Contribution of molecular diagnosis to the management of cutaneous leishmaniasis in travellers

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Abstract

Cutaneous leishmaniasis is one of the most frequent skin diseases occurring after travelling in endemic areas. Optimal management requires identification of the species of *Leishmania* involved. In this study we aimed to evaluate the use of molecular diagnosis as routine, in comparison with direct examination and culture. Thirty positive diagnoses were carried out between 2007 and 2013. Classical PCR enabled 11 positive cases to be identified that were found to be negative by conventional methods. Sequencing led to the identification of eight different species. Routine use of PCR and sequencing appears very efficient in the management of cutaneous leishmaniasis.

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Introduction

Dermatological complaints are the third reason for travellers to seek medical consultations. Cutaneous leishmaniasis (CL) is one of the ten most frequent skin diseases occurring after travelling [1] in North Africa, and Central and South America [2]. In recent years there has been an increase in the incidence of CL due to a greater number of international travellers, adventure holidays, migrations and military operations in endemic areas [3]. Because of the wide range of destinations there is a great variety in *Leishmania* species responsible for cutaneous lesions in travellers. In the Old World (OW), CL is mainly due to *L. tropica*, *L. major* and *L. infantum*, while *L. guyanensis*, *L. braziliensis*, *L. mexicana* and *L. panamensis* are found in the New World (NW). The physical aspects of the lesions can rarely allow the identification of the species involved, whereas each species has its own prognosis and treatment. Thus recent guidelines have been published for the management of CL that consider the lesion, the patient's status and the infecting species [4,5]. In this context, molecular tools could represent an interesting alternative to improve the diagnosis and management of CL.

Since 2007, in the Parasitology–Mycology Department of the Toulouse University Hospital (France), diagnosis of CL has been carried out by the combination of microscopic examination after May–Grünwald Giemsa staining, culture on Novy-MacNeal-Nicolle medium and molecular techniques. The molecular biology tools consist of a classical PCR that targets a conserved region (18S gene) [6] and for each positive sample, the species is then identified by sequencing a part of the cytochrome *b* gene as described by Foulet *et al.* [7]. If required, the PCR and sequencing are performed twice and once a week, respectively. In order to assess the value of molecular biology in comparison with conventional methods (microscopic examination and culture), we collected retrospectively all CL cases diagnosed in the department from January 2007 to July 2013.

During this period, of the 133 patients for whom physicians suspected CL, 17 samples were positive after microscopic examination and/or culture, while 30 were positive using PCR (Table 1). All positive samples found using conventional methods were positive after PCR analysis. Two samples were positive after culture whereas they were negative on microscopic examination, and conversely, four samples had a negative culture while they were positive by microscopy. The PCR enabled the identification of 11 (39%) positive cases that were found to be negative by conventional methods. Our study showed that diagnosis of CL by PCR was more sensitive than conventional techniques and faster than culture in routine

TABLE 1. Results of culture and microscopic examination of all 30 PCR-positive samples

Positive conventional techniques	17 (61%)
DE (+)/C (+)	5
DE (+)/C (−)	4
DE (−)/C (+)	2
DE (+)/C (NP)	6
Negative conventional techniques	11 (39%)
NP conventional techniques	2[a]
Total	30

DE, direct examination; C, culture; NP, not performed; (+), positive; (−), negative.
[a]Samples collected on a swab.

TABLE 2. Epidemiology and species identification of the 30 PCR-positive samples

	Sex	Age (years)	Country	Results of sequencing
1	M	13	Tunisia	*L. major*
2	M	57	Tunisia	*L. major*
3	M	65	Tunisia	Unknown
4	F	8	Algeria	*L. major*
5	F	81	Algeria	*L. major*
6	M	16	Morocco	*L. major*
7	M	55	Morocco	*L. major*
8	M	2	Ethiopia	*L. aethiopica*
9	M	72	North Africa[a]	*L. tropica*
10	M	59	French Guiana	*L. guyanensis*
11	M	19	French Guiana	*L. guyanensis*
12	M	26	French Guiana	*L. guyanensis*
13	M	27	French Guiana	*L. guyanensis*
14	M	21	French Guiana	*L. guyanensis*
15	M	21	French Guiana	*L. guyanensis*
16	M	22	French Guiana	*L. guyanensis*
17	M	20	French Guiana	*L. guyanensis*
18	M	20	French Guiana	*L. guyanensis*
19	M	20	French Guiana	*L. guyanensis*
20	M	19	French Guiana	*L. guyanensis*
21	M	23	French Guiana	*L. guyanensis*
22	M	21	French Guiana	*L. guyanensis*
23	M	26	French Guiana	*L. guyanensis*
24	M	25	French Guiana	*L. guyanensis*
25	M	34	French Guiana	*L. naiffi*
26	M	22	French Guiana	*L. brasiliensis*
27	M	29	Peru	*L. guyanensis*
28	F	21	Costa Rica	*L. panamensis*
29	F	23	No travel[b]	*L. infantum*
30	M	51	No travel[c]	*L. infantum*

