Journal of Microbiology, Immunology and Infection (2016) 49, 809—812

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

CASE REPORT

Diagnosis of *Leishmania* infection in a nonendemic area of South America

Diego Esteban Cargnelutti [a,b,*], Carlos Guillermo Borremans [a], Rosa Lydia Tonelli [a], Liliana Carmen Carrizo [a], María Cristina Salomón [a]

[a] *Área de Parasitología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina*
[b] *Instituto de Medicina y Biología Experimental de Cuyo (IMBECU), CCT-Mendoza, CONICET, Mendoza, Argentina*

Received 4 August 2014; accepted 29 November 2014
Available online 11 December 2014

KEYWORDS
Argentina;
leishmaniasis;
mucosal involvement;
nonendemic area

American tegumentary leishmaniasis is the generic name for a variety of cutaneous and mucocutaneous presentations of parasitosis caused by several species of the genus *Leishmania*. This is a widespread infection in the American continent, from the South of the United States to the North of Argentina. We herein describe the management of a patient with American tegumentary leishmaniasis in Mendoza, Argentina, a nonendemic area of South America, whose diagnosis and treatment were significantly delayed, because the patient did not report a recent history of travel to any known endemic areas. This case stresses the need for training health-care professionals in the diagnosis and treatment of not only endemic parasitosis within their work zones but also nonendemic parasitosis.

Introduction

Leishmaniasis is a parasitic disease caused by protozoa of the genus *Leishmania*, which affects a large population all over the world. Estimates suggest that more than 12 million people are infected by the protozoan *Leishmania*. There are different species of *Leishmania* that can cause the disease in different regions. American tegumentary

* Corresponding author. Área de Parasitología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Avenida del Libertador, Number 80, Mendoza, Argentina.
E-mail address: diegocargnelutti@hotmail.com (D.E. Cargnelutti).

http://dx.doi.org/10.1016/j.jmii.2014.11.014

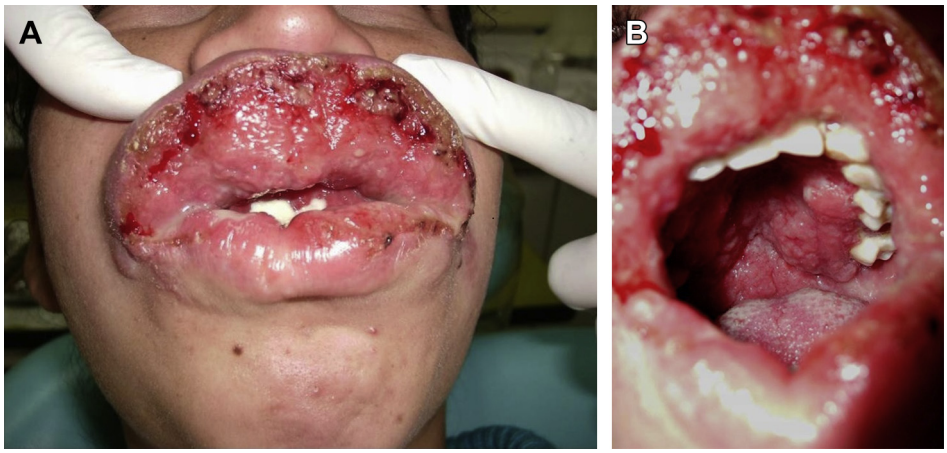

Figure 1. American tegumentary leishmaniasis. (A) Infection in the nose and lip regions that presented as edema and erythema. (B) Granulomatous lesion on the soft palate.

leishmaniasis is the generic name for a variety of cutaneous and mucocutaneous presentations of parasitosis caused by different species of *Leishmania*.[1] This infectious disease is a serious health problem. In endemic areas, high morbidity rates are reported, whereas in nonendemic areas it is usually misdiagnosed as some other similar granulomatous dermal diseases. Diagnosis of the infection is complicated because of the high costs of laboratory supplies and superinfection of the lesions. The main problem, however, is the insufficient training of health-care professionals in nonendemic areas.[2]

American tegumentary leishmaniasis is a widespread infection in the American continent, from the South of the United States to the North of Argentina.[3] The provinces of Salta, Jujuy, Tucumán, Catamarca, Santiago del Estero, Chaco, Formosa, Misiones, and Corrientes constitute the endemic area in Argentina. Although species of *Lutzomyia* (a vector of this disease) have been detected in the provinces of Santa Fe and Entre Ríos, so far there are no reports of infection in animals or humans.[4]

A common question asked by doctors whenever a patient presents with atypical clinical symptoms for the region is "Where have you been?" When investigating canine leishmaniasis cases, the veterinary surgeons usually inquire about the origin or movement of animals. A large proportion of leishmaniasis had been diagnosed in patients who either have been to or were born in an endemic area. Such questions should also be asked at the clinics of developing countries where nonautochthonous cases may appear.

