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Clinical and epidemiological features of leishmaniasis in northwestern-Argentina through a retrospective analysis of recent cases

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ABSTRACT

Leishmaniasis is a parasitic disease caused by hemoflagellates of the genus *Leishmania* and is transmitted to humans by the bite of infected phlebotomine sandflies. Depending on the *Leishmania* species, the disease has different clinical forms including cutaneous, mucocutaneous, and visceral manifestations. Previous studies performed in endemic zones of northwestern-Argentina, during epidemic outbreaks, have been important for detecting patients suffering from the acute phase of the disease, but have not given a complete representation of the clinical and epidemiological features in the region. Furthermore, due to the resurgence of leishmaniasis worldwide and in particular the large increase of international tourism to the region, it seems pertinent to update the current epidemiological and clinical profile of leishmaniasis in northwestern-Argentina. Here we present a retrospective analysis of 95 *Leishmania* positive cases, presenting between 2000 and 2014. Patients were derived from hospitals and diagnosed in our lab at the University of Salta, located in a non-endemic area in Salta, Argentina. We detected numerous extensive mucocutaneous cases (34/95, 35.8%) distinct from mucosal affected patients, some instances originating in locations with no previously reported human cases. Additionally patients suffering from concomitant diseases, besides leishmaniasis, were assessed. These included Chagas disease, syphilis, deep mycoses, tuberculosis, toxoplasmosis and intestinal parasitosis. This study updates the clinical and epidemiological features of leishmaniasis in northwestern-Argentina, and discusses the implications and management strategy for patients who acquire the disease in this region.

1. Introduction

Leishmaniasis is a neglected vector-borne disease caused by *Leishmania* hemoflagellates and is associated with poverty; 350 million people are considered at risk of contracting leishmaniasis, and some 2 million new cases occur yearly (World Health Organization, 2010). Depending on the parasite species and the immune response of the host, the disease can manifest as cutaneous (CL), mucocutaneous (MCL), and visceral (VL) forms (Convit et al., 1993; Silveira et al., 2004).

In the Americas, leishmaniasis endemic areas extend from Mexico to Argentina (Reveiz et al., 2013). CL and MCL forms, referred to as American Cutaneous Leishmaniasis (ACL), are variable in terms of their clinical presentation and course ranging from asymptomatic to localized, sometimes self-healing cutaneous

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lesions to severe mutilating (MCL) lesions or diffuse cutaneous lesions (Silveira et al., 2004). VL, also present in the region, is characterized by prolonged fever, hepatomegaly, splenomegaly, and it is usually fatal if not treated adequately (Romero and Boelaert, 2010).

ACL is endemic in ten provinces of northern-Argentina, and the incidence has been increasing annually since 1980 (Salomon et al., 2012). Orán and San Martín departments (Salta province), located in northwestern-Argentina (NWA), reported 53.1% of the total number of ACL cases recorded in the country, both are considered hyper-endemic areas (Salomon et al., 2008). Exposure to the sandfly vector has been positively associated with recent ecological disturbance (deforestation), and with periurban vegetation (Salomon et al., 2008). *Leishmania (Viannia) braziliensis*, *L (V.) guyanensis* and *L. (Leishmania) amazonensis* are commonly isolated from human ACL cases although *L. (V.) braziliensis* is the main agent associated with ACL outbreaks and subsequent MCL cases (Barrio et al., 2009; Frank et al., 2003; Marco et al., 2005). With respect to VL, most of the human cases are from Misiones province, where *L. (L.) infantum* (syn. *L. chagasi*) has been isolated from humans, dogs and the sandfly vectors (Acardi et al., 2010). In some northwestern-areas of the country (Santiago del Estero and Salta provinces), rare VL cases have also been reported and the permissive vector *Lu. longipalpis* described (Bravo et al., 2013), *My. migonei* has also been implicated as a putative vector (Salomon et al., 2010).

Except for scanty reports of ACL cases (Casero et al., 2010; Romero et al., 2004), there are very few accurate clinical descriptions of CL and MCL lesions in northern-Argentina. This could adversely affect case detection or lead to clinical misdiagnosis and inadequate treatment (particularly regarding MCL). In the light of the resurgence of disease worldwide, in areas not previously thought to be endemic, and specifically the increase of foreign tourists to the region, there is an urgent need to update the current clinical and epidemiological profile of leishmaniasis in NWA. In this context, we present a retrospective analysis of 95 confirmed *Leishmania* positive case-series. We describe the clinical forms, implicated species, and a differential diagnosis suggesting an optimized case detection and management strategy for diagnosed individuals. We also report on concomitant infections associated with patients infected with leishmaniasis.

2. Methods

2.1. Study population and data collection

The study population initially comprised 346 suspected leishmaniasis cases, referred from Señor del Milagro and San Bernardo hospitals in Salta city, Argentina. The patients were assessed at the laboratory of microbiology (lab-UNSa; Facultad de Ciencias de la Salud, Universidad Nacional de Salta), during the period 2000–2014, for a confirmatory diagnosis. Only patients with positive parasitological diagnosis (microscopy and/or culture) for *Leishmania* were included in the analysis. Patient data was collected by means of a semi-structured questionnaire and hospital clinical records. Structured questions derived personal, and demographic information, and open questions epidemiologic and clinical data. Briefly, questions encompassed the reason for consultation, place of exposure (contact with the vector), occupation at the time of infection, antecedents of the current disease, and other pathological antecedents. Particular note was taken of data collected outside of known endemic areas. All patients provided written informed consent and were subjected to a physical clinical examination. Suspected cases were referred for laboratory diagnosis (below), and a blood sample was also taken for Chagas' disease serodiagnosis. At the hospitals, further laboratory tests were undertaken to detect bacterial and fungal superinfections, and other concomitant pathologies (including mycoses, syphilis, toxoplasmosis and tuberculosis).

All confirmed cases were referred for treatment, provided by the Argentinian Ministry of Health. The first-line therapy in this region is meglumine antimoniate, (20–850 mgSb^{5+}/day) administered twice daily by intramuscular injections over a period of 3 weeks for CL or 4 weeks for MCL (Ministerio de Salud Pública, 2004).

2.2. Leishmania diagnosis

2.2.1. Microscopic examination

Dermal scrapings were taken from ACL suspected lesions, stained with May Grünwald-Giemsa, and amastigotes visualized microscopically according to published protocols (Barrio et al., 2007). All described diagnostic procedures (smears, cultures and PCR) were performed in triplicate.

