Cutaneous Leishmaniasis Due to *Leishmania infantum*

Case Reports and Literature Review

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**Background:** *Leishmania infantum* recently has been identified as a possible agent of cutaneous leishmaniasis (CL). This species has been isolated from cutaneous lesions of patients from the Mediterranean Basin. However, little is known about the clinical, biological, or therapeutic features of this newly recognized CL.

**Observations:** Six patients aged 9 months to 85 years in southeastern France were found to have autochthonous leishmaniasis. Parasitological identification showed that the agent was *L. infantum*, zymodemes Montpellier-1 for 2 patients and Montpellier-24 for 1 patient. Five patients who underwent testing with a Western blot assay were found to have antibodies against 4 antigens with molecular masses of 18, 21, 23, and 31 kd. Five patients were successfully treated with local injections of N-methylglucamine, and 1 patient was successfully treated with topical paromomycin sulfate. No patient had visceral disease at diagnosis or after follow-up.

**Conclusions:** Recent data provide increasing evidence that *L. infantum* is an important agent of CL. In southwestern Europe, this species is the only agent that has long been identified from autochthonous CL. *Leishmania infantum* should be considered an agent of CL in areas in which visceral leishmaniasis is endemic. Western blot assay could be a useful test for the diagnosis, but precise parasitological identification is important to having a better knowledge of the disease. The relationships between CL and the visceral disease have to be explored.


Most cases of cutaneous leishmaniasis (CL) in the Mediterranean Basin are caused by 2 *Leishmania* species: *Leishmania major* and *Leishmania tropica*.1 *Leishmania infantum*, the causative agent of visceral leishmaniasis (VL), was considered to cause only systemic disease. In 1980, Rioux et al2 identified *L. infantum* in 2 patients with CL. The responsibility of *L. infantum* as an agent of CL recently has been confirmed in several countries of the Mediterranean Basin. Although the visceral form of *L. infantum* leishmaniasis is well known, the cutaneous form has been poorly described. Visceral leishmaniasis is endemic in southern France, but CL is rare and occurs sporadically.3 We studied the clinical, biological, and therapeutic features of 6 patients with CL contracted in this region.

**RESULTS**

**CLINICAL MANIFESTATIONS**

The main characteristics of the patients are summarized in Table 1. The lesions occurred in apparently healthy individuals. No patient took immunosuppressive drugs, but patients 1, 2, and 3 used dermocorticoid ointments before the diagnosis as treatment, which resulted in deterioration of the lesions in 2 patients. Patient 5 was pregnant at the time of diagnosis. The face was the most affected site. The most usual clinical presentation was a chronic, symptomless, erythematous or lupoid papule (4 patients) (Figure 1, Figure 2, and Figure 3). Patient 1 presented with an ulcerative lesion, and patient 4 had an infiltrative lesion (Figure 4). No regional lymphadenopathy or clinical signs of visceral involvement were detected.

**DIAGNOSIS, PARASITOLOGICAL IDENTIFICATION, AND HISTOLOGY**

Microscopic examination of smears showed the presence of amastigotes in lesions from 5 patients. The identified parasites belonged to the *L. infantum* complex, zymodemes Montpellier (MON)-1 (2 patients) and MON-24 (1 patient). Western blot analysis of serum samples from

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PATIENTS AND METHODS

Between January 1986 and December 1995, 6 patients with autochthonous CL were investigated at Nice University Hospital, Nice, France (throughout this article, autochthonous CL will refer to CL acquired locally). All patients lived in southern France (Alpes-Maritimes and Var counties) and had not traveled during the past 3 years. The case of patient 1 was reported previously. Clinical data were recorded. Aspirate or skin biopsy specimens were smeared, fixed, stained (May-Grünwald-Giemsa), examined for Leishmania, and cultured in Nicolle-Nové-MacNeal medium. After isolation, organisms were typed by isoenzyme electrophoresis at the Laboratoire d’Ecologie Médicale et Pathologie Parasitaire, Montpellier, France.

The biopsy specimens were fixed in Bouin liquid and processed in the usual manner. Sections were stained with hematoxylin-eosin-safran, Giemsa, periodic acid–Schiff reagent, Grocott-Gomori, and Zielh-Neelsen. Serum samples were tested for indirect fluorescent antibody assay with promastigote antigens of L. infantum and for Western blot assay. The Montenegro test (leishmanin test) used antigens provided by the Istituto Superiore di Sanità, Rome, Italy. A positive test result was defined by the presence of palpable induration of 5 mm or greater at 48 hours. There were no attempts to locate parasites in the viscera or bone marrow.