M, male; F, female.
[a]Travel in North Africa.
[b]Native of Tunisia.
[c]Native of French Indies (Guadeloupe).

diagnosis as shown in previous studies [8–11]. More precisely, our data showed that only 54% (15/28) and 47% (7/15) of our samples were positive when assessed by microscopy and culture, respectively. Independently of the parasite load or species involved, the success of microscopy and culture may depend on the quality of the cutaneous sample. In our centre, travellers were usually cared for by non-dermatologist physicians who most often obtained a skin sample by simply scraping rather than a true biopsy, which may explain, in part, the low sensitivity of our conventional diagnosis.

Among the 30 positive samples sequenced, 29 identifications were carried out and eight different species were identified (Table 2). Only one sequencing failed due to a low amount of DNA. The two main species found were *L. guyanensis* from South America and *L. major* from North Africa. Although these two species represented 76% (22/29) of identified *Leishmania*, the diversity of species found showed that sequencing produced clinically relevant information. Among the eight cases of CL emanating from the OW, six were infected by *L. major*, one by *L. tropica* and one by *L. aetiopica*. Although the classical appearance of lesions caused by *L. major* or *L. tropica* allows these two species to in principle be distinguished, in practice it is not so easy. The need for an anti-leishmanial therapy is not systematic for *L. major* as lesions cure spontaneously within a few months. For *L. tropica*, lesions can evolve over 1 or 2 years and thus the management usually requires specific therapy [5]. Even if it is exceptional in travellers, CL caused by *L. aethiopica* can lead to diffuse CL in anergic patients. Among the 19 patients who travelled in the NW, 16 were infected by *L. guyanensis*, one by *L. braziliensis*, one by *L. panamensis* and one by *L. naiffi*. Seventeen patients were infected in French Guiana and 11 of them were military personnel who were infected during the same mission in a tropical forest. Interestingly, among this cluster of 11 patients, sequencing allowed one patient to be detected who was infected by *L. braziliensis*. *L. guyanensis* and *L. braziliensis* are clinically similar but may evolve differently. *L. guyanensis* causes almost exclusively cutaneous lesions that can be treated by a short course of pentamidine [4]. *L. braziliensis*, as *L. panamensis*, can lead to muco-cutaneous leishmaniasis, which is life-threatening and requires injections of pentavalent antimony for 20 days or liposomal amphotericin B if the first line of treatment fails [4].

Of the 30 positive samples, two patients were infected by *L. infantum*. An epidemiological investigation revealed that neither of them had travelled outside France for several years. The first case was a young Tunisian-born woman who did not return there for 7 years. The second case was a 50-year-old male native of the French West Indies (Guadeloupe) who also did not travel outside France for 3 years. The two patients had no clinical or biological evidence of visceral leishmaniasis. Even if it is not so common, it is well-known that *L. infantum* can also be responsible for skin lesions without systemic involvement. In south-eastern France, where *L. infantum* is the only endemic species, 39 cases of autochthonous CL have been reported between 1999 and 2012 [12], implying that CL must be considered in cases of chronic wounds. In North Africa and the Middle East, where *L. major* and *L. tropica* are the main species found in CL, CL may also be due to *L. infantum*. Considering all these clinical and therapeutic characteristics, the precise identification of species of involved *Leishmania* is important. Until now, the 'gold standard' to identify *Leishmania* was the isoenzyme characterization, described by Rioux *et al.* [13]. Nevertheless, this technique takes a long time due to the

need to culture the parasites and is available only in very few reference centres. Identification of species by sequencing has several advantages: achievable without culture, rapid execution and the availability of the technology now in many referral hospitals.

In conclusion, microscopic examination remains useful because it allows a quick and easy diagnosis in about 50% of cases of CL. Apart from the interest related to the isolation of strains, culture is not strictly speaking pertinent for diagnosis. In contrast, PCR and sequencing appear very relevant for the diagnosis and the management of CL, and for epidemiological surveillance.

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Transparency Declaration

The authors declare no conflicts of interest.

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