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First report of natural infection in hedgehogs with *Leishmania major*, a possible reservoir of zoonotic cutaneous leishmaniasis in Algeria

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ABSTRACT

We report here the first known cases of natural infection of hedgehogs with *Leishmania major*. Cutaneous leishmaniasis is an important public health problem in the area of M'sila, a semi-arid province in Algeria's northern Sahara, where two species of hedgehog live, *Atelerix algirus* and *Paraechinus aethiopicus*. The aim of this research was to survey *Leishmania* infection in these hedgehogs and evaluate whether they were reservoir hosts of *Leishmania* in an endemic zoonotic focus of leishmaniasis. Serological and molecular methods were used to determine the presence of *Leishmania* in 24 hedgehogs caught directly by hand and identified at species level as 19 *A. algirus* and 5 *P. aethiopicus*. Specific anti-*Leishmania* antibodies were detected in 29.2% of individuals by Western blot and in 26.3% by ELISA. The real-time PCR performed in spleen, ear and blood samples detected *Leishmania* spp. DNA in 12.5% of the individuals, one *A. algirus* and two *P. aethiopicus*. Three skin and two spleen samples of these animals were found to be parasitized and were identified by molecular test as *L. major*. Considering our results, it is suggested that hedgehogs have a potential epidemiological role as reservoir hosts of *L. major*.

1. Introduction

Leishmaniasis is one of the parasitic diseases frequent in the Mediterranean Basin. In Algeria they are the main parasitic disease, accounting for 35% of diseases with compulsory notification. This is one of the countries most affected by cutaneous leishmaniasis (CL), with 54,145 cases reported between 2000 and 2005 (Achour Barchiche and Madiou, 2009). CL in Algeria, known to be endemic since the beginning of the century (Lemaire et al., 1913), are caused by *Leishmania infantum*, *Leishmania tropica*, *Leishmania major* and a *Leishmania* close to *Leishmania killicki* (Harrat et al., 1996; Benikhlef et al., 2004; Mihoubi et al., 2008; Harrat et al., 2009). In the steppe areas of the center and south of the country (northern Sahara), zoonotic cutaneous leishmaniasis (ZCL), caused by the *L. major* zymodeme MON-25, is endemo-epidemic (Harrat et al., 1996). It is transmitted by *Phlebotomus papatasi* (Izri et al., 1992), with the rodents *Psammomys obesus* and *Meriones shawi* the main reservoir hosts identified (Belazzoug, 1983, 1986). This same author found *L. major* in ear margin lesions of *P. obesus* in 1983. Sporadic CL caused by *L. infantum* occurs on the northern coastline of the country (Benikhlef et al., 2004), with *Ph. perfiliewi* as vector (Izri and Belazzoug, 1993) and dogs involved as reservoir host (Benikhlef et al., 2004). A few cases caused by *L. tropica* have been related in the northern region of Constantine (Mihoubi et al., 2008). The study of an epidemic outbreak in an emergent focus in Ghardaïa (south Algeria) indicated the presence of a new dermotropic *Leishmania* close to *L. killicki*, which coexists sympatrically with *L. major* MON-25 (Harrat et al., 2009; Boubidi et al., 2011). It has been suggested that it is a zoonotic disease, with *Ph. sergenti* sand flies acting as vectors and the rodent *Ctenodactylus gundi* as reservoirs (Boubidi et al., 2011). Studies in Tunisia detected *L. killicki* in the rodent *C. gundi* by direct examination and PCR analysis, which was the first evidence of the potential role of this wild rodent as natural host of this parasite and of the involvement of *L. killicki* in CL cases in this area (Jaouadi et al., 2011).