5 patients showed the simultaneous presence of antibodies against 4 antigens with molecular masses of 18, 21, 23, and 31 kd.

Histological examination showed that the lesions predominated in the dermis. There was a massive, diffuse infiltrate throughout the dermis without a grenz zone. The infiltrate was composed predominantly of histiocytes and lymphocytes. Eosinophils and neutrophils were rare, and plasma cells were seen occasionally. Focally, the dermis was necrotic. In 4 patients, the histiocytes contained Leishmania species that were apparent with hematoxylin-eosin and Giemsa stains. When numerous, the parasites were also seen extracellularly. The kinetoplast was rarely detected with Giemsa stain. The microorganisms were readily differentiated from Histoplasma capsulatum and other small fungi by the negativity of periodic acid–Schiff and Grocott-Gomori stains. Minimal changes were observed in the epidermis: slight hyperkeratosis with parakeratosis and moderate acanthosis. In 2 patients, Leishman bodies were seen in the epidermal cells.

TREATMENT AND OUTCOME

Four patients were treated with a single local injection of meglumine antimoniate (1 mL/cm²). Patient 5 had a second injection 15 days later because of suspicion of incomplete healing. Patient 6 was treated with topical paromomycin for 10 days but showed local irritation a few days later. All patients were cured within 10 days to 1 month. No visceral involvement was clinically detected during long-term follow-up, which is ongoing.

Leishmania infantum is the causative agent of Mediterranean VL. Visceral leishmaniasis is endemic in southern France, where reservoirs are domestic dogs and vectors are mainly Phlebotomus perniciosus and Phlebotomus ariasi. The isolation of L. infantum in CL from our patients (southeastern France) and previous reports from the region of the Pyrenees-Orientales (southwestern France) confirm that this species is the only agent of sporadic autochthonous CL in France.

Leishmania infantum has long been considered exclusively to be an agent of VL, and L. major and L. tropica to be the only agents of CL in the Mediterranean Basin. However, in 1980, Rioux et al first isolated L. infantum from cutaneous lesions. Later, L. infantum was identified in CL in patients from other countries in the Mediterranean Basin: Spain, Italy, Algeria, Morocco, Tunisia, Malta, Cyprus, and Greece.

Leishmania infantum has been isolated and identified from approximately 100 patients with cutaneous lesions, however, few cases of L. infantum cutaneous leishmaniasis (LICL) are well documented. Most reports provide only a parasitological identification, but no clinical, biological, or therapeutic data have been provided and little is known about these aspects. In reviewing the literature we found only 11 previous patients with

Table 1. Pain Characteristics of the Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/ Age, y</th>
<th>No. of Lesions</th>
<th>Location</th>
<th>Size, mm</th>
<th>Clinical Form</th>
<th>Diagnostic Delay, mo</th>
<th>Montenegro Test Results</th>
<th>IFAT</th>
<th>Western Blot</th>
<th>Direct Examination</th>
<th>Parasite</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/56</td>
<td>2</td>
<td>Elbow</td>
<td>20</td>
<td>Ulcer</td>
<td>3</td>
<td>+</td>
<td>1/40</td>
<td>+</td>
<td>+</td>
<td>MON-24</td>
<td>NM gluc</td>
<td>Cure</td>
</tr>
<tr>
<td>2</td>
<td>F/3</td>
<td>3</td>
<td>Cheek</td>
<td>10</td>
<td>Papule</td>
<td>6</td>
<td>ND</td>
<td>1/160</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>NM gluc</td>
<td>Cure</td>
</tr>
<tr>
<td>3</td>
<td>F/3</td>
<td>1</td>
<td>Nose</td>
<td>10</td>
<td>Papule</td>
<td>6</td>
<td>ND</td>
<td>1/80</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>NM gluc</td>
<td>Cure (10 d)</td>
</tr>
<tr>
<td>4</td>
<td>F/85</td>
<td>1</td>
<td>Cheek</td>
<td>30×30</td>
<td>Infiltrate</td>
<td>3</td>
<td>+</td>
<td>1/40</td>
<td>+</td>
<td>+</td>
<td>MON-1</td>
<td>NM gluc</td>
<td>Cure (1 mo)</td>
</tr>
<tr>
<td>5</td>
<td>M/9 mo</td>
<td>1</td>
<td>Elbow</td>
<td>10</td>
<td>Papule</td>
<td>2</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>MON-1</td>
<td>NM gluc</td>
<td>Cure (1 mo)</td>
</tr>
<tr>
<td>6</td>
<td>M/9 mo</td>
<td>6</td>
<td>Face</td>
<td>5-10</td>
<td>Papule</td>
<td>6</td>
<td>ND</td>
<td>1/640</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>Topical</td>
<td>Paromomycin</td>
</tr>
</tbody>
</table>
documented LICL in 6 publications\textsuperscript{12,13,16,18,22,23} and the initial reports by Rioux and colleagues\textsuperscript{2,10,11} providing at least clinical, biological, histological, or therapeutic information.

