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Clinical Presentation of American Tegumentary Leishmaniasis in a *Leishmania* (*Viannia*) *braziliensis* Endemic Hotspot: A 35-Year History

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Abstract. American tegumentary leishmaniasis (ATL) is a neglected tropical disease affecting the skin and mucosa. American tegumentary leishmaniasis due to *Leishmania* (*Viannia*) *braziliensis* is endemic in Argentina, where the Department of Oran is a hyperendemic focus. All cases of ATL with laboratory confirmation evaluated at a referral center in Oran city between 1985 and 2019 were analyzed retrospectively. Information from cases included clinical form, lesion size and number, time of evolution, and anatomical location; sex, age, and geographic origin were also studied. The temporal distribution of cases was analyzed. A total of 3,573 cases were included in the analysis. The ratio of males to females was 3:1 and the median age was 33 years old. Eighty-seven percent of cases were from Oran city and its surroundings, highlighting the hyperendemic nature of the area. Regarding clinical forms, 92.5% of cases were cutaneous and 7.5% were mucosal, with a median evolution time until clinical evaluation of 30 days and 7 months, respectively. Single cutaneous lesions were more frequent, localized mainly on the exposed areas in the upper and lower limbs. Secondary events were observed and described in 140 (4%) cases, with a median interval of 3.8 years for the appearance of recurrent mucosal disease in previously cutaneous forms. This is the largest case series of ATL due to *L.* (*V.*) *braziliensis*. The most classic presentation is of adult males with single cutaneous ulcers in exposed body areas, with $<$ 10% of cases with mucosal complications. This comprehensive clinical characterization serves as a basis for future studies of the care and control of this neglected tropical disease.

INTRODUCTION

The leishmaniases are a group of vector-borne diseases endemic to 102 countries and caused by *Leishmania* parasites that are transmitted by female phlebotomines.[1] Globally, they are among the top 10 neglected tropical diseases, with more than 12 million infected people, 0.9–1.6 million new cases yearly, and 350 million people at risk of infection.[2] From a clinical perspective, there are three major forms of the disease: cutaneous, mucosal, and visceral. In the Americas, endemic areas extend from Mexico to Argentina, and American tegumentary leishmaniasis (ATL), which affects the skin and/or mucosa, is the most frequent clinical form.[3]

In Argentina, ATL is endemic to nine northern provinces; the northwestern province of Salta has historically had the greatest number of cases, reporting annually over 40% of the ATL cases in the country.[4] In Salta, most cases of ATL are from the Department of Oran, where cutaneous and mucosal disease is almost entirely caused by *Leishmania* (*Viannia*) *braziliensis*, with rare cases due to *Leishmania* (*Viannia*) *guyanensis* and *Leishmania* (*Leishmania*) *amazonensis*.[5,6] The epidemiological characteristics of the cases support forest transmission[7]; nevertheless, peridomiciliary transmission has also been proposed.[8] Finally, this highly endemic area has also been the focus of the two most important epidemic outbreaks of ATL in Argentina.[9,10]

The aim of this study was to characterize the ATL cases diagnosed over the course of 35 years (1985–2019) in a reference center located in a highly endemic area where *L.* (*V.*) *braziliensis* is prevalent. For this purpose, clinical and demographic characteristics at presentation were analyzed. The monthly and annual frequencies of cases were also determined to obtain valuable information for programmatic planning and serve as the basis for further epidemiological and implementation studies in the area.

MATERIALS AND METHODS

Study area. The Instituto de Investigaciones de Enfermedades Tropicales (IIET), located in the city of San Ramon de la Nueva Oran (SRNO) (23°08′S, 64°20′W; Department of Oran) in northwestern Argentina and 46 km south of the border with Bolivia, is a regional reference center for the diagnosis of leishmaniasis. This area is part of the Yungas ecoregion of a subtropical climate with a dry season in winter, and has been strongly affected by agricultural activities and forestry exploitation.[11,12] This reference center for the diagnosis of leishmaniasis, a reportable disease, integrates the network of the national system for health surveillance coordinated by the National Ministry of Health (https://www.argentina.gob.ar/salud/epidemiologia/notificacion).