2.2.2. Culture

Material from the peripheral edges of suspected CL and MCL lesions was aspirated by syringe containing 0.5 mL of sterile proline balanced salt solution supplemented with 100 U/mL penicillin and 50 μg/mL streptomycin, seeded into USMARU culture medium supplemented with 20% defibrinated rabbit blood, and incubated at 23 °C (Barrio et al., 2009).

2.3. Leishmania species identification

A sample of skin/mucosal lesion was placed in a microcentrifuge tube containing 300 μL TE, boiled for 10 min, and stored at −20 °C until use. Polymorphism Specific–PCR (PS-PCR) was performed for the identification of *Leishmania* species (Barrio et al., 2009). In more detail, samples were thawed prior to use, centrifuged at 14,000 rpm for 1 min, and 10 μL of supernatant used for the reaction. PS-PCR was performed in two-stages: Initially, using primers V1–V2 and L1–L2 for subgenera identification (*V. (Viannia)* and *L. (Leishmania))*, and secondly, species specific primers, as reported by Barrio et al. (2009). For suspected VL cases (based on clinical examination), bone-marrow aspirates were performed followed by microscopic examination of smears, culture and DNA extraction as described above. Nested-PCR was performed with VL specific primers, targeting the cytb-gene for confirmatory diagnosis and subsequent DNA sequencing for species identification (Barrio et al., 2012).

2.4. Anti-T. cruzi antibodies serology

Plasma samples were obtained by centrifugation of 20 mL EDTA anticoagulated blood and stored as 2 mL aliquots at −80 °C. Anti-T. cruzi antibodies analysis was performed using a recombinant enzyme-linked immunosorbent assay (recombinant ELISA v.3.0, Wiener lab, Argentina), following the manufacturers protocol.

2.5. Statistical analysis

Categorical variables are described in detail below (2.5.1) and included gender, clinical forms, activity in relation to exposure and infection, *Leishmania* species, geographical place of infection and presence of concomitant infectious pathologies. Frequencies and association of variables were assessed by Pearson's Chi-square test (IBM® SPSS® Statistics Version 21). Continuous variables (patient age and lesion age) described by means, medians, standard deviation and range were compared by the *T* test or Mann–Whitney test, depending the normality of distribution (GraphPad Prism® Software Version 5.01). Differences were considered statistically significant if *p*-values were <0.05.

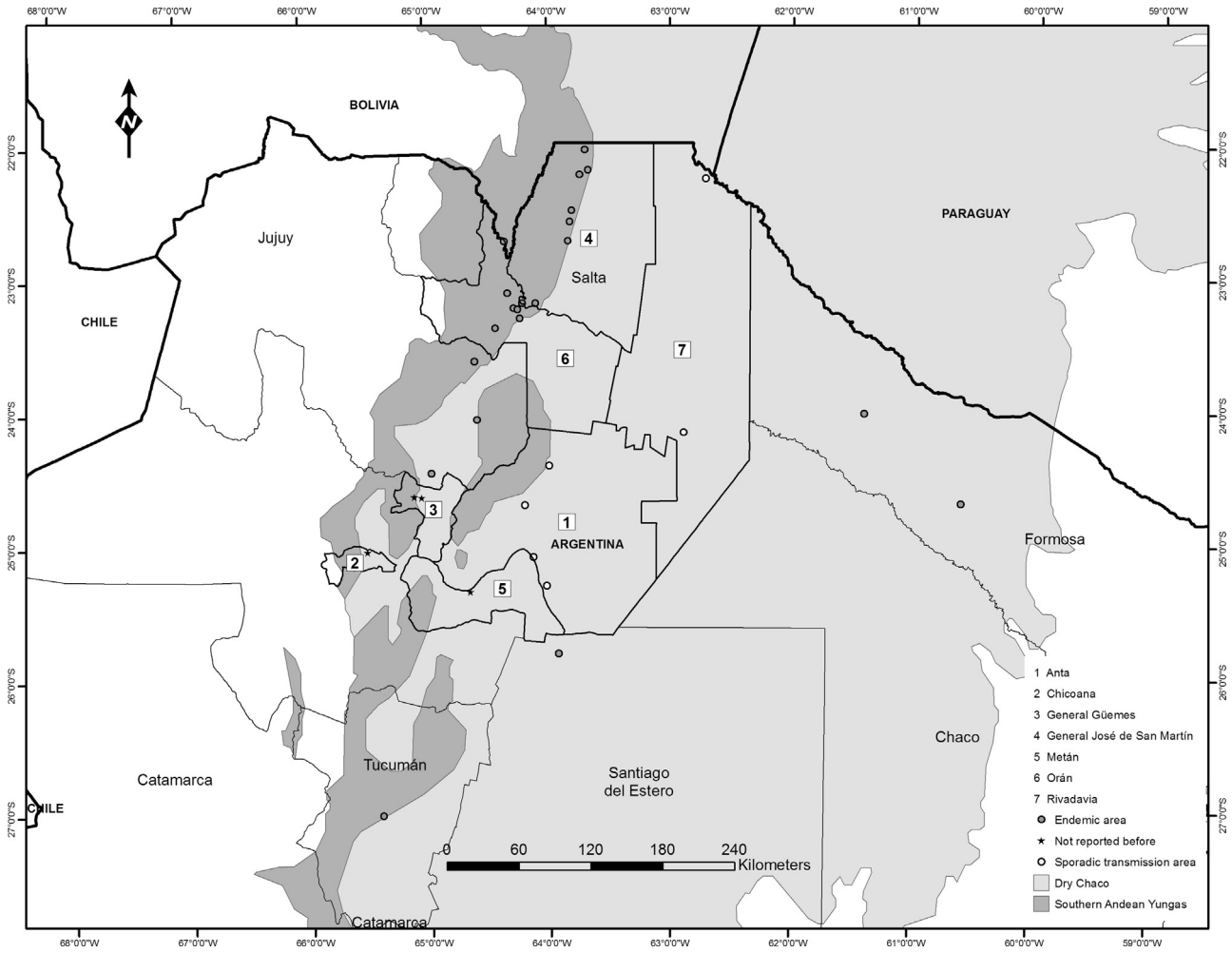

Fig. 1. Incidence of leishmaniasis in northwestern-Argentina. Map indicates location of study area. Light gray area indicate dry Chaco region, dark gray areas the Southern Andean Yungas region. Locations within Salta and other provinces showing the locations of case detection.

2.5.1. Clinical definition of variables

Age: Patients age at diagnosis.

Lesions age: For CL, defined here as the time the patient first reported the appearance of lesions until the time of consultation. For MCL forms, lesion age was considered as the time the patient first reported the appearance of lesions or mucosal symptoms until consultation.