In this report, we will describe the management of an American tegumentary leishmaniasis case that was detected in a nonendemic area. The diagnosis was significantly delayed because the patient did not report any recent history of travel to any known endemic areas.

Case report

Our case is a 19-year-old female patient who had never traveled outside the province of Mendoza, Argentina. As an antecedent to the present episode, at the age of 14, the patient suffered upper lip trauma while practicing sports, which caused edema and erosion in the affected area (i.e., the lips). At that time, she was diagnosed with *support granulomatous cheilitis*. She was treated with minocycline for a brief period in another institution, but the treatment was discontinued due to pharmacodermy. The patient mentioned that the lesions significantly increased in size during her two pregnancies, and that she did not receive any further treatment. In March 2013, she came to us for consultation because of severe granulomatous infiltration in the hard palate and soft upper and lower lips, which was associated with edema and mucosal erosions for 4 years (Fig. 1A and B). A nasal endoscopy showed granulomas in the nostrils, mouth, palate, and anterior and posterior pillars.

In April 2013, we performed a biopsy of the mucosa of the upper lip and the left posterior palate. Biopsy results showed intense inflammatory infiltrate with abundant lymphocytes, plasma cells with Russell bodies, and histiocytes with vacuolated cytoplasm. Rhinoscleroma was suspected.

Therefore, we requested additional tests to be performed on the patient: The patient's complete blood count was normal. The skin test for tuberculosis was negative. Her chest X-ray was normal. The venereal disease research laboratory result was negative (i.e., nonreactive). For the bacteriologic study, we collected mucus samples from the palate and lip. Neither alcohol-resistant acid bacillus nor fungal elements were observed in the sample. In addition, Giemsa and Ziehl—Neelsen staining were negative. Gram staining showed a few Gram-positive cocci. *Staphylococcus aureus* and *Streptococcus viridans* were isolated from the sample. She also underwent serology testing for deep mycoses. However, the mucus sample was negative for *Coccidioides*, *Paracoccidioides*, *Histoplasma*, and *Aspergillus*. The sample was negative for tumor markers as well.

In April 2013, despite failing to isolate *Klebsiella rhinoscleromatis* in culture, there was a clinical suspicion of rhinoscleroma based on biopsy results. The patient received ciprofloxacin (500 mg every 12 hours) for 4 months and cefixime (400 mg every 24 hours) for 1 month. A slight improvement in edema and erythema was observed in upper lip on the 1st month control visit. The patient remained stable on successive control visits.

In July 2013, the patient presented with hoarseness and persistent granulomatous lesions on the palate, infiltration of the lower third of the face, and protrusion of both lips without significant improvement despite treatment with ciprofloxacin for the past 4 months.

From a public health institution in the Mendoza province, the patient was referred to the Área de Parasitología at the School of Medicine of the National University of Cuyo (Mendoza, Argentina) to exclude *Leishmania* infection in differential diagnosis, because the patient did not show any signs or symptoms suggestive of this infection thus far.

Lesions were cultured by an aspirate technique in accordance with the procedure reported by Romero et al,[5] but with some minor modifications. For the culture, 10-mL capacity tubes with rubber caps filled with a biphasic culture medium were used. The solid phase consisted of 4 mL of a blood agar-based medium—Novy—MacNeal—Nicolle medium with blood agar base, to which 5% defibrinated rabbit blood was added at 40°C after the agar hardened. The liquid phase was 500 μL isotonic saline (0.9%) containing 100 U/mL penicillin and 100 μg/mL streptomycin.

Before sample collection, the puncture site was vigorously cleaned with 70% ethanol. A local anesthetic (100 μL of 2% lidocaine) was injected with a 1-mL syringe equipped with a 13-G needle. The aspiration puncture was made using a commercially available needle holder (Vacutainer). A 21-G needle at a 45-degree angle was then inserted into the dermis at the ulcer border by rotary movement. The sample was collected in the tube containing the biphasic culture medium by suction.