Study population. This retrospective analysis was performed on ATL cases that were diagnosed at IIET from January 1, 1985 through December 31, 2019. Case definition was established as any individual with lesions clinically and epidemiologically compatible with ATL who had a positive diagnostic result in smear and/or culture and/or Leishmanin skin test (LST). All skin lesions consisted of the typical ATL cutaneous ulcer. After diagnosis, patients were referred to the local reference hospital for clinical management including administration of antiparasitary treatment.

For this analysis, a database was constructed from the IIET archives including the following variables: age at presentation, sex, locality of residence, diagnosis date, evolution time of lesions, clinical form, anatomical location and sublocation of the skin lesions, largest lesion size, and number of lesions.

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Diagnostic procedures and operational definitions. Cutaneous lesions were categorized as "single" when there was a sole skin lesion and "multiple" when all lesions were on the skin without meeting the definition for the "disseminated" form that was defined as ≥ 10 lesions in ≥ 2 areas of the body.[13] Mucosal lesions were categorized as "late mucosal" when patients had a history of cutaneous leishmaniasis and/or typical scars; "concomitant mucosal/mucocutaneous" for simultaneous skin and mucosal lesions; "contiguous mucosal" for lesions in adjacent mucosal areas; and "undetermined mucosal" when no record of the cutaneous form was identified via history and physical examination.

For diagnosis, lesions are sampled by scraping their edges, which are fixed with methanol and stained with 10% Giemsa. Semiquantification of amastigote burden is performed as follows: P+, 1–10 parasites/1,000 fields; P++, 1–99 parasites/100 fields; and P+++, ≥ 10 parasites/10 fields. For culture, lesion edges are aspirated with a syringe containing sterile proline balanced salt solution. It is inoculated in USMARU medium supplemented by 100 U/mL penicillin and 50 g/mL streptomycin and incubated at 24°C.[14] LST is performed by injecting intradermally 0.1 mL of Leishmanin (40 μg of protein/mL) into the forearm; the reaction is read 48–72 hours post application, with indurations ≥ 5 mm considered positive.[15]

Some patients were referred back to the IIET by their physician because of "recidiva cutis" or recurrence. The former refers to reactivation of a healed lesion,[16] whereas the latter indicates new lesions located in different areas of the body.[17]

Data analysis. The data were analyzed via GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA). Continuous variables were analyzed by categories (sex, age class, or lesion location) via the Kruskal–Wallis test and Dunn's multiple comparison test. Correlations were estimated by Spearman's test. Positive cases were subdivided into confirmed (smear and/or culture positive) and suspected (smear and/or culture negative but LST positive), and these groups were compared with negative cases (smear and/or culture and/or LST negative) diagnosed during the same time period (1985–2019). Significance was defined at $P \leq 0.05$.

RESULTS

This analysis includes 3,573 ATL cases diagnosed at the IIET from 1985 through 2019. Of these, 75% (2,698/3,573) were considered "confirmed" as a result of parasitological confirmation, whereas the remaining 24% (566/3,573) were "suspected" for being only positive by LST. Over half of the smear-positive cases were P+ at semiquantification. Both groups were compared with negative cases ($N = 2,149$), revealing that in the three groups males were more frequent. Suspected cases were older than the other groups (37 versus 32 years old; $P = 0.025$) and also presented a higher proportion of mucosal cases (15 versus 10%). Negative cases showed smaller lesions (mean diameter, 16.8 mm; $P < 0.0001$) than positive and suspected cases, which did not differ in their size (mean diameter, 21.2 and 21.3 mm, respectively).

Regarding the case load over the study period, the years with the highest number of cases were 1997–1999 and 2002, with a total of 1,295 cases. Prior to 1997–1999 and between that period and 2002, the annual number of cases was < 100/year (Figure 1). Similarly, after 2002 until 2014, cases ranged between 50 and 100 per year. Throughout all those years there was a stable predominance of cutaneous cases (> 85%) over mucosal cases (< 15%). Exceptionally, the year 1993 ($N = 24$) presented almost the same proportion of cutaneous and mucosal cases (Figure 1). Considering the monthly distribution, August and September presented the highest frequency of cases, showing a significant positive correlation via Spearman's test ($P = 0.0014$) (Figure 2).

