

An outbreak of cutaneous leishmaniasis in Modena province (Northern Italy): report of 35 cases

A.M. CESINARO¹, S. NOSSEIR¹, E. MATAKA¹, M.C. MENGOLI¹, C. CAVATORTA¹, W. GENNARI²

¹ Department of Anatomic Pathology, Azienda Ospedaliero-Universitaria Policlinico, Modena, Italy; ² Department of Microbiology and Virology Unit, Azienda Ospedaliero-Universitaria Policlinico, Modena, Italy

Key words

Leishmania • Skin • Epidemiology • Histopathology • PCR

Summary

Canine Leishmaniasis is a disease endemic in many parts of Europe, carried by insects of phlebotomous species. Humans are occasional hosts of the parasites. Cases of human leishmaniasis have been registered in Italy, particularly in the southern and coastal regions.

In the period 1997-2016, we collected a series of 35 patients affected by cutaneous leishmaniasis, uncovered by skin biopsy and histological examination, 21 of them found in last 3 years. The patients, 28 males and 7 female, aged between 19 and 91, resided in a restricted area of Northern Italy, and none, but two, had travelled abroad. Lesions presented clinically mostly as single nodule or plaque, often ulcerated, and involved predominantly head-neck and upper extremities. Histology showed a diffuse, granulomatous inflammation including numerous plasma cells. Variable

numbers of amastigotes were visible, usually in the superficial part of the dermis, in all cases but two. In these two cases, highly suspicious by clinico-pathologic features, PCR analysis allowed to achieve the correct diagnosis. Our attention was then focused on the geographical residence of the patients, that turned out to be mostly in the piedmont area, whereas only one lived in the alluvial area corresponding to Padana plain.

These data underline the diffusion of phlebotomus in northern areas of Italy, and particularly on the hills, characterized by a type of soil more favorable to vector survival; also, they indicate the adaptation of leishmania to hosts other than dogs, such as foxes and small rodents. Histology alone resulted sufficient to make diagnosis in most cases, but PCR analysis is recommended in those cases showing a suspicious background, in absence of amastigotes.

Introduction

Leishmaniasis is a disease caused by an intracellular parasite of the genus *Leishmania*, transmitted by different species of phlebotomine sandflies¹. In Europe, leishmaniasis is endemic in the Mediterranean basin, it is caused mainly by *Leishmania Infantum*², and dogs represent the main reservoirs of parasites³. In recent years, an increased number of imported cases has been registered, due to immigration, tourism or military operations^{4,5}. Cutaneous involvement is the most common clinical manifestation of leishmaniasis⁶. A positive trend in reporting the disease has been observed, because of better awareness and consequent diagnosis, although probably many cases are still misdiagnosed or underdiagnosed⁴.

In Italy, canine leishmaniasis is endemic in central and southern regions, mainly in the Tyrrhenian coasts and the islands, but a spreading toward the northern

regions has been registered⁷, and a focus of infection in the hills has been reported in 2011 in Emilia Romagna region⁸.

In the present study, we describe 35 cases of cutaneous leishmaniasis, histologically diagnosed in patients resident in the province of Modena, Emilia Romagna region, Northern Italy, most of them living in the hills, or so-called piedmont area. The majority of cases were discovered in the last three years, suggesting an outbreak of the disease in this area.

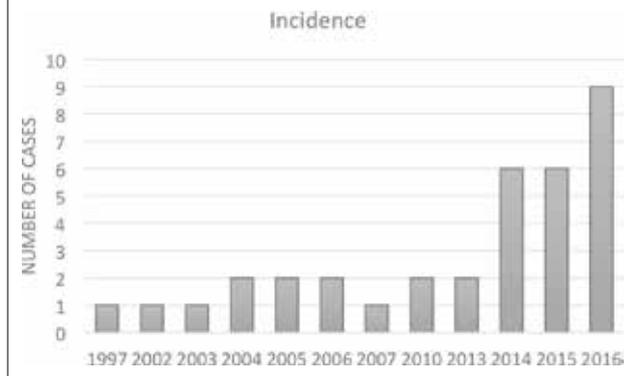
Materials and methods

Thirty-five cases of cutaneous leishmaniasis were consecutively diagnosed at the department of Anatomic Pathology of our tertiary-care hospital, in the period 1997-2016, 21 of them (60%) in the years 2014-2016 (Fig. 1). Patients were 28 males and 7