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Two mammal species, the North African hedgehog, *Atelerix algirus*, and the desert hedgehog, *Paraechinus aethiopicus*, members of the Erinaceidae family, Erinaceomorpha order, are widely distributed and have a seemingly stable population in North Africa, particularly in the ZCL endemic area of the M'sila region (Khaldi et al., 2012a,b). M'sila is an arid province in the northern Sahara desert that is an important focus of *L. major*, with peaks of annual incidence higher than 500 per 100,000 inhabitants (REM, 2003, 2009). Here, the worst outbreak of leishmaniasis in Algeria was reported since the historic outbreak in the Biskra region (Belazzoug, 1982). To our knowledge, there are no serological and molecular studies identifying these mammals as reservoir hosts of *Leishmania*. However, in the 1970s, the possible *Leishmania* reservoir role of hedgehogs was posed in research from the ex-USSR that had cited as a natural host of *Leishmania* the long-eared hedgehog (*Hemiechinus albulus major*), along with other small mammals (Petrisceva, 1971; Faizulin et al., 1975). Recently, *Leishmania* DNA has been detected in hair of one hedgehog (*Erinaceus europaeus*) studied in Spain (Muñoz-Madrid et al., 2013), but no other reports of hedgehogs as natural reservoir hosts of *Leishmania* are cited.

Due to the unknown role of these mammals as possible reservoirs of *Leishmania*, the aim of this research was to determine the presence of *Leishmania* in two species of hedgehog (*A. algirus* and *P. aethiopicus*) from the M'sila region (Algeria) and to study whether they were a wild reservoir in an endemic zoonotic focus of leishmaniasis. *Leishmania* infection was determined by serological and molecular methods and the research was completed with the identification of the *Leishmania* species by molecular tests.

2. Material and methods

2.1. Area and time of study

This study was conducted during the period April–September 2009 at 16 sampling sites (Fig. 1) in the area around Hodna (M'sila) (35°40′00″N, 4°31′00″E). The watershed Hodna has an area of 26,000 km^2 and is the fifth largest basin in Algeria. It is an open steppe area surrounded by mountains that contain various habitats (Hasbaia et al., 2012). The mean annual rainfall varied according to altitude between 370 mm in the higher mountains and 150 mm lower down and to the south of the salt lake of Hodna (Chott of Hodna), characterized by an upper arid climate with a mean annual temperature of 18 °C and cool winters.

2.2. Sampling of hedgehogs

The wild individuals were caught directly by hand with the aid of spotlights during walks at night, achievable due to these species' nocturnal activity, or retrieved as fresh road-kills. Later, they were weighed, measured and sexed; each individual was surveyed for the presence of skin lesions. The identification of species level was done in the Laboratoire d'Écologie at the University of M'sila, using morphological criteria keys (Aulagnier and Thévenot, 1986; Reeve, 1994; Aulagnier et al., 2008). The study was authorized by the local ethical committee and national legislation (le journal officiel n° 47 du 19 juillet 2006).

2.3. Studied samples

After the hedgehogs were killed painlessly, spleen and non-injured ear skin pieces and blood samples were collected at the Laboratoire d'Ecologie, University of M'sila. Tissue samples from each animal were placed in Eppendorf tubes and frozen at −40 °C; blood samples were kept on air-dried filter paper and preserved at room temperature; and sera samples were frozen at −40 °C. The serological and molecular studies were conducted at the Laboratory of Parasitology, Universitat de Barcelona.

2.4. Serological study

Anti-*Leishmania* antibodies were detected by ELISA and Western blot using whole *L. infantum* antigens (MHOM/FR/78/LEM75 zymodeme MON-1). Western blot (WB) was performed as described by Riera et al. (1999), with some modifications. It was done on 0.1% SDS–13% polyacrylamide gel on a Mini-gel Bio Rad System. Sera diluted at 1/50 were assayed and a protein A peroxidase conjugate (1/1000 dilution; Pierce) was used. We considered a serum positive when immunoreactivity against the 14 and/or 16 kDa *Leishmania* antigen fraction was observed. ELISA was performed as described by Riera et al. (1999). Sera were diluted at 1/50 and a protein A peroxidase conjugate (dilution, 1/30,000; Pierce) was used. The reaction results were measured in units (U) and the cut-off was established at 20 U.