Clinical presentation: CL is characterized by an ulcer with raised edges and a sharp crater. The ulcer is usually painless (unless superinfected with a secondary infection) and can take years to heal, leaving a scar with depressed rounded edges which can also be irregular. Depending on the *Leishmania* species and the immune response of the host, an ulcer may selfheal, spread or metastasize to the nasopharyngeal tract (MCL). MCL typically occurs after an initial skin lesion, usually presenting in the nasal mucosa and/or palate. Early lesions appear as infiltrated, ulcerated mucosa of the nasal septum and inferior turbinates. Extensive MCL is defined as the involvement of palate, pharynx and larynx (Soto et al., 2007). Mild MCL is defined as discrete involvement of the nasal skin and/or mucosa without perforation of nasal septum (Soto et al., 2007). Lesions are progressive and destruction of mucosa and even cartilage can sometimes occur concomitantly with skin lesions (concomitant mucosal form).

Location of infection: Geographical location where the patient may have been in contact with the vector was determined via questionnaire. Details covered a period of years, with particular regard to patients presenting with MCL.

Activity: Refers to occupation, place of abode, and related activities/occupation at the time of contracting the disease. Details relating to the fieldwork in rural zones (felling, agriculture and/or ranching, farming, etc.) or recreational activities (fishing, hiking, hunting, etc.) were also recorded.

Species: The *Leishmania* sp. involved, in cases where the molecular identification was possible.

2.6. Ethics statement

The study was conducted according the principles expressed in the declaration of Helsinki and approved by the authors' institutional review boards: Ethical Committee of Faculty of Health, National University of Salta, and Ministry of Public Health of Salta, Argentina. All adult subjects provided informed written consent, and a parent or guardian child participants provided written informed consent on their behalf.

3. Results

3.1. Demographic and clinical features

From a total of 346 suspected cases, 95 patients who had at least one positive parasitological test for leishmaniasis were included in the study. Table 1 shows the demographic and clinical peculiarities of these patients. The majority (78.9%) were male, the mean age was 39.1 years old, and the number of cases among 20

Table 1
Demographic and clinical features of leishmaniasis in Salta, Argentina, 2000–2014.

Characteristic	No. (%) patients
Gender	
Men	75 (78.9%)
Women	20 (21.1%)
Age	
Mean (SD)	39.1 (17.9%)[a]
00–19	16 (16.8%)
20–49	54 (56.9%)
≥50	25 (26.3%)
Clinical forms[b]	
Cutaneous (CL)	50 (52.6%)
Mucocutaneous (MCL)	43 (45.3%)
Visceral (VL)	2 (2.1%)
Occupation/housings/activities in relation to the transmission	
Rural work[c]	58 (61.1%)
Peridomestic/rural transmission[d]	20 (21%)
Recreational trips to endemic zone	4 (4.2%)
Not known	13 (13.7%)

[a] Data are as indicated in the left column.
[b] There was association between clinical forms regarding age ranges ($p = 0.015$). No association was found between CL and MCL regarding gender ($p = 0.691$) or activities in relation to the transmission ($p = 0.4595$). Pearsonís Chi square test.
[c] Children and women who accompany to the family to rural tasks are included.
[d] Persons who reside in rural/periurban areas.

and 49 years old (54/95, 56.9%) was higher in comparison with other age groups. The most frequent clinical presentation was CL (50/95, 52.6%), followed by MCL (43/95, 45.3%). There was a clear association between CL and MCL clinical forms regarding age ($p = 0.015$): Most patients aged 0–19 years (78.6%) presented with the cutaneous form, whereas the majority of patients older than 50 years (69.6%) presented with MCL. No association was observed ($p = 0.691$) between clinic presentation and gender. Only two VL cases (2/95, 2.1%) were identified.

Regarding the demographic circumstances of disease acquisition, most cases (58/95, 61.1%) were related with the place of work in rural zones (felling, agriculture and/or ranching, masonry etc. Twenty (21%) of 95 patients resided in known risk areas for leishmaniasis (rural/periurban housings), most of which were precarious, with corrals containing domestic animals including goats and pigs in close proximity to areas of leafy vegetation and/or waterways (according to questionnaireís data). Only 4/95 patients (4.2%) reported visiting previously known endemic zones. No information was available for 13 (13.7%) of 95 subjects studied.

3.2. Location of infection

Fig. 1 shows the study area and locations of detected cases. Seventy four individuals (77.9%) acquired the infection in Salta province. Forty-nine (66.2%) of the 74, acquired the disease in zones previously identified as endemic (Orán [22/74, 29.7%] and San Martín [27/74, 36.5%] departments). The remaining 33.8% became infected outside of endemic areas, some of them in places previously reported to be sporadic transmission's areas (Anta [18/74, 24.3%] and Rivadavia [2/74, 2.7%] departments). However 6.8% of cases were from locations which, according to the available literature, had no reported human cases. (General Güemes [2/74, 2.7%], Metán [1/74, 1.4%] and Chicoana [2/74, 2.7%] departments). Nine (9.5%) patients acquired the disease in Argentina, but outside Salta: Jujuy, 5.4% (5/95); Formosa, 2.1% (2/95); Tucumán, 1% (1/95) and Santiago del Estero, 1% (1/95). Three patients acquired the infection in other countries: Bolivia, 2.1% (2/95) and Brazil, 1% (1/95). In nine cases (9.5%) it was not possible to determine the location of exposure.

3.3. Diagnostic test

Of 95 positive patients, parasites were microscopically visualized in 87 and parasites recovered in culture in 37 samples; eight samples showed parasites only through culture.

3.4. Species identification

PS-PCR and cytb-gene amplification were performed in order to identify the causative agent to species level. Using a total of 53 specimens (25 clinical samples and 28 cultures), it was determined that *L. (V.) braziliensis* was responsible for 81.1% (43/53) of the cases analyzed, whereas *L. (L.) amazonensis*, *L. (L.) infantum* and *L. (V.) guyanensis* were the causative agents of 13.2% (7/53), 3.8% (2/53) and 1.9% (1/53) of cases, respectively.

Table 2 shows the distribution of *Leishmania* species regarding with different clinical forms of the disease and the location where the disease was acquired. *L. (V.) braziliensis* was apparent in all ATL patients from several geographical areas. *L. (L.) amazonensis* was only identified in patients from Anta and San Martín, and *L. (L.) infantum* was only present in VL patients from San Martín and Rivadavia, respectively. Eight individuals with *L. (V.) braziliensis* did not acquire their infections in Salta, 5 from Jujuy province in the localities of Aguas Calientes, Puesto Viejo, Ledesma and San Pedro respectively, 1 from Pellegrini in Santiago del Estero province, 1 from Las Lomitas, Formosa and 1 from Famaillá in Tucumán province. *L. (V.) guyanensis* was identified from an isolate from an American patient who acquired the disease in the Amazonian region, Brazil. In this instance it was not possible to establish associations of infective species, clinical form or place where the disease was acquired.