Correspondence

Cesinaro Anna Maria, Department of Anatomic Pathology, Azienda Ospedaliero-Universitaria Policlinico, via del Pozzo 71, 41124 Modena, Italy - E-mail: cesinaro.annamaria@policlinico.mo.it

Fig. 1. Incidence of cutaneous leishmaniasis cases in the period 1997-2016.



females, all Italian but one young man of Moroccan origin; the age ranged between 19 and 91 years. The most involved anatomical sites were head/neck and upper arm. Clinically, the lesions presented mostly as solitary nodule, or plaque, sometimes ulcerated. Complete clinical data are listed in Table I. Skin biopsies were sent for histological examination by clinicians with different diagnoses, including “epithelioma” (Fig. 2A), chronic ulcer, or “tumor”. In two cases, only, leishmaniasis was among the clinical suspects. Biopsies were routinely processed and hematoxylin-eosin (H&E) staining was performed, together with special stains, including PAS, Grocott methenamine and Giemsa.

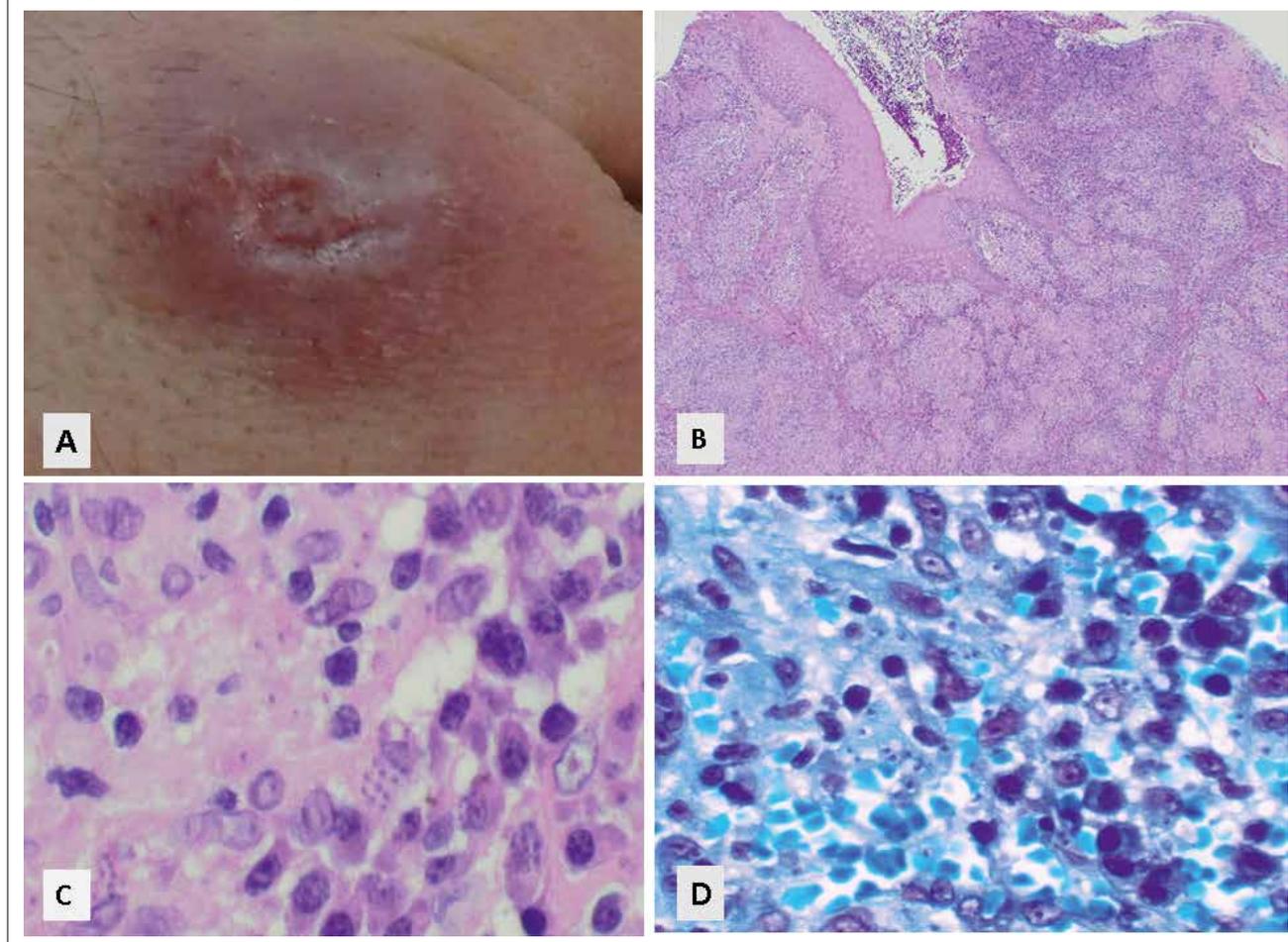
Tab. I. Clinical data of 35 patients with cutaneous Leishmaniasis.