2.5. Molecular study

2.5.1. DNA extraction

DNA was extracted from 25 to 50 mg of tissue (ear and spleen pieces) and two 6 mm-diameter filter papers by the isolation of nucleic acids with the mammalian tissue and the blood protocol of High Pure PCR Template Preparation Kit (Roche Applied Science, Mannheim, Germany). It was eluted in 200 μL of elution buffer, following the manufacturer's instructions.

2.5.2. Real-time PCR

The detection and quantification of *Leishmania* spp. DNA was analyzed by amplification of kinetoplast minicircle DNA sequence by real-time PCR (qPCR) (Martín-Ezquerra et al., 2009). Each amplification was performed in triplicate, in a 20 μL reaction mixture containing 1X iTaq supermix with Rox (Bio-Rad, Hercules, CA, USA), 15 pmol of direct primer (CTTTTCTGGTCCTCCGGGTAGG), 15 pmol of reverse primer (CCACCCGGCCCTATTTTACACCAA), 50 pmol of the labeled TaqMan probe (FAM-TTTTCGCAGAACGCCCCTACCCGC-TAMRA) and 5 μL of sample DNA. The ABI Prism 7700 system (Applied Biosystems) at 94 °C and 55 °C cycling over 40 cycles was used. A non-template control was used in each run as the qPCR negative control. A 10-fold dilution series of DNA from promastigotes (MHOM/ES/04/BCN-61, *L. infantum* zymodeme MON-1) was used as calibrators (serial dilution from 10^5 parasites/ml to 10^{-3} parasites/ml), allowing plotting of a standard curve. The qPCR was considered positive for *Leishmania* when the threshold cycle (tC) was <40 in all replicates.

2.5.3. PCR-RFLP and PCR-FFL analysis of the ribosomal ITS-1 gene

For the identification of *Leishmania* species, the ribosomal internal transcribed spacer 1 (ITS-1) region was amplified with the primers LITSR (5′-CTGGATCATTTTCCGATG-3′) and L5.8S (5′-TGATACCACTTATCGCACTT-3′), previously described by Schönian et al. (2003), which were fluorescently labeled for the fluorescent fragment length (FFL) analysis with blue and green fluorochromes (6-FAM and VIC), respectively (Applied Biosystems, UK) (Tomás-Pérez et al., 2013). PCR products were digested with the restriction enzyme *BsuR*I (*Hae*III) (Fermentas, Life Sciences, Germany) without prior purification, in accordance with the manufacturer's recommendations. For the restriction fragment length polymorphism analysis (PCR-RFLP), PCR products were separated by electrophoresis in 3% Wide Range Agarose (Sigma, USA) at 150 V in SGTB 1X buffer (GRISP LDA; Portugal). A solution of SYBR safe DNA gel stain (Invitrogen, UK) was used to view the separated DNA fragments under ultraviolet light. In PCR-FFL analysis, digestion products were pooled with the internal lane size standard GeneScan™-500 LIZ®