3.5. Clinical pathology

Table 3 shows the clinical characteristics of CL patients (50/95, 52.6%), who possessed lesions with a median evolution time of 2.5 months (range 0.5–18 months). Thirty-three (34.7%) of the total patients presented single cutaneous lesions, affecting the hands, feet and face. Seventeen (17.9%) patients presented with multiple cutaneous lesions at different sites, and one revealed a disseminated clinical form (100 or more quickly spreading cutaneous ulcers and acne-like lesions, which appeared to metastasize from a single or few bite-lesions).

Table 4 shows the clinical characteristics of 43 MCL patients analyzed. The median evolution time of lesions was 19 months (range 2–360 months), significantly different respect to evolution time of CL lesions ($p < 0.001$). Thirty four (79.1%) patients presented extensive disease (Fig. 2). Two patients subsequently died due to the severity of the pathology (both had severe involvement of the pharyngolaryngeal mucosa). One patient, with significant inflammation of the larynx, suffered death by asphyxiation, the other died of airway obstruction due an excess of mucosal secretions. Only 9/43 (20.9%) patients presented with mild disease. Nine (20.9%) of 43 patients had concomitant mucosal pathology and active cutaneous lesions. Two (2.1%) of the 95 patients presented with VL, a 1-year-old child and a 44-year-old male (Barrio et al., 2012). Both were diagnosed several months after the appearance of symptoms, which included a long-lasting fever, hepatomegaly, splenomegaly, pancytopenia, and increased levels of gamma-globulins. These patients did not present CL or MCL concomitant lesions.

3.6. Secondary microbial infections

Forty ACL patients subjected to additional microbiological analyses revealed *Staphylococcus aureus* as the most frequently isolated microorganism at the site of the lesion (24/40, 60%), fol-

Table 2
Species specific *Leishmania* infection, clinical forms and location of exposure.

Species	*L. (V.) braziliensis*	*L. (L.) amazonensis*	*L. (L.) infantum*	*L. (V.) guyanensis*
No. (%)	43 (81.1%)	7 (13.2%)	2 (3.8%)	1 (1.9%)
Clinical form				
Single	13/43 (30.3%)	5/7 (71.4%)	–	1/1 (100%)
Multiple	10/43 (23.2%)[a]	–	–	–
Mucocutaneous	20/43 (46.5%)[b]	2/7 (28.6%)	–	–
Visceral	–	–	2/2 (100%)	–
Place where the disease was acquired				
Argentina				
Salta province				
Orán	14/43 (32.6%)	–	–	–
San Martín	10/43 (23.3%)	3/7 (42.9%)	1/2 (50%)	–
Anta	8/43 (18.5%)	4/7 (57.1%)	–	–
Chicoana	2/43 (4.5%)	–	–	–
Rivadavia	1/43 (3.0%)	–	1/2 (50%)	–
Other provinces				
Jujuy	5/43 (11.5%)	–	–	–
Formosa	1/43 (2.2%)	–	–	–
Santiago del Estero	1/43 (2.2%)	–	–	–
Tucumán	1/43 (2.2%)	–	–	–
Brazil	–	–	–	1/1 (100%)

[a] One patient displayed the disseminated clinical form.
[b] Four patients displayed concomitant CL and MCL forms.

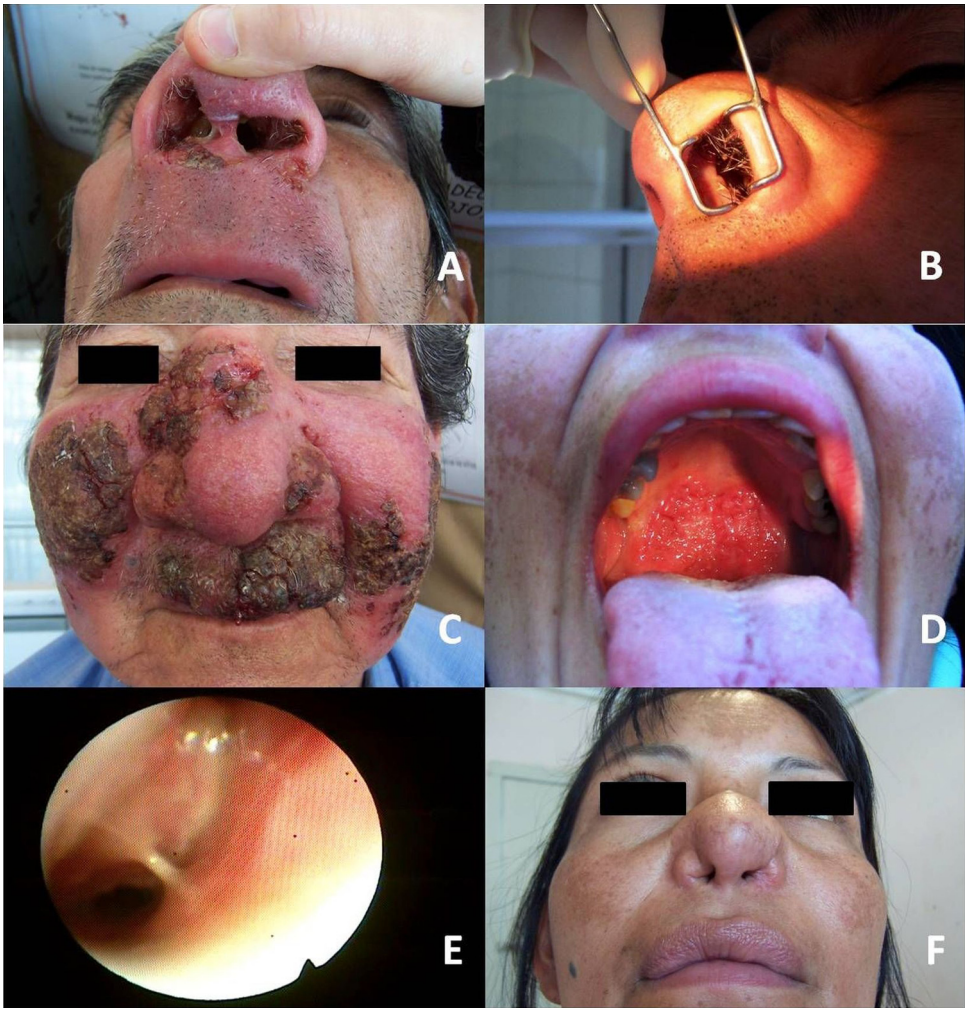

Fig. 2. Extensive mucocutaneous leishmaniasis (MCL) in patients from Salta-Argentina. (A) Characteristic nasal mucosal involvement, with extension to nasal epidermis and to upper lip. (B) MCL sequelae: necrosis in the cartilaginous tissue and perforation of nasal septum. (C) MCL concomitant with extended lesions in face skin. (D) Typical granulomatous MCL lesion in palate. (E–F) Disfiguring synechiae of MCL, causing obstruction in the pharynx and nasal passage respectively. (E) Videofluoroscopy of nasal synechiae (patient F). (F) Stenosis of nasal cavity in the patient E.