Patient	Age (years)/gender	Anatomic location	Clinical features	Type of biopsy
1	56/F	Left leg	NA	Incisional
2	24/M	Left arm	Centrally ulcerated nodule	Excisional
3	29/M	Nasolabial fold	NA	Incisional
4	38/M	Right hand	Centrally ulcerated nodule	Excisional
5	32/F	Forehead	NA	Incisional
6	29/M	Right elbow	Crusted erythematous papule	Incisional + radical excision
7	25/M	Lumbosacral region	NA	Incisional
8	19/F	Back	Itching papule	Incisional
9	38/M	Right arm	Ulcerated nodule	Incisional
10	72/M	NA	Exudative squamous patch	Incisional
11	50/F	Nose	Infiltrated erythematous plaque	Incisional
12	71/M	Neck (tracheostomy)	NA	Incisional
13	76/M	Forehead	Desquamative ulcerated lesion	Excisional
14	68/M	Neck	Erythematous-edematous crusted nodule, with previous suppuration	Incisional + radical excision
15	75/M	Forehead	Fixed erythematous-pomphoid lesion	Incisional
16	36/M	Right hand (back)	NA	Incisional
17	48/M	Scalp	Ulcerated lesion	Incisional
18	71/M	Left shoulder	Ulcerated lesion	Excisional
19	63/M	Right forearm	Ulcerated nodule	Incisional + radical excision
20	19/M	Left arm	Grouped reddish papules	Incisional + radical excision
21	62/M	Right arm	NA	Incisional
22	56/M	Nose	NA	Incisional
23	91/F	Nose	Erythematous-edematous plaque with central ulcer	Incisional
24	68/M	Left arm	Extensively inflamed nodule	Incisional + radical excision
25	55/M	Right leg	Non itching, crusted plaque	Incisional (*)
26	53/M	Nasal mucosa	NA	Incisional
27	72/M	Right arm	Non itching, reddish nodules	Incisional + radical excision
28	29/F	Forehead	NA	Incisional
29	61/M	Right hand	Ulcer	Excisional
30	71/M	Scalp	Nodule	Incisional + radical excision
31	27/F	Right leg	NA	Incisional + radical excision
32	65/M	Nose	Persistent edematous plaque	Excisional
33	42/M	Right leg	Erythematous nodule	Excisional (*)
34	79/M	Scalp	Ulcerated lesion	Incisional
35	87/M	Scalp	Hyperkeratotic plaque	Incisional

(*): cases diagnosed by PCR

NA: not available

Fig. 2. Clinical presentation of cutaneous leishmaniasis as an ulcerated lesion (A); diffuse dermal granulomatous inflammation (H&E) (B), and amastigotes inside macrophages (H&E) (C); amastigotes stained by Giemsa (D).



PCR analysis was performed on paraffin embedded tissue in two cases, characterized by a clinico-pathological background highly suspicious, but devoid of unequivocal amastigotes at H&E examination and after Giemsa staining. Briefly, DNA's from formalin-fixed, paraffin-embedded samples were purified by MasterPure™ Compete DNA and RNA Purification Kit (Epicentre, Madison, WI, USA) according to the manufacturer's protocol. For the first round PCR, 1.5 µg of each DNA was used. External and nested-PCR primers used were CSB2XF/CSB1XR and 13Z/LiR respectively, as previously described⁹.

