

Co-infection by *Leishmania amazonensis* and *L. infantum/L. chagasi* in a case of diffuse cutaneous leishmaniasis in Bolivia

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Abstract

We present the first report of a co-infection by *Leishmania amazonensis* and *L. infantum/L. chagasi* isolated in 1993 from a patient with diffuse cutaneous leishmaniasis (DCL), living in the sub-Andean region of Bolivia. This is the third reported case of DCL in Bolivia, but the first one with isoenzymatic identification of the aetiological agents involved and the first one giving evidence for a mixed infection by 2 *Leishmania* parasites in the same lesion.

Keywords: leishmaniasis, diffuse cutaneous leishmaniasis, *Leishmania amazonensis*, *Leishmania infantum/chagasi*, co-infection, case report, Bolivia

Introduction

Visceral and cutaneous leishmaniasis are endemic diseases in Bolivia, occurring at a higher frequency in the sub-Andean area below 1800 m above sea level (LE PONT *et al.*, 1992). While visceral leishmaniasis (*Leishmania infantum/L. chagasi*) seems restricted to the Yungas of La Paz Department (1000–1800 m), as well as to the eastern lowlands of Santa Cruz Department (LE PONT *et al.*, 1992), cutaneous and mucocutaneous leishmaniasis (*L. braziliensis*) extends from 1800 m to the lowlands of Beni Department. *L. amazonensis* may produce a particular form of cutaneous leishmaniasis named 'diffuse cutaneous leishmaniasis' (DCL), which was reported (2 cases) only from the Yungas region but without formal identification of the parasite (PRADO BARRIENTOS, 1948a, 1948b; VALDA, 1980). This latter species has been identified occasionally in humans presenting cutaneous leishmaniasis in the Santa Cruz Department (LA FUENTE *et al.*, 1986; DUJARDIN *et al.*, 1987; GRIMALDI *et al.*, 1987; DESJEUX, 1991), and recently a new focus, very active for cutaneous leishmaniasis due to *L. amazonensis*, was reported in the sub-Andean region of La Paz (MARTINEZ *et al.*, 1998).

We present the third known case of DCL in Bolivia, the first one with aetiological confirmation (co-infection by *L. amazonensis* and *L. infantum/L. chagasi*), in a child living in a settlement located in the sub-Andean region.

Patient and Methods

Clinical history

The patient (E.Q.Z.), detected in 1993, was a 5-year-old Aymara girl, born in the colony Unión Barea II (Caranavi Province, La Paz Department), a sub-tropical mountainous area located at 900–1000 m, without evidence of travel to other regions. The disease began 2 years previously, with an ulcer on the right arm, followed by infiltrated satellite lesions, some with superficial ulceration. One month later, lesions appeared on the cheeks, nose and left ear, followed by multiple and confluent lesions on the thigh, leg, hand, thorax and abdomen. Some lesions evolved with periods of total or partial healing followed by reactivation. Three months later a mucocutaneous nasal lesion flared up. Topical antibiotics and pentavalent antimonials (meglumine antimonate) were previously administered at an inadequate dose, without any favourable result.

Clinical examination showed multiple infiltrated plaques, scars and seric crusts principally on the face and left ear, mucocutaneous nasal lesion and facial oedema (Fig. 1). The patient had scars, hypopigmentation,



Fig. 1. A 5-year-old girl in the sub-Andean region of Bolivia, diagnosed with diffuse cutaneous leishmaniasis: (A) infiltrative plaques, scars, seric crusts, mucocutaneous nasal lesion and facial oedema; (B) ulcerated and infiltrative lesions; and (C) confluent cheloid plaques, crusts and reactivated lesions.

cheloid and infiltrated plaques on the right shoulder and elbow, and reactivated lesions on the right elbow. There were scars on the thorax and abdomen, 2 united ulcers (3 × 5 cm each) and infiltration on the left hand and wrist. There were also confluent and cheloid plaques, some with superficial ulceration, on the left buttock, thighs and left leg (Fig. 1) together with oedema in both legs. Additional signs included dry, rough hair with loss of pigmentation, weight loss, moderate swelling of the abdomen, irregular fever, anaemia, accelerated erythrocyte sedimentation, lymphocytosis, hypoproteinaemia, hypoalbuminaemia and inversion of the albumin/globulin ratio. Characteristic

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clinical manifestations of visceral leishmaniasis such as splenomegaly and hepatomegaly were not detected.