Regarding demographic characteristics, the male:female ratio was 3:1 and the median (interquartile range; IQR) age was 33 years old (20–49), showing significant differences between sexes with median ages of 34 years (22–49) and 28 years (13–46) for males and females, respectively ($P < 0.0001$). Children < 15 years old represented 16% of cases ($N = 554$), with a median age of 9 years (5–12).

Of 3,521 cases with information on place of residence, 98% were from Argentina (97% from the Province of Salta and 1% from the provinces of Jujuy and Formosa) and 2% were from Bolivia (Figure 3). Within Salta ($N = 3,412$), cases came from eight departments: 91% from the Department of Oran, 9% from the neighboring department of General Jose de San Martin, and the rest from six other departments. In turn, within the Department of Oran ($N = 3,057$), 62% of cases came from the town of SRNO, 13% from the locality of Hipolito Yrigoyen, 12% from the locality of Pichanal (both within 25 km of SRNO), and the remaining 13% from 18 other localities in the department.

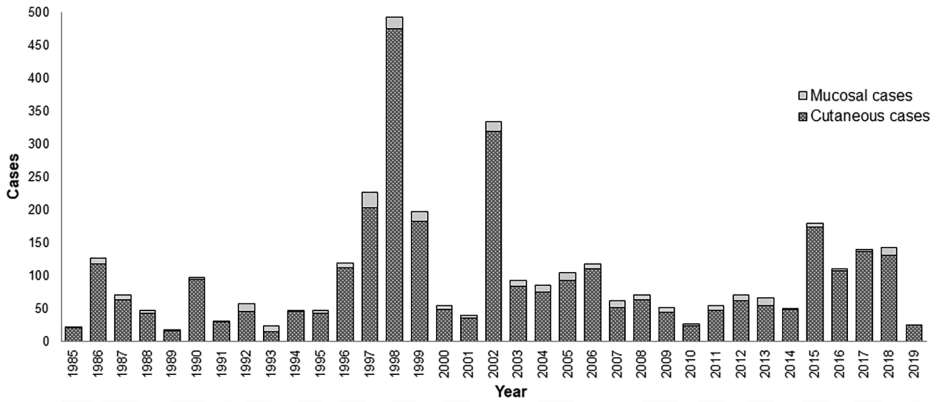

FIGURE 1. Annual number of cases of American tegumentary leishmaniasis and proportion of clinical forms during the study period (1985–2019). This figure appears in color at www.ajtmh.org.

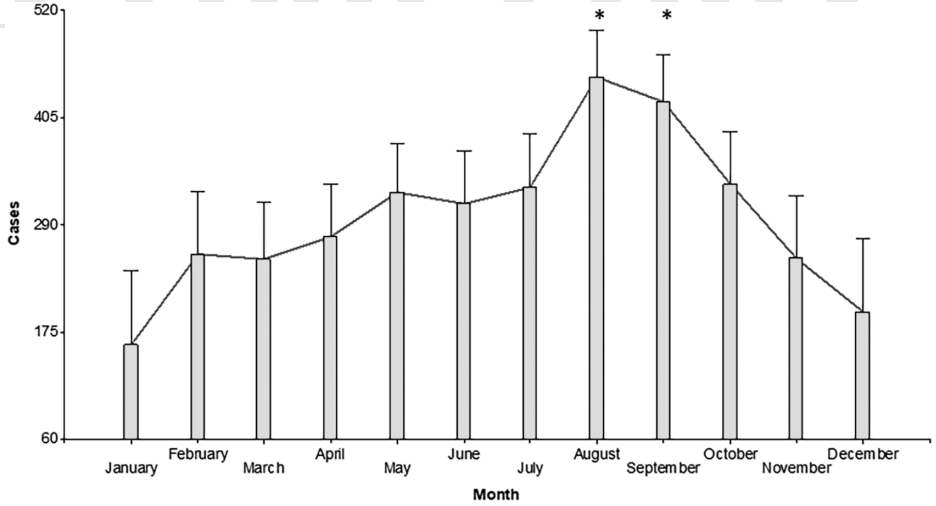

FIGURE 2. Monthly distribution of cases throughout the study period (1985–2019). Error bars indicate the standard error of the mean. *Significant positive Spearman's correlation between the number of cases and the month. This figure appears in color at www.ajtmh.org.