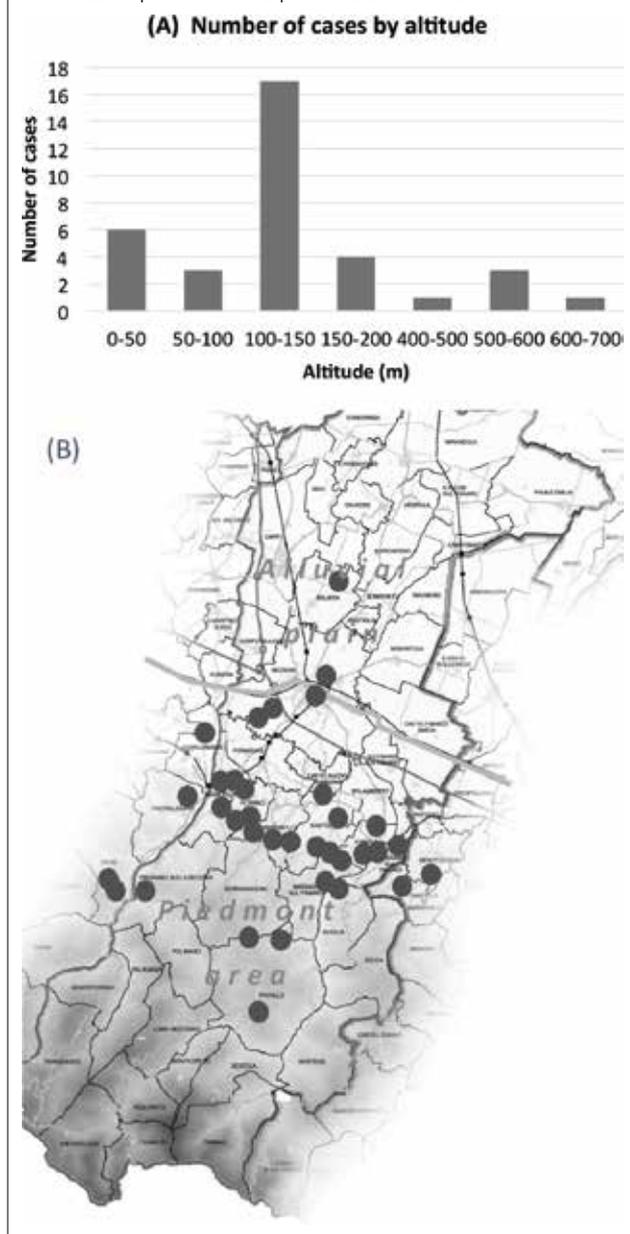
Results

With the exception of a patient of Moroccan and one of Italian origin, all others had never travelled abroad in endemic areas; moreover, all patients were immunocompetent. Histologic examination showed in all cases a diffuse pattern of inflammation, characterized by granulomas, lymphocytes and several plasma cells. Ulceration was observed in 16 cases. In all cases, but two, amasti-

gotes were visible inside the cytoplasm of macrophages, predominantly in the papillary dermis (Fig. 2B, C), and were highlighted by Giemsa stain (Fig. 4D). According to the modified Ridley's index¹⁰, 2 cases were classified as 0,5 as 1+, 23 as 2+ and 5 as 3+. PCR analysis confirmed the presence of *Leishmania* DNA in the two cases with parasite index 0. PAS and Grocott methenamine stains resulted negative for fungi. Interestingly, one patient had a previous biopsy with a diagnosis of lymphoproliferative disease, because of massive lymphoid infiltrate; the diagnosis was revised and amended after a second biopsy.

Focusing on the geographical distribution of cases throughout the territory of the province, we found out that patients lived in places at altitudes that ranged between 28 and 682 meters (Fig. 3A); interestingly, 18 of them resided on the hills, between 100 and 200 meters, corresponding to the southern part of the province, the so-called *piedmont area*; 5 patients lived in areas less than 50 meters in altitude, and only one resided in the northern part of the province, corresponding to the Padana plain, the so-called *alluvial area* (Fig. 3B).