The patient received 2 courses of treatment with meglumine antimonate for 20 d, at a higher dose (28.7 mg antimony/kg daily) than recommended (WHO, 1990; BARRAL-NETTO *et al.*, 1995) for the severe form of the disease. There was a discrete improvement but no global satisfactory result. The patient returned with her parents to her home region and died in March 1994.

Parasitological diagnosis

Smears were taken from lesions, fixed with ethanol and stained with Giemsa's stain at 1:10 to search for parasites. A Montenegro skin test was carried out. Biopsies were obtained from a thigh lesion by skin puncture, triturated with a sterile saline solution and the suspension was subsequently inoculated into the legs and nose of 2 hamsters. With a syringe containing a sterile saline solution, material from the granuloma that developed in the hamsters was aspirated and cultured in tubes of diphasic medium (NNN/Schneider) at 24°C.

Isoenzyme electrophoresis

Parasite stocks were submitted to multilocus enzyme electrophoresis (MLEE) on cellulose acetate plates as described by BEN ABDERRAZAK *et al.* (1993). Eight enzyme systems were assayed: alanine aminotransferase (EC 2.6.1.2, ALAT), glucose phosphate isomerase (EC 5.3.1.9, GPI), isocitrate dehydrogenase (EC 1.1.1.42, IDH), malate dehydrogenase NAD⁺ (EC 1.1.1.37, MDH), mannose phosphate isomerase (EC 5.3.1.8, MPI), nucleoside hydrolase, substrate deoxyinosine (EC 2.4.2.*¹, NHD), nucleoside hydrolase, substrate inosine (EC 2.4.2.*², NHI) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44, 6PGD).

Five reference strains were used: *L. infantum/L. chagasi* (MHOM/BR/74/PP75), *L. amazonensis* (IFLA/BR/67/PH8), *L. mexicana* (MNYC/BZ/62/M379), *L. braziliensis* (MHOM/BO/83/LPZ155) and *L. guyanensis* (MHOM/BR/78/M5378).

Results

Diagnosis

The smears showed abundant free amastigotes, as well as many vacuolated histiocytes containing numer-

ous parasites. The Montenegro skin test was negative (0 mm) after 48 and 72 h. After 4 weeks, the 2 inoculated hamsters exhibited moderate granulomas at the inoculation site that evolved in a few months to voluminous, un-ulcerated and serious lesions showing tendency to ulceration. These lesions contained abundant intracellular and extracellular amastigotes, vacuolated histiocytes and little host-cell reaction, similar to observations for samples from the patient. The hamsters died after 9 months without further study.

Isoenzyme electrophoresis

The 8 enzyme systems allowed the analysis of 9 enzymatic loci (the NHI system revealed 2 different loci). The electrophoretic profiles were in agreement with the presence of 2 *Leishmania* species. Indeed, except for NHD locus, all loci showed superposition of 2 isoenzymatic profiles without evidence of heterozygote forms (Figs 2 and 3). A comparison with the reference strains allowed recognition of the typical profiles of *L. amazonensis* (IFLA/BR/67/PH8) and of *L. infantum/L. chagasi* (MHOM/BR/74/PP75) (Figs 2 and 3). The 2 species were detected in the primary culture (NNN/Schneider's media); when subcultured, *L. infantum/chagasi* disappeared and only *L. amazonensis* remained.