From 3,498 cases with information on clinical presentation, 92.5% presented cutaneous lesions that had a median (IQR) evolution time of 30 days (30–60), and the single form predominated for both sexes (Figure 4). Mucosal lesions were present in 7.5% of cases, with a median evolution time of 7 months (2–24); the late mucosal was the most frequent form (Table 1). In cutaneous cases, the most affected age class was 21–30 years old, whereas in mucosal cases it was 41–50 years old. For both clinical forms, in each decade of adult age, the number of male patients was significantly higher than that of females (Figure 5).

Only 4% of patients (140/3,573) had more than one clinical event, and for most of them the first event was of the cutaneous type (Table 2). Of these, 26% (36/137) experienced recidiva cutis with a median (IQR) interval of 8 months (4.5–14.4) between events. The remaining 74% (101/137) had a recurrence, including cutaneous recurrent and mucosal recurrent lesions with an interval of 1.6 years (0.5–6.7) and 3.8 years (1.2–6.9), respectively, between primary and secondary lesions. The recurrent lesions were significantly older than relapsing lesions ($P = 0.0001$).

Anatomically, for both sexes, cutaneous lesions predominated in the lower limbs, followed by the upper limbs (Figure 6A). Considering their sublocation, lesions predominated in leg and ankle and in forearm and wrist (Figure 6B). No significant differences were observed between the distribution of single and multiple lesions. Among patients with two lesions, fewer than 5% had both in the same body area.

The mean lesion diameter of cutaneous lesions was 20 mm (IQR, 10–30); it was uniform in all anatomical locations, except in the head and neck, where lesions were smaller (median, 10 mm; $P < 0.0001$). Pediatric cases presented smaller lesions in the lower limbs (10 mm for pediatrics versus 20 mm for adults; $P < 0.0001$).

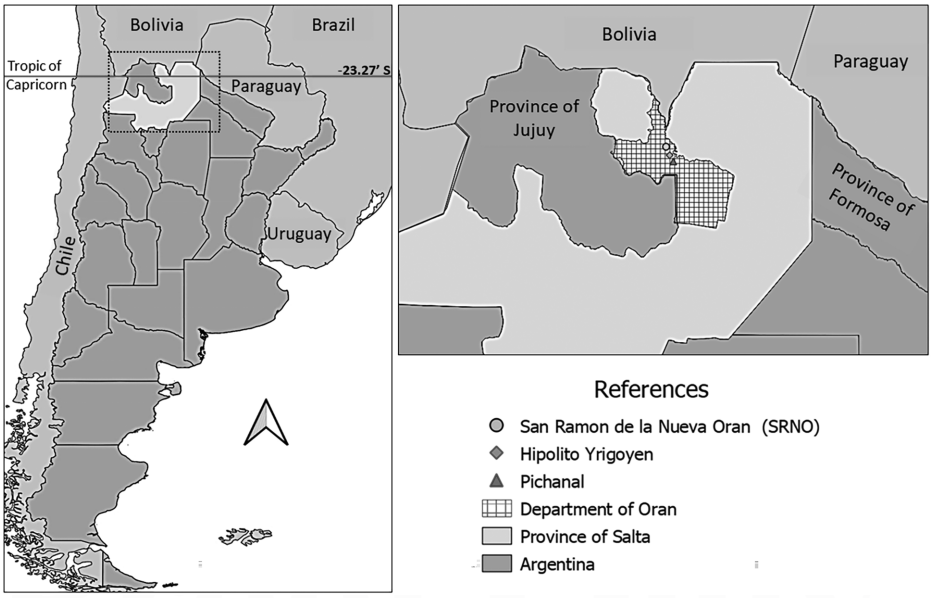

FIGURE 3. Study area: the main places of origin of the cases of American tegumentary leishmaniasis diagnosed at the Instituto de Investigaciones de Enfermedades Tropicales. This figure appears in color at www.ajtmh.org.