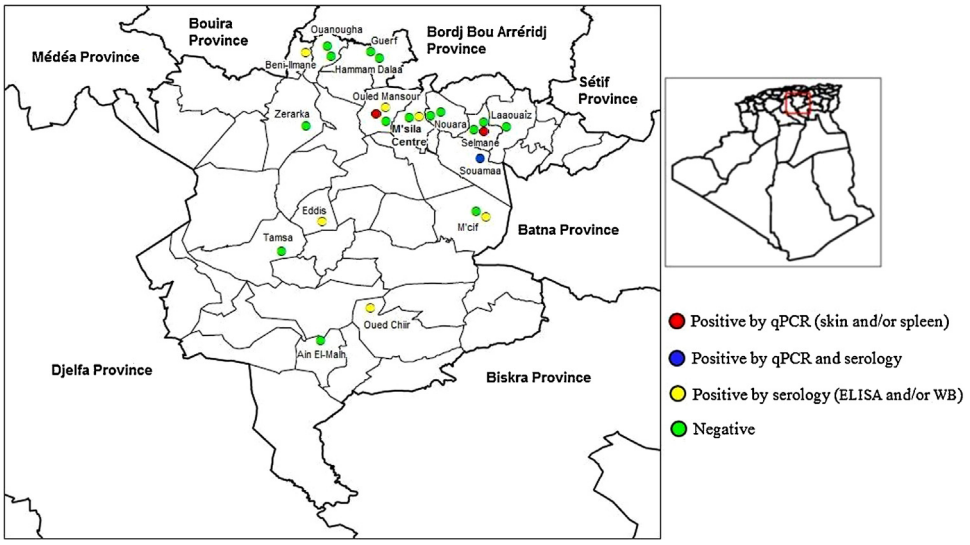

Fig. 1. Geographical distribution of the capture sites of the 24 hedgehogs studied in the M'sila region (Algeria) with the molecular and serological results obtained.

(Applied Biosystems, USA) for the analysis on the DNA sequencer ABI Prism 3730 (Applied Biosystems). Fragment sizes were determined using the fragment analysis program, Peak Scanner Software v1.0 (Applied Biosystems).

2.5.4. Nested PCR

For the identification of the *Leishmania* species, a nested PCR was performed with the external primers CSB2XF (C/GA/GTA/GCAGAAAC/TCCCGTTCA) and CSB1XR (ATTTTTCG/CGA/TTTT/CGCAGAACG), and the internal primers 13Z (ACTGGGGGTTGGTGTAAAATAG) and LiR (TCGCAGAACGCCCCT), previously designed by Noyes et al. (1998) around conserved kDNA regions. The PCR mix contained 3 μL of 10X Roche Buffer, 5 mM dNTP, 0.3 U of *Taq* Roche and 0.3 μM of each primer in a final volume of 30 μL. The mixture was incubated in the thermocycler under the following conditions: 94 °C for 120 s, then 40 cycles of 94 °C for 30 s, 54 °C for 60 s and 72 °C for 90 s. The product was diluted in water 9:1 and used as template for the second PCR under the same conditions. The PCR products were viewed under ultraviolet light after separation by electrophoresis in 1.5% agarose gel (TAE 0.5%).

3. Results

3.1. Captured individuals

During the study, 24 wild adult hedgehogs were captured in 16 sampling sites between 412 and 974 meters of altitude. All were found near human settlements at distances ranging from 50 to 150 m in gardens, palm groves, olive groves and apricot orchards. They were identified as two different species: 19 individuals of *A. algirus* (7 males, 12 females) and 5 of *P. aethiopicus* (1 male, 4 females) (Table 1). No skin lesions were observed except in one individual (H40) who had one lesion on the snout.

3.2. Serological study

Immunoreactivity from the *Leishmania* antigen was detected by WB in 6 of the 19 *A. algirus* tested, which came from the localities of M'cif, Oued Chiir, Eddis, Ouled Mansour, M'sila center and Beni-Ilmane (31.6%, 95% CI: 15.16–54.20%), and in 1 of the 5 *P. aethiopicus* from Souamaa (20%, 95% CI: 2.03–64.04%). Specific antibodies were detected by ELISA in sera from 5 *A. algirus* with 26.3% seropositivity (95% CI: 11.45–49.14%). Overall, antibodies were detected in 7 of 24 individuals, with a seropositivity of 29.2% (95% CI: 14.71–49.37%). All ELISA-positive results were also WB-positive (Tables 2 and 3).

3.3. Detection of Leishmania spp. by qPCR

Leishmania spp. DNA was detected in one male of *A. algirus* (5.3%, 95% CI: 0.0–26.48%) from Selmane (H44); and in two *P. aethiopicus* (40%, 95% CI: 11.60–77.09%), one female and one male from Souamaa (H40) and Ouled Mansour (H49) respectively. These results account for 3 of the 24 individuals studied (12.5%, 95% CI: 3.51–31.84%), with 5 positive samples out of the 67 studied (3/24 skin samples, 2/24 spleen samples and 0/19 blood samples) (Tables 2 and 3). PCR threshold cycles among positive samples ranged from 23 to 32, with the highest parasite load found in skin (Table 2). One qPCR positive *P. aethiopicus* was also positive by WB analysis but not by ELISA analysis. The other two qPCR positives individuals presented negative results in WB and ELISA analysis (Table 2).