Table 3
Clinical characteristics of CL patients in Salta, Argentina, 2000–2014.

Characteristic	No. (%) patients
Number of CL-patients	50/95 (52.6%)
Single lesion	33/95 (34.7%)
Head	10 (10.5%)
Face	9 (9.5%)
Ear	1 (1%)
Upper limb	12 (12.6%)
Upper arm	2 (2.1%)
Forearm	3 (3.2%)
Hand	7 (7.3%)
Lower limb	11 (11.6%)
Above knee	1 (1%)
Below knee	4 (4.2%)
Foot	6 (6.4%)
Multiple lesions[a]	17/95 (17.9%)
2	9 (9.5%)
≥3[b]	8 (8.4%)
Lesions age in months (median, min–max)[c]	2.5 (0.5–18)[d]

[a] The presence of multiple lesions includes other locations in addition to those described for single lesions: scalp, neck and trunk.
[b] One patient displayed the disseminated clinical form (Silveira et al., 2004).
[c] Significant differences was found between lesions age in CL and MCL ($p < 0.001$). Mann–Whitney test.
[d] Data are as indicated in the left column.

Table 4
Clinical characteristics of MCL patients in Salta, Argentina, 2000–2014.

Characteristics	No. (%) patients
Number of patients	43/95 (45.3%)
CL active concomitant lesions	9/95 (9.5%)
Mucosal severity[a]	
Mild disease	9/43 (20.9%)
Extensive disease	34/43 (79.1%)
Lesions age in month, median (min–max)[b]	19 (2–360)[c]

[a] Mild disease: involvement of the nasal skin and mucosa only. Extensive disease: involvement of the palate, pharynx and larynx (Soto et al., 2007).
[b] Significant differences was found between lesions age in CL and MCL ($p < 0.001$). Mann–Whitney test.
[c] Data are as indicated in the left column.

lowed by *Candida albicans* (5/40, 12.5%), *Pseudomonas aeruginosa* (4/40, 10%), *Streptococcus pyogenes* (2/40, 5%) and *Proteus mirabilis* (2/40, 5%). Less frequently, other bacterial included *Staphylococcus epidermidis*, *Staphylococcus intermedius*, *Enterococcus faecalis*, *Escherichia coli*, *Enterobacter* sp., *Acinetobacter* sp. and *Citrobacter freundii*. Patients with MCL also exhibited co-infections with *Streptococcus pneumoniae* (2/40, 5%), *Klebsiella pneumoniae* (2/40, 5%), *Haemophilus* sp. and *Moraxella catarrhalis* (1/40, 2.5%). Eleven patients (11/40, 27.5%) had no associated secondary infections.

3.7. Concomitant pathologies

We recorded 39 patients with concomitant pathologies. Regarding infectious pathology these included Chagas disease (64.1%, 25/39), toxoplasmosis (2/39, 5.1%), syphilis (4/39, 10.2%), tuberculosis (3/39, 7.7%), coccidioidomycosis (*Coccidioides posadasii*, 1/39, 2.6%), paracoccidioidomycosis (*Paracoccidioides brasiliensis*, 2/39, 5.1%), and intestinal parasitosis (2/39, 5.1%). Of these patients 4 exhibited multiple infectious pathologies: syphilis and Chagas disease (1/39, 2.6%); toxoplasmosis, tuberculosis and coccidioidomycosis (1/39, 2.6%); syphilis and tuberculosis (1/39, 2.6%); tuberculosis and intestinal parasitosis (1/39, 2.6%). Most patients with concomitant infectious pathologies presented with the mucosal form of the disease (62.5%; $p = 0.0320$). Regarding non-infectious pathology, alcoholism was present in (11/39, 28.2%) of cases and diabetes in 7.7% of cases (3/39).

4. Discussion

This study was based on the retrospective analysis of a case-series, updating the clinico-epidemiological features of 95 clinically confirmed leishmaniasis cases in NWA from 2000 to 2014. The study reveals a high prevalence of extensive MCL forms from endemic locations and also from locations not previously thought to be endemic.

Previous studies in our region, revealed positive results for ELISA and/or the Montenegro skin test (Gil et al., 2010), both of which lack specificity and have the potential of cross-reaction with other infectious agents producing false positive results (Barrio et al., 2007; Frank et al., 2003), in contrast with the diagnostic approach employed in this study. The unknown incidence of asymptomatic infections, and the ambiguity of individuals infection history can often lead to flawed epidemiological assumptions. In the present study, each patient was subjected to an exhaustive case history, using a semi-structured questionnaire where particular attention was paid to areas where cases were not reported previously. In many cases, using this approach, we were able to establish the specific location where the infection was acquired. The north of Salta province has been considered geographically at risk and an endemic for leishmaniasis (Salomon et al., 2001a; Sosa-Estani et al., 2001, 2000, 1998). The majority of patients acquired the disease in this endemic area (Orán and San Martín departments, corresponding to Yungas region). However, we determined that 33.8% (25/74) of the patients examined from Salta acquired the disease outside of the endemic area, and some of them in places without previous reports of human cases. These sites of concern are from the Chaco-region (Dry Chaco) and Yungas region including the department of Chicoana (El Carril, 30 km close to the capital city) (Fig. 1).