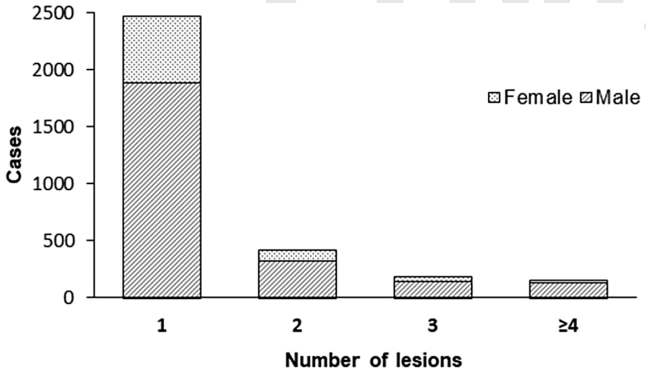

FIGURE 4. Number of lesions by sex. This figure appears in color at www.ajtmh.org.

DISCUSSION

In this study, 3,573 ATL cases from an endemic area for *L.* (*V.*) *braziliensis* are described,[5,18–20] contributing to a comprehensive clinical characterization of *L.* (*V.*) *braziliensis* infection, the species most frequently responsible for mucosal leishmaniasis.[2]

When comparing positive (including confirmed and suspected cases) with negative cases, no notable differences were found among the groups. The suspected cases were older and the LST result could be attributed to sensitization due to higher exposure, although we have previously demonstrated the strength of the relationship between smear and LST across all age classes.[15] Although there were no differences in the median age of skin lesions of confirmed and suspected cases, the mean time was longer for the latter (2 versus 5 months), hence suggesting the lack of parasitological confirmation related to the low parasite load in chronic and mucosal lesions,[21] highlighting the potential usefulness of immunological tests in those cases.

At presentation, > 90% of cases were cutaneous and nearly 8% had mucosal involvement, in agreement with previous reports from the study area in smaller studies.[18] The single lesion cutaneous form was the most frequent, as reported in other endemic areas of *L.* (*V.*) *braziliensis* in Bolivia and Brazil,[22,23] whereas the disseminated form was infrequent (< 1%), as in other studies from Brazil.[13,24]

TABLE 1
Clinical forms and evolution time of the cases

Clinical presentation		Median of evolution time at presentation (interquartile range)
Cutaneous form 3,234/3,498 (92.5%)	Single 2,449/3,234 (75.7%)	30 days (25–60)
	Multiple 760/3,234 (23.5%)	30 days (30–60)
	Disseminated 14/3,234 (0.4%)	60 days (30–150)
	Not determined* 11/3,234 (0.3%)	Not determined*
Mucosal form 264/3,498 (7.5%)	Late mucosal 114/264 (43.2%)	12 months (3–24)
	Undetermined mucosal 93/264 (35.2%)	6 months (2–14)
	Concomitant mucosal 37/264 (14.0%)	12 months (3–24)
	Contiguous mucosal 20/264 (7.6%)	12 months (4–24)

* Cases without complete clinical information other than diagnostic results.

The mean size of cutaneous lesions of 20 mm (IQR, 10–30) was similar to that of *L.* (*V.*) *braziliensis* lesions (IQR, 15–33) in other reports.[22,25] Its lesions are larger than those caused by *L.* (*V.*) *guyanensis*, *Leishmania* (*Leishmania*) *mexicana*, and *Leishmania* (*Leishmania*) *venezuelensis*, which are < 10 mm.[21,26,27] Also species dependent, the parasite load of the skin lesions was low (P+ smear predominance). Generally, these lesions have lower amastigote density than those caused by the *Leishmania* subgenus[21]; even within the *Viannia* subgenus, lesions of *L.* (*V.*) *braziliensis* have fewer parasites than those of *L.* (*V.*) *guyanensis*.[28,29]

For both sexes, lesions were more frequent in the lower limbs, as previously reported for *L.* (*V.*) *braziliensis*.[22,25] There, lesions predominated in the legs and ankles, which is clinically relevant because it was suggested that lesions located below the knee might be prone to delayed healing.[30] This differs from those caused by *L.* (*V.*) *guyanensis* and *L.* (*L.*) *mexicana* because their lesions are more frequently above the waist,[21,29] whereas *L.* (*L.*) *venezuelensis* and *Leishmania* (*Viannia*) *peruviana* produce mainly facial lesions.[27,31] In any case, lesions in populations residing in tropical areas were more frequent in exposed body areas.