Discussion

The clinical characteristics described were similar to the first case reported by PRADO BARRIENTOS (1948a, 1948b), with regard to the beginning of the disease with an ulcerative lesion, the evolution to posterior satellite lesions with secondary ulceration and partial healing followed by reactivation, the slow dissemination, the involvement of the nasal mucous membrane and the poor response to treatment. These clinical manifestations of DCL were also described by other authors (VALDA, 1980; VELASCO *et al.*, 1989; CONVIT *et al.*, 1993; BARRAL-NETTO *et al.*, 1995). The atypical characteristics of the skin lesions were probably due to the co-infection by 2 *Leishmania* species. However, the typical description with nodular lesions without ulceration is not common to all cases, and the presence of ulcerative lesions is relatively frequent (PRADO BARRIENTOS, 1948a, 1948b; BITTENCOURT *et al.*, 1992; COSTA *et al.*, 1992, 1995b; BONFANTE-GARRIDO *et al.*, 1996), as well as mucosal lesions (PRADO BARRIENTOS, 1948a, 1948b; MENEZES *et al.*, 1978; VALDA, 1980; VELASCO *et al.*, 1989; COSTA *et al.*, 1992). The spontaneous partial healing has also been described (COSTA *et al.*, 1995a). The abundance of parasites and vacuolated histiocytes in the lesions, and the negative skin test, were in agreement with the diagnosis of DCL. The important development of the infection in hamsters was characteristic of *L. amazonensis* (LA FUENTE *et al.*, 1986).

The isoenzyme electrophoresis revealed the presence of 2 *Leishmania* species (*L. amazonensis* and *L. infantum/L. chagasi*) in the same lesion. The presence in the stock of 2 different species cannot be due to a laboratory contamination. Indeed, the parasites isolated directly from hamsters were not cultivated in parallel with any reference stocks, neither *L. infantum/L. chagasi* nor *L. amazonensis*. From the isoenzymatic profiles the presence of 2 *Leishmania* species in the culture was confirmed (2 different profiles) and the existence of a hybrid parasite was easily discarded (absence of hybrid profiles). Previously *Leishmania* hybrids have been identified by different techniques, but in each study the MLEE technique was always used as the basic methodology (KELLY *et al.*, 1991; BELLI *et al.*, 1994; DUJARDIN *et al.*, 1995; BAÑULS *et al.*, 1997). It was not possible to clone the 2 species because *L. infantum/L. chagasi* was eliminated in the subcultures. This phenomenon is well known when co-cultures of *Leishmania* are carried out (PACHECO *et al.*, 1987). The better

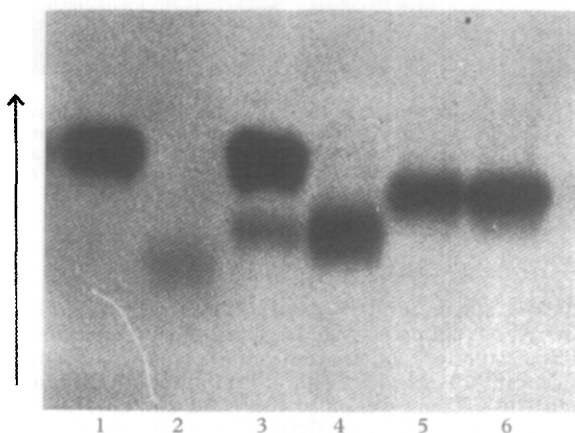


Fig. 2. Comparative isoenzyme electrophoretic profiles of glucose phosphate isomerase (GPI) between the stock isolated from the Bolivian patient and the reference strains. Key: 1, *L. infantum/L. chagasi* (MHOM/BR/74/PP75); 2, *L. mexicana* (MNYC/BZ/62/M379); 3, patient stock; 4, *L. amazonensis* (IFLA/BR/67/PH8); 5, *L. braziliensis* (MHOM/BO/83/LPZ155); 6, *L. guyanensis* (MHOM/BR/78/M5378).