3.4. Identification of Leishmania species by PCR-RFLP, PCR-FFL and nested PCR

The five positive qPCR samples were analyzed by ITS-1 PCR-RFLP and PCR-FFL and only the ear skin sample from *A. algirus* (H44) was identified as infected by *L. major* by both methods, with fragment lengths of 135 and 204 bp by PCR-FFL, which fall within the lengths previously described for *L. major* species (Tomás-Pérez et al., 2013). To increase the sensitivity of the identification, a kDNA nested PCR was performed being able to identify the five *Leishmania* DNA-positive samples as *L. major* (Table 2).

4. Discussion

In the area of M'sila studied there are two species of hedgehog: *A. algirus* and *P. aethiopicus* (Khaldi et al., 2012a,b) which are widely distributed in the same geographical territory as the rodents *P. obesus* and *M. shawi*, considered the main reservoir hosts of *L. major* in this area (Belazzoug, 1983, 1986). It is not currently known whether hedgehogs might be infected by the parasite and their possible epidemiological role as reservoir hosts of *Leishmania*.

Serology has been used in several epidemiological studies for detection of *Leishmania* infection in reservoir hosts (Abranches

Table 1
Hedgehog species, date of capture, location of capture site, altitude of capture site, sex, weight and body length of the 24 individuals studied from the area of M'sila (Algeria).

Sample	Species	Date of capture	Procedence	Altitude (m)	Sex	Weight (g)	Body length (cm)
H20	*A. algirus*	26/04/2009	M'cif	412	F	535	23.5
H24	*A. algirus*	29/04/2009	Nouara	472	M	701.1	26
H25	*A. algirus*	03/05/2009	Oued Chiir	535	F	560.2	21
H26	*A. algirus*	04/05/2009	M'cif	412	F	425	18
H27	*A. algirus*	05/05/2009	Hammam Dalaa	767	F	797.6	27
H28	*A. algirus*	05/05/2009	Selmane	528	F	649.3	25.5
H29	*P. aethiopicus*	09/05/2009	Zerarka	477	F	364.6	18
H30	*A. algirus*	10/05/2009	Nouara	472	F	539.2	22
H32	*A. algirus*	13/05/2009	Ouanougha	944	M	858.4	30
H33	*A. algirus*	17/05/2009	Ouanougha	944	F	590.5	25
H34	*A. algirus*	19/05/2009	Eddis	536	M	692.6	25
H35	*A. algirus*	31/05/2009	Ouled Mansour	486	F	712	22
H36	*A. algirus*	03/06/2009	Guerf	966	M	562.2	25
H38	*A. algirus*	13/06/2009	M'sila centre	479	M	414	20
H39	*P. aethiopicus*	13/06/2009	Ouled Mansour	486	F	360	19
H40	*P. aethiopicus*	14/06/2009	Souamaa	476	F	215.2	14.5
H41	*A. algirus*	15/06/2009	Laaouaîz	552	F	694.1	23
H42	*A. algirus*	16/06/2009	M'sila centre	479	M	661	24
H43	*P. aethiopicus*	16/06/2009	Tamsa	707	F	325.7	10
H44	*A. algirus*	17/06/2009	Selmane	528	M	424	19
H45	*A. algirus*	17/06/2009	Selmane	528	F	662.5	22
H46	*A. algirus*	28/06/2009	Beni-Ilmane	860	F	615.6	22.5
H47	*A. algirus*	08/07/2009	Ain El-Malh	974	F	922.5	26.5
H49	*P. aethiopicus*	30/09/2009	Ouled Mansour	486	M	352.1	21

A. algirus: *Atelerix algirus*; *P. aethiopicus*: *Paraechinus aethiopicus*; F: female; M: male.

Table 2
Results obtained in 9 out of the 24 hedgehogs studied that were positive in any of the techniques used: ELISA, WB or qPCR, and molecular *Leishmania* species identification.