The culture tubes were incubated at 26°C. For parasite examination, the contents of each tube were gently homogenized, and approximately 10 μL of the suspension was observed under an optical microscope (40× magnification). The cultures were examined every 3 days to detect promastigotes under the optical microscope. After 6 days, *Leishmania* spp. promastigotes were observed (Fig. 2A and B).

Discussion

The diagnosis of American tegumentary leishmaniasis based on clinical aspects is usually inaccurate because many other conditions such as fungal and mycobacterial infections, sarcoidosis, and neoplasms imitate the behavior of this type of leishmaniasis. In addition, serological and histopathological methodologies have a limited diagnostic certainty, because of their low sensitivity levels. Cell-mediated immune response measured by delayed-type hypersensitivity often identifies positive cases more accurately. Nevertheless, the results depend on the immunological status of the patient, the period since the patient acquired the infection, the kind of *Leishmania* species, and the quality of the reagents used. Despite the high specificity of the skin test, it is not possible to differentiate current from past infections using this method.[6]

Although polymerase chain reaction has been proved to be highly effective in the diagnosis of leishmaniasis,[7] the disadvantages to this approach are the high cost, the availability of reagents and equipment, and the poor adaptability to field conditions.

Currently used parasitological diagnostic methodologies involve the direct observation of amastigotes in stained smears or cultures of samples from skin lesions. The traditional culture methods consist of a biphasic culture system of blood agar with liquid overlay.[8] Therefore, there is a need to establish simpler procedures for obtaining the samples from cutaneous leishmaniasis patients, because the complexity of the collection significantly restricts its massive use in public health institutions. Culturing of aspirates from the American tegumentary leishmaniasis lesion is an alternative methodology because it is easily performed, economical, and had a high positivity rate, ranging from 28.6% to 89.0% in studies that evaluated patients infected with different *Leishmania* species.[5,9]

In the Argentine Republic, the endemic area corresponds with the tropical and subtropical region north of the country in a broad geographical region crossing Argentina from east to west. This territory covers nine provinces of the Argentine Republic (Salta, Jujuy, Tucumán, Catamarca, Santiago del Estero, Chaco, Formosa, Misiones y Corrientes) with an approximate extension of 497,000 km^2.

The endemic area extends from a longitude of 66° W to 54° E; and from a latitude of 22° N to 29° S. A recent study reported the presence of *Lutzomyia* (a vector of this

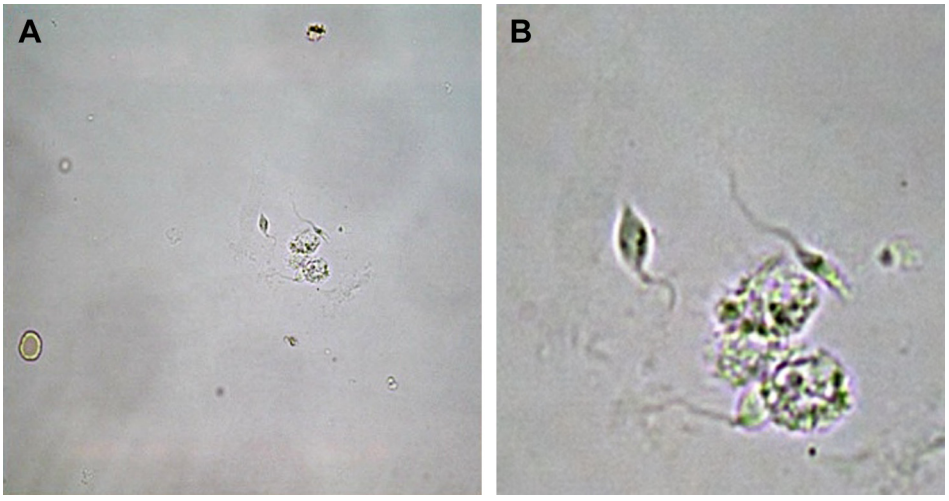

Figure 2. *Leishmania* spp. promastigotes: (A) under optic microscopy (40× magnification). (B) Zoom of image A.

disease) in the provinces of Santa Fe and Entre Ríos, but so far there are no reports of infection in animals or humans.[4]

Mendoza city (32°53′ LS, 68°49′ LW), capital of the Mendoza province, is located in the foothills that extend to the east of the final slopes of the Andes and is bordered by Chile. The city is 750 m above sea level. It is a semiarid temperate zone, although the mountain ranges provide a certain degree of aridity with scarce rainfall (average annual rainfall, 250 mm; average annual temperature, 18.8°C). The city of Mendoza is more than 800 km away from the area of transmission of the American tegumentary leishmaniasis and lies in a completely different ecological region to those where sand fly insect vectors are found.