We did not find previous reports of clinical cases from General Güemes, Metán and Chicoana departments. Bernasconi described an area of possible transmission between parallels 22 and 25, and meridians 63 and 66, within Salta and Jujuy (Bernasconi, 1928). While General Güemes is situated inside this possible transmission area, Metán and Chicoana are located outside of this area. The implication is that potential areas for active transmission may be more extensive than previously reported. A recent study of the distribution and abundance of sandflies in Argentina (Quintana et al., 2012) concluded that within the Dry Chaco there are sporadic transmission "hot spots". The same authors used distribution models to predict the "potential" presence of permissive vectors throughout the Chaco region (Quintana et al., 2013). Our findings of human infections corroborate that active vectorial transmission occurs in these areas. Concerning VL, two cases were reported, one infant (1 year) from Santa Victoria Este (Rivadavia department), and a 44 year old male rural worker (Barrio et al., 2012) who was frequently bitten in a deforested area on the jungle periphery. These two human cases were infected in deforested regions. Although no firm conclusions can be drawn from two VL cases, it is known that deforestation and other ecological modifications can affect transmission dynamics in the enzootic cycle, leading to an increase in human exposure to infected vectors (Salomon et al., 2010, 2008). In contrast, the Misiones province (the most affected area of the country), with approximately 80% of total VL cases, is characterized by foci of urban transmission primarily affecting children between 0 and 15 years of age (Gould et al., 2013).

The majority of affected individuals were men between 20 and 49 years of age, in agreement with previous reports (Sosa-Estani et al., 2001) as infection is strongly associated with male dominated labour oriented activities. Indeed, 61.1% of the present patients were rural workers performing various field activities, including treefelling, farming, and bricklaying. We also report a low occurrence of the disease among women, youngsters and children, who normally do not perform rural work activities, but accompanied

men to their place of work or lived in periurban zones close to rural areas. These observations suggest that the risk of human-vector contact is still associated with a rural transmission cycle (Salomon et al., 2008, 2001b).

Fifty patients presented with CL. The disseminated CL form (Silveira et al., 2004) was infrequent in the studied group (1/50, 2%; Table 2). The prevalence of MCL was similar to CL and not significantly different between genders ($p = 0.691$). Previous reports from Salta, indicated that CL was more frequent than MCL. Sosa Estani et al. (1998) and Frank et al. (2003) reported a 2.6% and 2.4% of patients with MCL, respectively. However, these previous studies focused on hyperendemic zones and/or during epidemic outbreaks and detected patients suffering the acute phase of the disease (where CL is typical), so may not describe the complete epidemiological picture in nonepidemic scenarios. Some reports from other areas of Argentina (provinces of NEA) also show a predominance of CL over the MCL form, 10.5% MCL in Formosa between 1992 and 2001 (Salomon et al., 2002), and 10% MCL in Corrientes, Formosa and Chaco, diagnosed between 1988 and 2000 (Borda et al., 2002). The higher number of MCL cases in our data series may be explained, in part, as many CL patients are diagnosed and treated locally for acute stages while most patients referred for definitive diagnosis to our lab, are in the chronic phase of the disease (≥50 days). Alternatively, Salta Ministry of Healthís data suggests that the relative number of MCL cases has increased, from 3% to 27% during the period 2002–2012 (Garcia Bustos et al., 2014). Salomon et al. (2012) explain that the decrease of incident cutaneous cases, and the past cutaneous epidemics, resulted in an increase in the ratio of mucosal to cutaneous cases. However, this implies that these patients were not treated or they had treatment failure.

The relative lack of data is in part due to the scarcity of reporting and consequence of a lack of proper diagnosis as MCL may concur with other pathologies. In the current study 43 patients were diagnosed with MCL, 34 with involvement of the palate, pharynx, and larynx (extensive disease, Fig. 2). Our data support the notion that MCL is more common than previously thought, and also suggests that the extensive MCL form is significantly more frequent than the mild MCL form. This is important when taking into account the severity of extensive MCL form which results in disfigurement, disability with associated psychological and social repercussions. Tragically in our study group two patients died as consequence of the severity of MCL pathology. In the same way, due to a lack of proper diagnosis, the number of VL cases could be higher than the cases reported up to now. It is clear that more details regarding different clinical variants and severity of leishmaniasis in NWA are needed. One specific consequence of this work is the contribution to the increase of case detection and of patients receiving treatment.

Concerning the implication of *Leishmania* species, *Leishmania (V.) braziliensis* was identified in the majority (81.1%) of cases of ACL, followed by *L. (L.) amazonensis* (Table 2) consistent with previous studies from our group (Barrio et al., 2009) and in accordance with Frank et al., who identified *L. (V.) braziliensis* and *L. (L.) amazonensis* by MLEE (Frank et al., 2003). However, unlike of the present work, Frank et al. did not specify the geographical location where the patients acquired infection. Marco et al. (2005) identified *L. (V.) braziliensis* and *L. (V.) guyanensis* also by MLEE, in patients from Salta and Corrientes respectively. The patient identified by us with *L. (V.) guyanensis* did not acquire the infection in Salta, but in Brazil. All our previous studies identified *L. (V.) braziliensis* as responsible for all MCL cases (Barrio et al., 2009), and *L. (L.) amazonensis* was only present in CL patients (Barrio et al., 2009; Garcia Bustos et al., 2011). In this study, we detect two patients with mucosal involvement infected with *L. (L.) amazonensis* (Table 2). Nested-PCR (cytb-gene) revealed that the two VL cases were due to the infection with *L. (L.) infantum* (Barrio et al., 2012). Of note, six *L. (V). braziliensis* cases, identified from cultures, originated from patients who acquired the disease outside of Salta, in Jujuy, Santiago del Estero, and Formosa. Interestingly, for the first time we report *L. (V). braziliensis* species, in Formosa.

A distinctive aspect of the present work is the identification of other microorganisms responsible for concomitant pathologies. Regarding bacterial infections, patients were often secondarily infected with *S. aureus* (60%), *C. albicans* (12.5%), *P. aeruginosa* (10%), *S. pyogenes* (5%) and *P. mirabilis* (5%). Secondary bacterial infection can increase tissue degradation and lead to delays in lesions healing and prolong the evolution of the disease (Ziaie and Sadeghian, 2008). Although the microbiological analysis of the lesions should be a routine practice followed up by antibiotics treatment we frequently observed that it is not performed as a matter of routine.