Positive individuals	Serology		*Leishmania* DNA qPCR (tC)[a]			Species identification
	ELISA (U)	WB (kDa)	Skin	Spleen	Blood	
H25	22	16	40	40	40	–
H26	13	14, 16, 18, 24, 31	40	40	ns	–
H34	20	14, 16, 18, 20	40	40	40	–
H35	20	16	40	40	40	–
H38	28	14, 16, 18, 24, 31, 40	40	40	40	–
H40	13	16	32	40	40	*L. major*
H44	10	nb	23	32	ns	*L. major*
H46	21	16, 18, 20	40	40	40	–
H49	9	nb	32	32	ns	*L. major*

ELISA cut off: 20 U; WB positive: 14 and/or 16 kDa; nb: no band detected; tC: threshold cycle; qPCR positive: tC values <40; ns: no sample.
[a] Mean values of the three replicates of each sample.

et al., 1984; Fisa et al., 1999; Di Bella et al., 2003; Sastre et al., 2008; Millán et al., 2011), but there have been no serological studies to detect specific antibodies in hedgehogs. Data obtained in this study show that specific antibodies were detected by ELISA and WB in 20.83% and 29.17% of hedgehogs tested, respectively. The seroprevalence obtained in our study with Algerian hedgehogs corroborates serological studies made in central Tunisia of the main *L. major* reservoirs, *P. obesus* and *M. shawi*, which reported 20% and 33% seropositivity, respectively (Ghawar et al., 2011). The detection of specific antibodies in individuals widely distributed in the endemic region of M'sila shows a contact between the parasite and the hedgehog population that could indicate present or past infection. For this reason, confirmation of active infection by direct techniques or molecular methods is required to detect the parasite in the possible reservoir hosts and to determine the species involved.

Molecular biology techniques were used in studies to detect new reservoirs of *Leishmania* because of their high specificity and sensitivity (Chitimia et al., 2011; Millán et al., 2011). In our research, parasites were studied by qPCR in three different samples: skin, spleen and blood. Of the 24 hedgehogs tested, *Leishmania* DNA was detected in 2 *P. aethiopicus* (40%) and in 1 *A. algirus* (5.3%). The three infected hedgehogs were from the north of M'sila region, in Ouled Mansour, Souamaa and Selmane, between 476 and 528 m of altitude, where most of the individuals were captured. In all these positive animals, *Leishmania* DNA was detected in non-injured skin;

Table 3
Results of serology and qPCR studies of skin and spleen samples from the two hedgehog species studied: *A. algirus* and *P. aethiopicus*.

Species	Serology		qPCR		Contact evidence[a]
	ELISA	Western blot	Skin sample	Spleen sample	
A. algirus 95% CI	5/19 (26.3%)11.45–49.14	6/19 (31.6%)15.16–54.20	1/19 (5.3%) 0.0–26.48	1/19 (5.3%) 0.0–26.48	7/19 (36.8%)19.05–59.06
*P. aethiopicus*95% CI	0/5 (0.0%) 0.0–48.91	1/5 (20.0%) 2.03–64.04	2/5 (40.0%)11.60–77.09	1/5 (20.0%)2.03–64.04	2/5 (40.0%) 11.60–77.09
Total 95% CI	5/24 (20.8%) 8.80–40.91	7/24 (29.2%)14.71–49.37	3/24 (12.5%)3.51–31.84	2/24 (8.3%)1.16–27.00	9/24 (37.5%)21.09–57.36

Results expressed as number of positives/total studied; CI: confidence interval.
[a] Positive either for serology or qPCR.

and in two of them, in the spleen sample as well. Quantitative results indicated a higher parasite load in the ear skin than in the spleen samples. All blood samples analyzed were qPCR-negative, which is not unexpected as only one blood sample of a positive animal was analyzed, one *P. aethiopicus* with *Leishmania* DNA detected only in the skin. The detection of *Leishmania* DNA in the skin and spleen of the hedgehogs studied and the significant parasite load observed suggest genuine proliferation of *Leishmania*, which could indicate a reservoir capacity for these animals.

