly vector and the mammalian reservoir host.

Vulnerability of CL was independent to sex, indicating that both genders were equally affected, which is in elsewhere (**Sharma et *al* 2005a; Fazaelia et *al*. 2009**)**.** While the weak observed difference was usually due to the unequal sex-ratio in the population, and most of these researchers found that feminine sex was slightly more represented. Repartition of CL positive cases according to age was also in agreement with previous findings (**Aytekin et *al*., 2006; Sharma et *al*., 2005b**). Generally, CL affect more children of ≤10 than adult, other studies showed that most highly infected age group with CL is person of ≤20 years old (**Sharma et *al*., 2005a**). This can be explained by the fact that immunity system of children is still not well-developed resulting inability of the body to fight CL infection and children tissues are too sensitive.

The number of dogs was a significant risk factor. Stray and domestic dogs were equally frequent; domestic dogs are not properly maintained; therefore, both are the main reservoir host of zoonosis in the FJ region, indeed the domestic dog was the main reservoir host of leishmaniasis (**Ready, 2008**). This disease threatens a large number of dogs in endemic areas, and it is difficult to control since no efficient vaccine exists and the chemotherapeutic agents have a limited efficacy and a high cost (**Dedet and Pratlong, 2009**).

Because of the growing urbanization, the following elements (number of dogs, breeding and garbage), have strongly become factors of risk to the public health in FJ region. Thus, positivity is not only dependent on family lifestyle and the patient's environment since the limits of passive dispersion of vector species are too large than the relative geographic distance between localities. In FJ region, a heterogeneous environment in which, the coexistence of modern and traditional lifestyle is noticed. This transitory step between rural and city life created an unstable environment.

The characterization of the infecting species based on clinical symptoms is not crucial, since clinical spectrum of CL is broad, symptoms can vary and may be confused with other etiologic agents. On the other hand, the characterization of *Leishmania* species in clinical infections is important, as different species may require distinct treatment regimens, hence the necessity to identify *Leishmania* parasite with certainty.

ITS1-PCR-RFLP used in this study enables easy species identification in all PCR-positive cases. This molecular method is sufficiently sensitive to detect *Leishmania* directly in stained smears (**Schonian *et al*., 2003**). The appearance of CL due to *L. tropica* has become increasingly important due to ecological and demographic changes (**Ashford, 1999**). The increasing urbanization processes in the settlements that have poor housing conditions and sanitation and which are usually over-crowded may facilitate the expansion of the disease. We have also demonstrated that *P. sergenti* is the most abundant sand fly species in FJ (57%). *Phlebotomus sergenti* is a main vector of *L. tropica* in Morocco, although in some specific areas (Saudi Arabia; Palestinian Territories) other Sand flies can play a role in *L. tropica* transmission (**Al Zahrani *et al*., 1988; Svobodova *et al*., 2006**). Researches should be continued to find out if the disease in this region is due to a single or a variety of *L. tropica* genotypes. The disease control requires local stakeholders and health authorities' interference.

During 6 months, twice a month, in an altitude ranging between 625 and 810 m, the study in FJ region shows high biodiversity of phlebotomine fauna, with six species among 22 identified in Morocco (**Maroli et *al*., 2009**). This result is consistent with the study in the High-Atlas Mountains of Morocco, that's (**Guernaoui *et al*., 2006**) found the sand fly fauna to be more diverse at altitudes between 800 and1199 m.

Species presence and diversity appeared to be affected by several factors. It seems unlikely that altitude and aspect have a simple and direct effect on sand fly diversity. Instead, they probably act via their relationship with climate (temperature, precipitation, humidity etc) and human behavior.