PARASITOLOGICAL IDENTIFICATION

Six zymodemes have been isolated from CL in immunocompetent hosts: MON-11, MON-24, MON-29, MON-33, MON-78, and MON-111.\textsuperscript{6} The reservoirs of these dermotropic zymodemes are not identified, and, regarding vectors, only \textit{L. infantum} MON-24 has been isolated from \textit{P. ariasi}\textsuperscript{24} and \textit{Phlebotomus perfiliewi}.\textsuperscript{25} Some zymodemes have been found in VL and CL in immunocompetent hosts: MON-1, MON-34, and MON-80.\textsuperscript{6}

GEOGRAPHICAL DISTRIBUTION AND EPIDEMIOLOGY

\textit{Leishmania infantum} cutaneous leishmaniasis is present in the Mediterranean Basin in 2 distinctive areas. (1) In northern Africa, different \textit{Leishmania} species are responsible for CL. \textit{Leishmania infantum} and \textit{L. major} are present in Algeria,\textsuperscript{23} whereas \textit{L. infantum}, \textit{L. major}, and \textit{L. tropica} are found in Tunisia and Morocco.\textsuperscript{23,26} \textit{Leishmania major} and \textit{L. tropica} are present in the arid zone of northern Sahara, but \textit{L. infantum} is observed in the northern part of these countries, in the subhumid bioclimatic zone, which is a region endemic for VL.\textsuperscript{15,16} (2) However, in southwestern Europe, including Spain, Italy, Malta, Cyprus, and France, \textit{L. infantum} is the only species that has long been identified in patients with autochthonous VL, mucocutaneous lesions,\textsuperscript{26} and CL. It is likely that \textit{L. infantum} is the agent of CL in Portugal since the dermotropic zymodeme MON-24 has been isolated from \textit{P. ariasi}\textsuperscript{24}

In contrast to the rare reports of LICL, CL has been known for many years in the different countries of the Mediterranean Basin.\textsuperscript{27,28} However, the difficulty in interpretation of earlier reports is that identifications of the parasite were not as precise as they are now. The incidence of LICL is variable in each focus. In France before 1975, fewer than 40 patients with CL had been reported.\textsuperscript{11,42} During 1986 and 1987, a survey of patients with leishmaniasis found 4 with autochthonous CL, whereas VL was diagnosed in 95 patients in the same period.\textsuperscript{3} In the counties we studied, the finding of 6 patients in 10 years is equivalent to a rate of 0.5 cases per million population per year, whereas the rate of VL is estimated to be 10 per million.\textsuperscript{9} In 1985, Rioux et al\textsuperscript{10} identified \textit{L. infantum} from 21 patients with CL in the Pyrenees-Orientales,\textsuperscript{21} which represents the most important series of isolation of \textit{L. infantum} from CL. In Spain or Italy, CL has been diagnosed in hundreds of patients for years in different foci.\textsuperscript{20,28,29} The incidence of LICL in northern Africa is difficult to assess because of the presence of other species of \textit{Leishmania}. The most important series of presumptive LICL was reported by Belazzoug et al,\textsuperscript{30} with 114 cases from northern Algeria supposedly due to \textit{L. infantum} MON-24. It is likely that the true incidence of LICL...
is underestimated because of the undetected or unreported cases, because small lesions are not considered to be a priority for medical care, and because of the lack of precise knowledge of *L. infantum* in patients with CL.

Most foci show an enzymatic polymorphism. In southern France, 5 zymodemes have been identified in patients with CL: MON-1, MON-11, MON-24, MON-29, and MON-33. In Italy, despite an enzymatic polymorphism, *L. infantum* MON-24 is responsible for about 70% of the cases of CL. In countries in which a low polymorphism has been observed, it is difficult to draw conclusions because of the small number of strains studied.