Fig. 3. Distribution of cases by altitude (A) and geographical residence (B) of patients in the province of Modena.



Discussion

Leishmaniasis is a zoonosis endemic in many regions of old world, including the coastal zones of Italy. In Italy it constitutes an emerging problem also for humans, partly as a consequence of imported cases⁴, partly because of the diffusion of canine leishmaniasis, particularly in Northern Italy¹¹. We observed a dramatically increased number of cases of cutaneous leishmaniasis diagnosed in our department of Anatomic Pathology in last three years, accounting for 60% of all cases diagnosed in a 20 years period. Interestingly, all patients were Italian, but one Moroccan, and none but two had travelled abroad in endemic places. The infection was unsuspected in the

large majority of cases, the patients were immunocompetent and developed mostly a single cutaneous lesion. Our data are in contrast with those reported in a recent paper by Giavedoni et al.⁵, in which roughly the same number of cases of cutaneous leishmaniasis has been reported in a tertiary-care hospital in Spain in two consecutive decades, between 1992 and 2012. Moreover, 35% of cases were imported, and immunocompromised patients accounted for almost 30% of total number, being particularly numerous in the native group; finally, in 59% of cases multiple lesions were observed, particularly in immunocompromised patients. A previous study, performed in another tertiary-care hospital in Emilia Romagna region, Northern Italy, evaluated the number of visceral and cutaneous leishmaniasis in the period 1992-2013. Fifteen out of 134 patients analyzed were found to be affected by leishmaniasis; 8 had cutaneous leishmaniasis, three of them came from endemic area of Northern Africa and one had worked as missionary in Africa; only four patients did not have travelled abroad¹².

The cases of cutaneous leishmaniasis reported in the present study were diagnosed by histologic examination, and the large majority were unsuspected by the clinicians. All skin biopsies were characterized by a granulomatous infiltrate with lymphocytes and several plasma cells. The presence of amastigotes was documented in all cases but two. Although immunostaining with anti-CD1a has been proposed as a useful ancillary technique to enhance amastigotes¹³ in cases without unequivocal parasites, we performed PCR analysis that allowed to confirm the diagnosis of leishmaniasis in both cases with parasitic index 0. Of note, another case showed a massive lymphoid infiltrate, that was misinterpreted in a first biopsy as lymphoproliferative disease, a potential pitfall reported in the literature¹⁴.

Given the high number of cutaneous leishmaniasis registered among the native population of our province in last three years, we sought to explain the reason of this outbreak. The great majority of our patients resulted to live in the so-called piedmont area. In this area the soil is characterized by a lesser degree of humidity, since it is constituted by a higher percentage of sand and gravel and a lower percentage of clay, as compared to the so-called alluvial plane¹⁵. These geological properties of the soil make the piedmont area more suitable for the survival and proliferation of phlebotomus, whose diffusion seems to be inversely correlated to the level of ambient humidity¹⁶. In fact, a recent study carried out in Iran¹⁷ revealed that cases of cutaneous leishmaniasis were more frequent in regions with desert and dry climate. The prevalence of our cases of cutaneous leishmaniasis in the hilly region can be correlated, also, to a study conducted on canine population in Emilia Romagna region¹¹. This study demonstrated the diffusion of canine leishmaniasis in last decade, due to increasing number of vectors of phlebotomous genre, mainly restricted to the piedmont area. Analogously, another study performed in north-western Italy¹⁸ documented the presence of *Phlebotomus perniciosus*, the main

vector of leishmaniasis in dogs, particularly in hilly zones, between 100 and 300 meters of altitude. All these data could justify the outbreak registered also in our province. On the other hand, the sanitary surveillance and prevention of vectors' diffusion made in last years by Emilia Romagna region in kennels, has lowered the risk of infection in dogs ¹¹, therefore it is plausible that wild mammals, such as foxes, as documented in southern Italy ¹⁹, or even small rodents ²⁰, have become new hosts of parasites also in this area.

In conclusion, cutaneous leishmaniasis is an emerging problem also in immunocompetent and non-travelers patients, resident in certain areas of Northern Italy. The observation of a granulomatous infiltrate with numerous plasma cells in a skin biopsy should prompt the search of amastigotes, also with PCR techniques in cases without visible parasites.

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