Prior studies of *P. obesus*, the main reservoir host of *L. major*, by microscopy and/or PCR methods on ear skin samples showed high prevalence, with 51.1% in spring and winter seasons in Israel (Wasserberg et al., 2002), similar to the 65% infestation for *P. obesus* observed in the north of Africa also by PCR analysis of ear skin samples (Boudrissa et al., 2011). In our case, the samples were mainly obtained from April to June, with only one from September that was *Leishmania*-positive. This suggests that greater prevalence of infection might have been found if the study had been conducted in late summer months or autumn, which are more favorable for *Leishmania* infection detection. It cannot be ruled out that hedgehogs could be suffering a transient infection episode at the sand flies transmission period. In addition, the low numbers of desert hedgehogs (*P. aethiopicus*) analyzed, accounting for the positivity observed, might also misrepresent the real positivity.

Given the different species of *Leishmania* present in Algeria, molecular techniques were used to identify the species involved in the infected hedgehogs studied. The ITS PCR-RFLP and PCR-FFL methods, often used for species identification, allowed the identification of *L. major* in the skin sample of the *A. algirus* infected, which is related to the important parasite number observed by qPCR. The other *Leishmania* DNA-positive samples, with a lower parasite load observed, could not be identified by the cited techniques. The more sensitive nested PCR performed with the five *Leishmania* DNA-positive samples identified all of them as *L. major*. The results obtained with these techniques confirmed the infection of the hedgehogs with *L. major*, which was reported in prior studies in Algeria as the principal *Leishmania* species responsible for the ZCL recorded in this semi-arid area (Harrat et al., 1996).

Twenty-three of the 24 hedgehogs included in this study showed no lesions on their exposed skin (ears, nose or eyelids) and only one case (H40) had a lesion on the snout, which unfortunately could not be studied. This hedgehog was PCR-positive in non-injured skin and seropositive by WB analysis. Prior studies of natural hosts' infection with *L. major* gave as a valid indicator of infection the presence of ear lesions, but they concluded that it is not a definitive sign because 35% of the proven infected specimens had no skin lesions (Fichet-Calvet et al., 2003).

Control programs of the main reservoir host, *P. obesus*, in the endemic *L. major* area of M'sila, recorded a decrease in CL cases from 1391 in 2003 to 965 in 2004 (31% reduction), achieved by removing the Chenopodiaceae plants that are the exclusive feed of these gerbils in 3600 hectares (Cherif et al., 2012). In Tunisia, Derbali et al. (2013) showed that insecticide-treated rodent baits could be used to reduce the population of *Ph. papatasi* in ZCL endemic areas. The decrease in a main reservoir host in the area usually promotes the increase of other reservoir hosts, like *M. shawi* (Ben Salah et al., 2007; Ghawar et al., 2011). It also could have favored the adaptation of *L. major* to new reservoirs, like hedgehogs, which are not affected by the destruction of this kind of vegetation, because they feed mainly on arthropods and a few mollusks, with Formicidae and Coleoptera as their most appreciated prey (Derdoukh et al., 2012). Hedgehogs are abundant in rural areas between stones, rocks and cactus, in our case they were captured close to human settlements in gardens, palm groves, olive groves, and apricot orchards. Our results indicate the importance of the identification of the reservoir hosts of *L. major* in the area as key to establishing a more efficient control strategy.

In conclusion, the number of seropositive hedgehogs, together with the detection of *L. major* in the skin and spleen of the hedgehog species *A. algirus* and *P. aethiopicus* in the *L. major* endemic area studied, suggest for the first time the involvement of these hedgehog species in the life cycle of this parasite. Our results indicated that both hedgehog species could be reservoir hosts of leishmaniasis in the area, playing a role in the epidemiology of ZCL and interacting with the wild cycle maintained basically by the rodents *P. obesus* and *M. shawi*. To confirm the role of hedgehogs as reservoir hosts of the parasite is necessary to investigate the ability of sand flies to get infected through feeding on hedgehogs. Furthermore, it would be interesting to increase the number of hedgehogs in future studies. The serology and molecular test used in this study are good methods to continue, in future research, the search for reservoirs and for species identification.

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