*Leishmania infantum* cutaneous leishmaniasis is a sporadic disease that has been described in most patients in foci where the visceral disease is endemic. The *L. infantum* complex has a wide geographic distribution, extending in the Old World from northeastern China to the western Mediterranean Basin and from southern Europe to Africa. Therefore, LICL is expected to exist in these regions. In the New World the agent of VL is *Leishmania chagasi*. Most authors agree that LICL is identical to *L. infantum*. The finding of *L. chagasi* in a cutaneous lesion was first recognized by Oliveira Neto et al in Brazil. Later, *L. chagasi* was identified in patients with CL from Honduras, El Salvador, and other foci of Central America. Zeledon et al reported an outbreak of 200 patients with CL attributed to *L. infantum* in Costa Rica. Therefore, there is increasing evidence that LICL is present in the Americas.

**CLINICAL FEATURES**

All age groups are represented within a range of 9 months to 85 years, but mostly children are affected in northern Africa. The male-to-female ratio is 1. The initial lesion is a small erythematous papule that slowly increases in size to form a nodule or a plaque that may ulcerate and become crusted. The most usual clinical feature is a single lesion that consists of small, crusty ulcers surrounded by a notable erythematous reaction or nonulcerated papules that, when ulcerated, are recovered with a discrete crust. Lesions are located on the face (80%), are generally unique or few in number (1-3), and are symptomless. The medium diameter is 10 mm, but it varies from a few to 30 mL. The delay before diagnosis varies from 1 month to 2 years, with a mean of 3 to 6 months. The relatively long period before diagnosis is due to the progressive and symptomless evolution of the lesion. The diagnosis is usually made in autumn or winter in Europe because patients are contaminated in summer, the period of activity of phlebotomine sandflies.

The lesions may show polymorphism in their clinical features. They are composed of a variable degree of elementary clinical lesions: papule, erythema, ulceration, or infiltration. The clinical aspect results in the predominance or the association of these elementary compounds. According to the initial description proposed by Rioux et al, it is possible to distinguish 4 main clinical forms (Table 2): papulous, impetiginoid, ulcerative, and infiltrative. These clinical characteristics are those of a localized CL (LCL). Data are limited to show a correlation between the zymodeme and the clinical feature. Draining regional lymph nodes and solitary subcutaneous nodules representing lymphatic dissemination have not been reported. Results of the general physical examination are normal.

If untreated, the lesions usually persist longer than 1 year. Sometimes they persist for 3 years, but spontaneous healing occurs, leaving a flat, atrophic scar. Rioux et al reported 2 episodes of LICL in the same patient due to 2 different zymodemes that occurred 3 years apart. The absence of protective immunity after the first infection suggests that there is no crossover immunity between 2 different zymodemes. However, the authors stated that the rapid healing of the second infection suggested that the first infection gave partial immunity.

*Leishmania infantum* cutaneous leishmaniasis shares most clinical and biological features with LCL due to *L. major* and *L. tropica*, the concurrent species in the Mediterranean Basin. Belazzoug et al consider that LICL may be clinically distinguished from LCL due to *L. major*, which produces larger and multiple lesions with a heavy crust and unsightly scars. However, the only way to differentiate the diseases is by isolation and identification of the parasite.

**DIAGNOSIS**

As for other patients with CL, the diagnosis is based on the demonstration of the parasite in smears or in skin biopsy specimens. In patients with LICL, direct examination of smears is difficult because the parasites are usually rare; however, it remains the most sensitive technique for the diagnosis of CL. Specimens from biopsy or aspirate may be cultured on Nicolle-Novy-MacNeal blood agar. The growth of promastigotes allows subsequent species identification by means of isoenzyme analysis. However, cultivating *Leishmania* species from LICL in conventional blood-agar medium has proved difficult. Unconventional semisolid blood-agar medium and an in vivo method using inbred hamsters treated with cortisone have been used to solve these difficulties. Identification of the *Leishmania* species by use of a diagnostic DNA probe has been proposed. Results of the Montenegro test usually become positive after 2 months but provide little information. Classic serological tests (indirect fluorescent antibody test, enzyme-
linked immunosorbent assay, and direct agglutination) do not provide determinant informations. \cite{10,11} When these antibodies are present, the titers are usually low. Marty et al.\cite{12} recently reported that the Western blot analysis from patients with VL due to *L infantum* showed the simultaneous presence of antibodies against 4 antigens with molecular masses of 18, 21, 23, and 31 kd. This Western blot assay used in 5 of our patients also showed the presence of antibodies against these 4 antigens. Therefore, if these results are confirmed by larger studies, the Western blot assay could be a useful diagnostic test for VL or CL. However, this technique, using promastigotes derived from culture of strains of *L infantum* MON-1,\cite{13} cannot distinguish between dermotropic strains and viscerotropic strains. Studies are ongoing to determine whether this Western blot is specific to *L infantum*.