NWA is known as an endemic region for several infectious diseases (Frank et al., 2003; Romero et al., 2004) and some display clinical presentations superficially similar to leishmaniasis. Differential diagnoses for each patient, allowed us to identify co-infection of leishmaniasis with other pathologies including Chagas disease, tuberculosis, syphilis, paracoccidioidomycosis, coccidioidomycosis, toxoplasmosis and/or enteroparasitosis. To avoid cross reaction between Chagas disease and leishmaniasis, an Anti-T. cruzi antibodies analysis was performed using a commercial kit based on the use of recombinant T. cruzi specific proteins as antigens (Chiaramonte et al., 1996; Frank et al., 2003; Vega Benedetti et al., 2013). Three patients with tuberculosis also suffered from other infections in addition leishmaniasis (syphilis, toxoplasmosis and coccidioidomycosis, and intestinal parasitosis, respectively). Coinfection with *Mycobacterium tuberculosis*, is known to inhibit the host immune system exacerbating leishmaniasis; conversely, infection with *Leishmania* parasites can alter the protective immune response to BCG vaccination (Li and Zhou, 2013). Furthermore, different factors play a role in the development of concomitant pathologies, among them malnutrition, housing and educational. Poverty is often associated with malnutrition and a higher burden of infectious diseases, potentially increasing the severity of individual clinical manifestations (Alvar et al., 2006; Werneck et al., 2011). Nutritional intervention could enhance the effectiveness of treatments for ACL (Oliveira et al., 2013).

Currently the World Health Organization and National Argentinian guidelines for the control of leishmaniasis do not recommend vector control campaigns, as they are not cost-effective. Early detection of ACL is at present the main prophylactic measure. Therefore, prevention relies mainly on rapid diagnosis and early treatment (Ministerio de Salud Pública, 2004). Here we described the clinico-epidemiological features of leishmaniasis in Salta, Argentina, and neighboring areas. Knowledge of the clinical presentation and current geographic distribution of the disease are necessary for a robust confirmation of diagnosis without which the patients cannot receive appropriate treatment. The present study contributes to the clinical manifestations presented and the *Leishmania* species circulating in the region including cases from areas previously considered free from vectorial transmission.

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References

Acardi, S.A., Liotta, D.J., Santini, M.S., Romagosa, C.M., Salomon, O.D., 2010. Detection of *Leishmania infantum* in naturally infected *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae) and *Canis familiaris* in Misiones, Argentina: the first report of a PCR-RFLP and sequencing-based confirmation assay. Mem. Inst. Oswaldo Cruz 105, 796–799.

Alvar, J., Yactayo, S., Bern, C., 2006. Leishmaniasis and poverty. Trends Parasitol. 22, 552–557.

Barrio, A., Garcia Bustos, M.F., Mora, M.C., Parodi Ramoneda, C.M., Ramos, F., Moreno, S., Basombrio, M.A., 2009. Identification by PS-PCR of *Leishmania* species and its correlation with clinical epidemiologic. Rev. Argent. Salud Pública 1, 30–33.

Barrio, A., Mora, M.C., Ramos, F., Moreno, S., Samson, R., Basombrio, M.A., 2007. Use of kDNA-based polymerase chain reaction as a sensitive and differentially diagnostic method of American tegumentary leishmaniasis in disease-endemic areas of northern Argentina. Am. J. Trop. Med. Hyg. 77, 636–639.

Barrio, A., Parodi, C.M., Locatelli, F., Mora, M.C., Basombrio, M.A., Korenaga, M., Hashiguchi, Y., Garcia Bustos, M.F., Gentile, A., Marco, J.D., 2012. *Leishmania infantum* and human visceral leishmaniasis, Argentina. Emerg. Infect. Dis. 18, 354–355.

Bernasconi, V.E., 1928. Contribución al estudio de la distribución geográfica de la leishmaniosis en la República Argentina. Bol. Inst. Clin. Quirug B. Aires 15, 325–328.

Borda, C.E., Rea, M.J.F., Rosa, J.R., 2002. Diagnóstico y tratamiento de la leishmaniasis tegumentar Americana en el Nordeste Argentino. Rev. Divulg. Cient. Tecnol. 2002, M-055.

Bravo, A.G., Quintana, M.G., Abril, M., Salomon, O.D., 2013. The first record of *Lutzomyia longipalpis* in the Argentine northwest. Mem. Inst. Oswaldo Cruz 108, 1071–1073.

Casero, R., Laconte, L., Fraenza, L., Iglesias, N., Quinteros Greco, C., Villablanca, M., 2010. Recidivant laryngeal leishmaniasis: an unusual case in an immunocompetent patient treated with corticosteroids. Rev. Argent. Microbiol. 42, 118–121.

Convit, J., Ulrich, M., Fernandez, C.T., Tapia, F.J., Caceres-Dittmar, G., Castes, M., Rondon, A.J., 1993. The clinical and immunological spectrum of American cutaneous leishmaniasis. Trans. R. Soc. Trop. Med. Hyg. 87, 444–448.

Chiaramonte, M.G., Zwirner, N.W., Caropresi, S.L., Taranto, N.J., Malchiodi, E.L., 1996. *Trypanosoma cruzi* and *Leishmania* spp. human mixed infection. Am. J. Trop. Med. Hyg. 54, 271–273.

Frank, F.M., Fernandez, M.M., Taranto, N.J., Cajal, S.P., Margni, R.A., Castro, E., Thomaz-Soccol, V., Malchiodi, E.L., 2003. Characterization of human infection by *Leishmania* spp. in the Northwest of Argentina: immune response, double infection with *Trypanosoma cruzi* and species of *Leishmania* involved. Parasitology 126, 31–39.

Garcia Bustos, M.F., Barrio, A., Parodi, C., Beckar, J., Moreno, S., Basombrio, M.A., 2014. Miltefosine versus meglumine antimoniate in the treatment of mucosal leishmaniasis. Medicina (B. Aires) 74, 371–377.

Garcia Bustos, M.F., Barrio, A.B., Parodi Ramoneda, C.M., Ramos, F., Mora, M.C., Convit, J., Basombrio, M.A., 2011. Immunological correlates of cure in the first American cutaneous leishmaniasis patient treated by immunotherapy in Argentina. A case report. Invest. Clin. 52, 365–375.

Gil, J.F., Nasser, J.R., Cajal, S.P., Juarez, M., Acosta, N., Cimino, R.O., Diosque, P., Krolewiecki, A.J., 2010. Urban transmission of American cutaneous leishmaniasis in Argentina: spatial analysis study. Am. J. Trop. Med. Hyg. 82, 433–440.

Gould, I.T., Perner, M.S., Santini, M.S., Saavedra, S.B., Bezzi, G., Maglianese, M.I., Antman, J.G., Gutierrez, J.A., Salomon, O.D., 2013. Visceral leishmaniasis in Argentina cases notification and distribution of vectors (2006–2012). Medicina (B. Aires) 73, 104–110.

Li, X.X., Zhou, X.N., 2013. Co-infection of tuberculosis and parasitic diseases in humans: a systematic review. Parasit. Vectors 6, 79.