Cutaneous lesions had a median evolution time of 1 month (IQR, 1–2), as reported for *L.* (*V.*) *braziliensis* lesions,[29] which is shorter than that reported for patients from northwestern Argentina, whose lesions were 2.5 months old.[5] This discrepancy may be related to sample size because that study included < 100 patients.

In this study, the prevalence of the mucosal form (8%) is similar to that reported for different parts of Brazil that presented < 10% of mucosal cases.[22,25,32] The median age of mucosal lesions was 6 months (IQR, 2–24), notoriously lower than in another report that registered a chronicity > 2 years at presentation.[33]

Furthermore, 43% of the mucosal cases were of the late mucosal type and its prevalence was lower than in Brazil, where > 70% of mucosal cases showed cutaneous scars.[22,25,32] On the other hand, 14% of mucosal cases were mucocutaneous, which is similar to that reported for *L.* (*V.*) *braziliensis* (10–15%)[22,25,32] but differs from that reported for *Leishmania* (*Viannia*) *panamensis*, which caused 61% of mucocutaneous cases in a very small study of 23 cases.[34] According to our data, 75% of the mucocutaneous cases presented multiple cutaneous lesions and 60% of the concomitant skin lesions were located above the waist; this is compatible with previous proposals suggesting that multiple skin lesions above the belt would present a higher risk of mucosal development.[35]

Regarding the demographic features of the cases, the male:female ratio was 3:1 for both the cutaneous and mucosal forms, which differs from that found in other work in which that ratio was higher for mucosal forms.[22] The age class 21–30 years old was the most frequent among cutaneous cases, whereas mucosal cases were mostly concentrated in those ≥ 40 years old, in agreement with other reports.[32] The predominance of male cases in the working age group, which has been widely reported for areas where *L.* (*V.*) *braziliensis* prevails, indicates that transmission in this area would be associated with wild environments during work and recreational activities.[7,18]

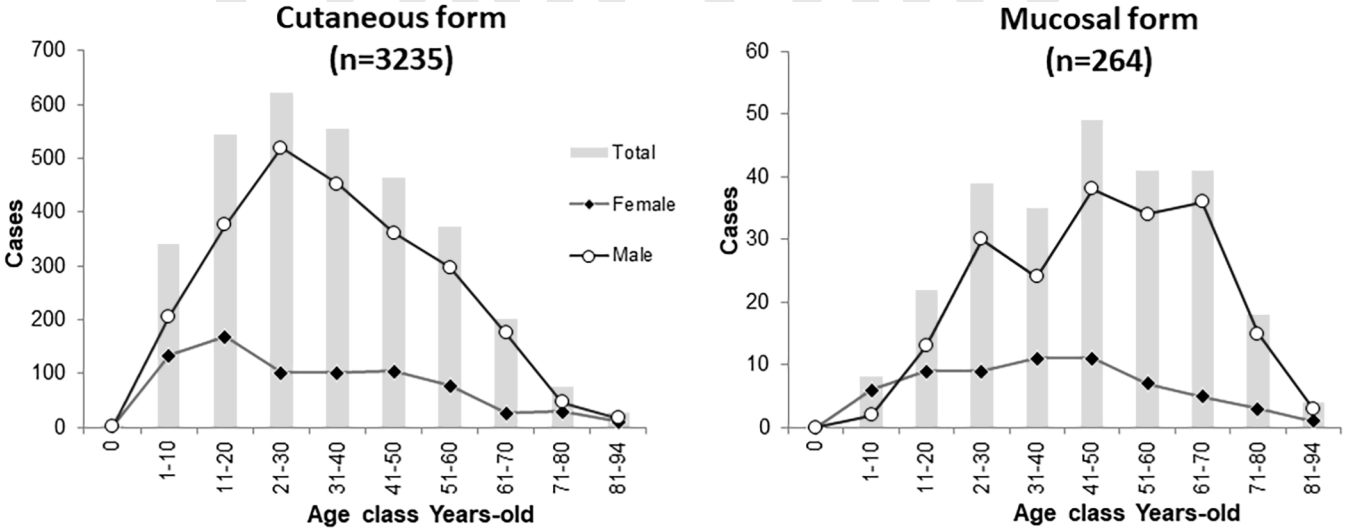

FIGURE 5. Clinical form by sex and age class. This figure appears in color at www.ajtmh.org.