In order of abundance, *P. sergenti* comes the first with 57%. The study area is known to contain the highest genetic diversity of this species (**Yahia *et al*., 2004**). The second more abundant species was *P. longicuspis* representing 23% of the total capture. The relative abundance of these two species, all of which can act as vectors of parasites that cause human leishmaniasis, is cause for concern. *Phlebotomus sergenti* is not only highly susceptible to *L. tropica* infection (**Killick-Kendrick *et al*., 1995**) but also a proven vector of this parasite (**Pratlong et *al*., 1991**). Anthroponotic cutaneous leishmaniasis is restricted to the Old World and largely caused by *L. tropica* transmitted by

P. sergenti (**Desjeux, 2001**). *P. sergenti* is believed to have a marked 'preference' for semi-arid habitats (**Rioux et al., 1984**). The taxon known as *P. sergenti* shows considerable genetic variation over its large area of distribution (**Depaquit *et al.*, 2002**; **Yahia *et al.*, 2004**; **Moin-Vaziri *et al.*, 2007**). The possibility that it represents several distinct lineages, possibly including cryptic species, that differ in their vectorial capacities (**Depaquit *et al.*, 2002**) and/or mechanisms of transmission for *leishmania* parasites (**Yahia et *al.*, 2004**) needs to be considered. In Morocco, three mitochondrial- DNA haplotypes of *P. sergenti* were identified among the sandflies collected in Azilal, Essaouira and Taza, which are *L. tropica* foci (**Yahia *et al.*, 2004**). These lineages could be markers for (regionally distributed) cryptic species. In Spain, two *P. sergenti* lineages were identified a typically Spanish mitochondrial lineage and another one that is common in Morocco (**Baro´n *et al.*, 2008**).

The method used to collect sandflies can also affect the numbers, sex ratios and species of the flies caught, and thus the known distributions of particular species (**Lucientes-Curdi et *al.*, 1991**).

Sticky papers are one of the standard surveillance techniques that have been used to collect adult phlebotomin Sand flies. Collectors are less exposed to the risk of *leishmania* infection. However, these traps often yield small or no catches (**Hogsette *et al., 2008*),** so FJ region could include a greater density than that observed in our study. **Aroli *et al.*, 2009,** used sticky traps, hand aspiration and CDC traps, and they choose to analyze sticky paper data to determine the seasonal trends in the sandfly density. Sand flies collection with sticky traps caught more males than females, this confirms data obtained elsewhere using the same type of traps (**Boussaa *et al.*, 2007**).

Sand fly seasonal trends in density expressed as the number of sand flies collected has showed two peaks, one in June and another late in August. This bimodal profile was nearly the same as those observed in other previous studies in different parts of the world; in Syria for *P. sergenti* and *P. papatasi* (**Maroli *et al.*, 2009**)**,** and in Morocco for *P. papatasi*, *S. minuta* and *S. fallax* (**Boussaa *et al.*, 2005**)**.** The summer of 2010 in Moroccan was very hot, maximal temperature recorded in July reached 50°C and above; this explains the fall of population density of sand flies. When extreme temperatures were registered during the survey days or the month prior to sampling, vector activity and thus vector captures were limited (**Galvez et *al.*, 2010**). The temperature is one of the main factors preventing the spread of leishmaniasis to Northern Europ (**Kuhn, 1999**).

Sand flies occurrence according to biotope shows that the animal shelter was the favorite biotope at least for *P. sergenti* and *P. longicuspis*. The limited number of catch does not allow to clarify the preferred biotope of the other species. *P. longicuspis* is also an anthropophilic species that prefers biotopes characterized by dense vegetation, close to human dwellings (**Guernaoui *et al.*, 2009**). In addition to the endophilic character, the presence of attractive vegetation, such as cactus (**Junnila *et al.*, 2011**), and the rural lifestyle enhance the presence of the resting and the breeding sites of the vector sand flies around the human dwelling. Further investigations on isolating *leishmania* strains from vectors are needed to clarify the CL transmission risk, and to confirm the role of *P. sergenti* and probably the involving of *P. longicuspis* in the transmission of *Leishmania* in this CL focus.

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