Marco, J.D., Barroso, P.A., Calvopina, M., Kumazawa, H., Furuya, M., Korenaga, M., Cajal, S.P., Mora, M.C., Rea, M.M., Borda, C.E., Basombrio, M.A., Taranto, N.J., Hashiguchi, Y., 2005. Species assignation of *Leishmania* from human and canine American tegumentary leishmaniasis cases by multilocus enzyme electrophoresis in North Argentina. Am. J. Trop. Med. Hyg. 72, 606–611.

Ministerio de Salud Pública, 2004. Resolución 386/2004: Manual de Procedimientos para el Nivel Gerencial y Profesional sobre Leishmaniosis. Buenos Aires, 5 de Mayo de 2004. http://www.msal.gov.ar/pngcam/resoluciones/msres386_2004.pdf. (accessed 29.08.15.).

Oliveira, A.G., Brito, P.D., Schubach, A.O., Oliveira, R.V., Saheki, M.N., Lyra, M.R., Salgueiro, M.M., Terceiro, B.F., Pimentel, M.I., Vasconcellos, E.C., Valete-Rosalino, C.M., 2013. Influence of the nutritional status in the clinical and therapeutical evolution in adults and elderly with American tegumentary leishmaniasis. Acta Trop. 128, 36–40.

Quintana, M., Salomon, O., Guerra, R., De Grosso, M.L., Fuenzalida, A., 2013. Phlebotominae of epidemiological importance in cutaneous leishmaniasis in northwestern Argentina: risk maps and ecological niche models. Med. Vet. Entomol. 27, 39–48.

Quintana, M.G., Fernandez, M.S., Salomon, O.D., 2012. Distribution and abundance of phlebotominae, vectors of leishmaniasis, in Argentina: spatial and temporal analysis at different scales. J. Trop. Med. 2012, 652803.

Reveiz, L., Maia-Elkhoury, A.N., Nicholls, R.S., Romero, G.A., Yadon, Z.E., 2013. Interventions for American cutaneous and mucocutaneous leishmaniasis: a systematic review update. PLoS One 8, e61843.

Romero, G.A., Boelaert, M., 2010. Control of visceral leishmaniasis in latin america-a systematic review. PLoS Negl. Trop. Dis. 4, e584.

Romero, H.D., Taranto, N.J., Malchiodi, E.L., 2004. American integumentary leishmaniasis associated with AIDS in Argentina. Medicina (B. Aires) 64, 47–50.

Salomon, O.D., Quintana, M.G., Bezzi, G., Moran, M.L., Betbeder, E., Valdez, D.V., 2010. *Lutzomyia migonei* as putative vector of visceral leishmaniasis in La Banda, Argentina. Acta Trop. 113, 84–87.

Salomon, O.D., Quintana, M.G., Mastrangelo, A.V., Fernandez, M.S., 2012. Leishmaniasis and climate change-case study: Argentina. J. Trop. Med. 2012, 601242.

Salomon, O.D., Quintana, M.G., Zaidenberg, M., 2008. Urban distribution of Phlebotominae in a cutaneous leishmaniasis focus, Argentina. Mem. Inst. Oswaldo Cruz 103, 282–287.

Salomon, O.D., Sosa Estani, S., Canini, L., Cordoba Lanus, E., 2001a. Tegumentary leishmaniasis in area with epidemic levels of transmission Salta Argentina, 1998. Medicina (B. Aires) 61, 284–290.

Salomon, O.D., Sosa Estani, S., Dri, L., Donnet, M., Galarza, R., Recalde, H., Tijera, A., 2002. Tegumental leishmaniasis in Las Lomitas Province of Formosa, Argentina, 1992–2001. Medicina (B. Aires) 62, 562–568.

Salomon, O.D., Zaidenberg, M., Burgos, R., Heredia, V.I., Caropresi, S.L., 2001b. American cutaneous leishmaniasis outbreak, Tartagal city, province of Salta, Argentina, 1993. Rev. Inst. Med. Trop. Sao Paulo 43, 105–108.

Silveira, F.T., Lainson, R., Corbett, C.E., 2004. Clinical and immunopathological spectrum of American cutaneous leishmaniasis with special reference to the disease in Amazonian Brazil: a review. Mem. Inst. Oswaldo Cruz 99, 239–251.

Sosa-Estani, S., Segura, E.L., Gomez, A., Salomon, O.D., Peralta, M., Coutada, V., Ruiz, L.M., 2001. Cutaneous leishmaniasis in Northern Argentina: identification of risk factors in a case-cohort study of three municipalities in Salta. Rev. Soc. Bras. Med. Trop. 34, 511–517.

Sosa-Estani, S., Segura, E.L., Salomon, O.D., Gomez, A., Peralta, M., Coutada, V., Ruiz, L.M., 2000. Tegumentary leishmaniasis in Northern Argentina: distribution of infection and disease, in three municipalities of Salta, 1990–1992. Rev. Soc. Bras. Med. Trop. 33, 573–582.

Sosa Estani, S., Campanini, A., Sinagra, A., Luna, C., Peralta, M., Coutada, V., Medina, L., Riarte, A., Salomon, D., Gomez, A., Segura, E.L., 1998. Clinical features and diagnosis of mucocutaneous leishmaniasis in patients of an endemic area in Salta. Medicina (B. Aires) 58, 685–691.

Soto, J., Toledo, J., Valda, L., Balderrama, M., Rea, I., Parra, R., Ardiles, J., Soto, P., Gomez, A., Molleda, F., Fuentelsaz, C., Anders, G., Sindermann, H., Engel, J., Berman, J., 2007. Treatment of Bolivian mucosal leishmaniasis with miltefosine. Clin. Infect. Dis. 44, 350–356.

Vega Benedetti, A.F., Cimino, R.O., Cajal, P.S., Juarez Mdel, V., Villalpando, C.A., Gil, J.F., Marcipar, I.S., Krolewiecki, A.J., Nasser, J.R., 2013. Performance of different Trypanosoma cruzi antigens in the diagnosis of Chagas disease in patients with American cutaneous leishmaniasis from a co-endemic region in Argentina. Trop. Med. Int. Health 18, 1103–1109.

Werneck, G.L., Hasselmann, M.H., Gouvea, T.G., 2011. An overview of studies on nutrition and neglected diseases in Brazil. Cien Saude Colet. 16, 39–62.

World Health Organization, 2010. Control of the leishmaniasis: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, 22-26 March 2010.

Ziaie, H., Sadeghian, G., 2008. Isolation of bacteria causing secondary bacterial infection in the lesions of cutaneous leishmaniasis. Indian J. Dermatol. 53, 129–131.