The histological pattern of LICL is similar to that of other LCLs. However, the finding of parasites in the epidermis has been exceptionally reported.\cite{14} In our patients, the transepithelial elimination of *Leishmania* organisms was seen in histiocytes in patients with exocytosis and in epidermal cells. The significance of the phenomenon is not clear. Some authors suggest that *Leishmania* organisms can be differentiated from *Histoplasma capsulatum* using hematoxylin-eosin-safran staining sections.\cite{15} In our 4 patients, the clear-capsule–like zone around individual organisms, given as a histological clue for *H capsulatum*, was a constant feature of Leishman bodies. Therefore, in our experience, special stains were necessary in all cases for distinguishing *Leishmania* organisms from *Histoplasma* organisms and other small fungi.

## THERAPY

Systemic therapy with meglumine antimoniate or stibogluconate sodium has been efficient in all patients with presumptive LICL.\cite{16} However, our opinion is that systemic therapy is not indicated for patients with lesions usually of limited size that heal spontaneously. Local therapies is a more attractive approach. Local injection of meglumine antimoniate has been used successfully as in our patients.\cite{17} There are isolated reports of other systemic or local therapy being used in patients with presumptive LICL, but data are limited. After therapy, the lesions heal slowly within 10 days to 1 month, leaving a depressed, atrophic scar.\cite{18,19}

### LICL IN HUMAN IMMUNODEFICIENCY VIRUS–SEROPOSITIVE PATIENTS

Hundreds of cases of VL have been reported from southern Europe in human immunodeficiency virus–infected patients.\cite{20,21} In contrast, cutaneous lesions have been reported rarely in these patients.\cite{22,23,24} It has been suggested that immunocompromised patients tend to develop VL rather than LCL.\cite{25} Some cutaneous lesions have been reported in patients with VL, and seem to be related to secondary cutaneous location of parasites due to the dissemination of the *Leishmania* species.\cite{26,27} Apparent primitive LCL has been reported rarely, and only a single case has been proved to be due to *L infantum*.\cite{28} In none of these patients has a secondary visceral localization been proved.

## RELATIONSHIPS WITH VL

Manson-Bahr\cite{29} suggested in 1955 that in some patients, a primary skin lesion could occur at the site of the sandfly bite, with lymphatic spread of the parasites to the regional glands and eventually to the viscera. It is likely that this hypothesis cutaneous lesion, called *leishmania* in some textbooks or reports, has been assimilated to LICL.\cite{30} However, this hypothesis seems to be exceptional.\cite{31} No documented case of LICL has been complicated by a visceral disease in an immunocompetent patient. The only report of a possible LICL that has preceded VL was of a child who had VL 4 months after CL.\cite{32} However, the parasites had not been identified, and it has been shown that the same patient may be infected with different zymodemes a few months apart.\cite{33}

The reason some zymodemes may cause either cutaneous lesions or visceral disease and others may cause both cutaneous and visceral disease is unclear. It has been suggested that the zymodeme type might affect the clinical response.\cite{34} Another hypothesis is that the host immune response affects the clinical response.\cite{35} The different tropism observed in immunocompetent hosts is not observed in patients with human immunodeficiency virus infection in whom VL may be caused by dermotropic zymodemes in the absence of cutaneous lesions.\cite{36,37} It has been proposed that the immunodeficiency related to human immunodeficiency virus infection favors the dissemination of parasites that are normally dermotic.\cite{38} Immunosuppression with corticosteroids in hamsters provokes visceralization of dermotic *Leishmania* species.\cite{39} In 1957, André et al.\cite{40} reported the case of a newborn who had VL after exsanguinotransfusion from a donor with LCL.

Localized CL suggests a strong, specific, cell-mediated response, demonstrated by Montenegro testing, and VL is characterized by the absence of cell-mediated immunity.\cite{41} Our patients confirm that *L infantum* is a causative agent of CL in the Mediterranean Basin. Its responsibility in CL from other areas where VL is endemic may be suspected. Our opinion is that CL in many patients is undetected because of the poor knowledge of the disease. Identification of the parasites isolated from CL from these regions is necessary for a better understanding of the disease and to elucidate its relationships with the visceral form.

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