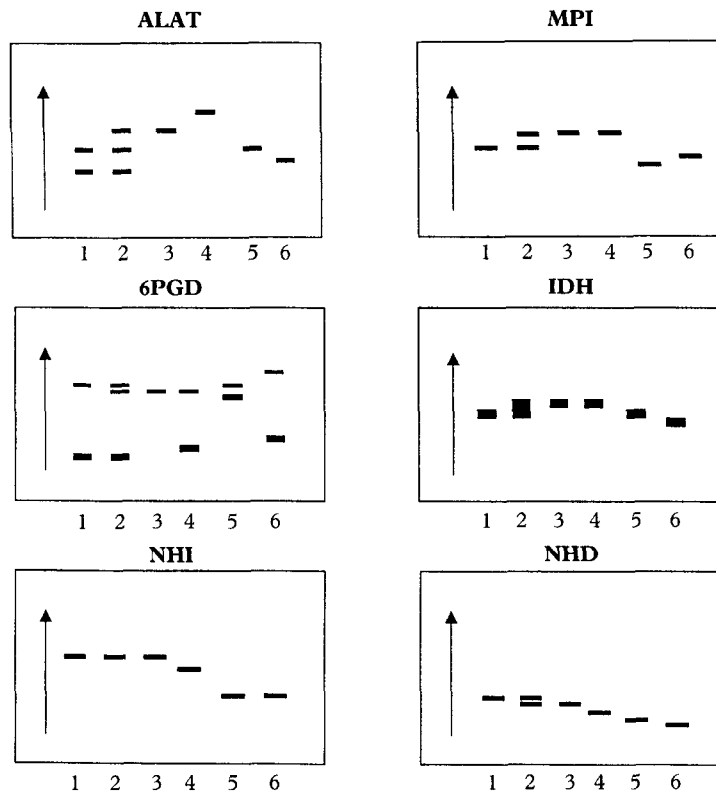


Fig. 3. Multilocus enzyme electrophoresis gel patterns obtained for the stock isolated from the Bolivian patient and the reference strains. Lane 1, *L. infantum/L. chagasi*; lane 2, patient stock; lane 3, *L. amazonensis*; lane 4, *L. mexicana*; lane 5, *L. guyanensis*; lane 6, *L. braziliensis*. For definition of the enzyme abbreviations see the main text.

adaptation and growth of *L. amazonensis* in relation to *L. infantum/L. chagasi* in culture media was probably the reason for *L. amazonensis* remaining in the subcultures. Unfortunately the hamsters died and were cremated and no other isolation of parasites nor additional studies were possible. As far as we know, there is no previous report of such mixed infection in an individual lesion. The 2 cases of co-infection reported by SILVEIRA *et al.* (1984) and by OLIVEIRA-NETO *et al.* (1986a) concerned different parasite associations (*L. braziliensis-L. amazonensis*, and *L. braziliensis-L. infantum/L. chagasi*, respectively) which were not isolated from an individual lesion. BARRAL *et al.* (1991) suspected but did not find mixed infection in foci where *L. infantum/L. chagasi* and *L. amazonensis* were circulating in sympatry.

In patients with visceral leishmaniasis, *L. infantum/L. chagasi* is usually found in bone-marrow, internal organs or ganglions. In some cases *L. infantum/L. chagasi* may be isolated from normal skin or skin lesions (VASCONCELOS *et al.*, 1993), especially in patients with post kala-azar dermal leishmaniasis (BARRAL *et al.*, 1991), or in patients showing cutaneous lesions without clinical symptoms of visceral leishmaniasis (OLIVEIRA-NETO *et al.*, 1986b).

In the clinical examination, visceral involvement was not detected, but we did not exclude this possibility because other clinical or laboratory examinations were not carried out to discard this clinical form of leishmaniasis. The reason why these assays were not performed was that the presence of *L. infantum/L. chagasi* and a co-infection was detected after the patient died. The clinical signs, including the loss of weight, swelling of the abdomen, alterations in the hair, fever, anaemia, accelerated erythrocyte sedimentation, lymphocytosis, hypoproteinaemia, hypoalbuminaemia and inversion of the albumin/globulin ratio, could have been associated with this clinical form.

Infection with *L. infantum/L. chagasi* is known to depress the immune response (MARSDEN & JONES, 1985). In this context, possibly the patient was infected previously with *L. infantum/L. chagasi* that induced an immunosuppression, and a secondary infection with *L. amazonensis* occurred as DCL. In fact, in our experience we have detected only cutaneous cases in a very prevalent focus of leishmaniasis due to *L. amazonensis* in a similar region in the same department.

In spite of repeated clinical investigations, neither suspected cases of visceral leishmaniasis nor other cases of DCL were found in the area, but several cases of typical cutaneous leishmaniasis were observed. However, in neighbouring areas cases of visceral leishmaniasis were detected. The sandfly *Lutzomyia nuneztovari anglesi* was an abundant anthropophilic species detected in the area; this species is the vector of *L. amazonensis* in another region of La Paz (MARTINEZ *et al.*, 1999).

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