During discussions, the patient denied several times having traveled outside the province of Mendoza. Once the diagnosis was established, the patient had to be hospitalized for treatment. The patient's mother mentioned that the girl had been born and remained in the province of Salta until the age of 3 years. The infection in our case was thus asymptomatic until 9 years of age, after which she developed upper lip trauma as a manifestation of this disease.

This parasitic disease is an exotic infection in Mendoza, and in some cases, the provincial public health system has not been able to establish the diagnosis of the infection accurately.[10] However, the present case of American tegumentary leishmaniasis that had an unconventional mucocutaneous presentation for the Argentine Republic was diagnosed and treated in an efficient and accurate way by the academic—scientific staff at the School of Medicine of the National University of Cuyo, Mendoza province.

This case demonstrates the importance of teamwork between the health and academic institutions that allowed for the correct diagnosis of a nonendemic parasitosis in Mendoza. In addition, this case stresses the need for training health-care professionals in the diagnosis and treatment of not only endemic parasitosis within their work zones but also nonendemic parasitosis. Globalization of parasitosis due to the migration of populations increases the need for continuous training in the approach to and management of nonendemic parasitosis.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

This work was carried out with the financial support of the Secretaría de Ciencia, Técnica y Posgrado of the Universidad Nacional de Cuyo (Project No. 06/J417).

References

1. WHO Expert Committee on the Control of Leishmaniases. *Control of the leishmaniases*. Geneva, Switzerland: WHO Press; 2010. p. 5—12.
2. Escobar MA, Martinez F, Scott Smith D, Palma GI. American cutaneous and mucocutaneous leishmaniasis (tegumentary): a diagnostic challenge. *Trop Doct* 1992;**22**:69—78. 63—4.
3. Salomón OD, Sosa-Estani S, Ramos K, Orellano PW, Sanguesa G, Fernández G, et al. Tegumentary leishmaniasis outbreak in Bella Vista City, Corrientes, Argentina during 2003. *Mem Inst Oswaldo Cruz* 2006;**101**:767—74.
4. Salomón OD, Orellano PW, Quintana MG, Perez S, Sosa Estani S, Acardi S, et al. Transmission of tegumentary leishmaniasis in Argentina. *Medicina (B Aires)* 2006;**66**:211—9.
5. Romero GA, Sampaio RN, Macedo Vde O, Marsden PD. Sensitivity of a vacuum aspiratory culture technique for diagnosis of localized cutaneous leishmaniasis in an endemic area of *Leishmania (Viannia) braziliensis* transmission. *Mem Inst Oswaldo Cruz* 1999;**94**:505—8.
6. Berman JD. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. *Clin Infect Dis* 1997;**24**:684—703.
7. Toz SO, Culha G, Zeyrek FY, Ertabaklar H, Alkan MZ, Vardarlı AT, et al. A real-time ITS1-PCR based method in the diagnosis and species identification of *Leishmania* parasite from human and dog clinical samples in Turkey. *PLoS Negl Trop Dis* 2013;**7**:e2205.
8. Marzochi MC, Teixeira PC, Marzochi KB, da Conceição NF, Coutinho W, de Brito DB. Vacuum aspiratory puncture system for *Leishmania* culturing, isolation and transport. Preliminary report. *Rev Inst Med Trop Sao Paulo* 1993;**35**:301—3.
9. Weigle KA, Labrada LA, Lozano C, Santrich C, Barker DC. PCR-based diagnosis of acute and chronic cutaneous leishmaniasis caused by *Leishmania (Viannia)*. *J Clin Microbiol* 2002;**40**:601—6.
10. Martín-Sánchez J, Navarro-Mari JM, Pasquau-Liaño J, Salomón OD, Morillas-Márquez F. Visceral leishmaniasis caused by *Leishmania infantum* in a Spanish patient in Argentina: what is the origin of the infection? Case report. *BMC Infect Dis* 2004;**4**:20.