The geographic origin of the cases indicates that > 90% were from the Department of Oran, Province of Salta, Argentina. Within the department, 87% of the cases came from the neighboring localities of SRNO, Hipolito Yrigoyen, and Pichanal, with over half coming from the first locality. This reveals that cases reach the IIET from a reduced area and, according to data from the Ministry of Health, in 2019 the IIET reported 50% of cases in the Province of Salta and 22% of cases in Argentina, which highlights the hyperendemic nature of the area.[4]

In our results, secondary events due to relapsing lesions had a median interevent time of 8 months, which coincides with case reports stating that recidiva occurs between 3 and 24 months after clinical cure.[16,36] The presence of persistent parasites has been reported in scars of clinically healed lesions several years after the primary event[37]; therefore, relapsing could be explained by reactivation of persistent amastigotes in those scars.[38] Regarding recurrent lesions, they were more frequent and occurred with a longer interevent interval than lesions of recidiva cutis, as previously reported.[17] The classification of recurrent cases was based solely on the remote location of the new lesions in relation to primary lesions and has no molecular support; hence, parasite dissemination could have occurred instead. However, when recurrent ATL lesions of a patient from our study area were molecularly characterized, two genotypes of *L.* (*V.*) *braziliensis* were found, suggesting that these lesions indicate different infection events and the lack of protective immunity after a primary infection.[39]

TABLE 2
Clinical forms in cases with two clinical events

First clinical event	Second clinical event	Median of time interval between events (interquartile range)
Cutaneous form 98% (137/140)	Recidiva cutis 26.3% (36/137)	8 months (4.5–14.4)
	Cutaneous recurrent form 51.8% (71/137)	1.6 years (0.5–6.7)
	Mucosal recurrent form 21.9% (30/137)	3.8 years (1.2–6.9)
Mucosal form 2% (3/140)	Cutaneous recurrent form 100% (3/3)	1.6 years (0.7–17.8)

Interevent times are shown.

Regarding the annual distribution of ATL cases, epidemic periods were observed interspersed with interepidemic periods but without a defined pattern (Figure 1), which was attributed to plotting all cases from the IIET database without distinguishing among localities. When analyzing the case annual distribution for the main localities (SRNO, Hipolito Yrigoyen, and Pichanal), still no pattern was found. For these three neighboring localities, the 1997–1999 period stood out for presenting the highest number of cases, which, in fact, belonged to the largest ATL epidemic in Argentina.[10] Regarding the monthly case distribution, the highest frequency occurred in August and September. Considering an estimated incubation period of 2 months[7] together with the reported evolution time, the rough estimations indicate that infections could have occurred at least 3 months before presentation for clinical evaluation.

Limitations of this analysis are related to its retrospective approach, which might introduce bias in certain clinical and demographic variables, most notably the lack of thorough physical examination in some patients, which prevented the identification of scars from previous cutaneous lesions. Also, the time elapsed between recognition of the lesion and presentation for evaluation has the limitation of the patient's recollection. The number of cases for each town and year, as well as the incidence of recurrences and recidiva cutis, has the limitation posed by the fact that some patients might have sought care in other centers or even in the provincial capital, Salta city; however, the incidence of this is likely to be rather small, as shown by the reports by the National Ministry of Health of this disease of mandatory communication and, in any case, our analysis focuses on clinical characteristics with programmatic implications rather than full epidemiological analysis. The suboptimal sensitivity of the diagnostic methods used in this group of patients might have introduced a limitation in the misclassification due to false-negative results in some cases; this was partially resolved by the inclusion of suspected cases that had positive LST results.

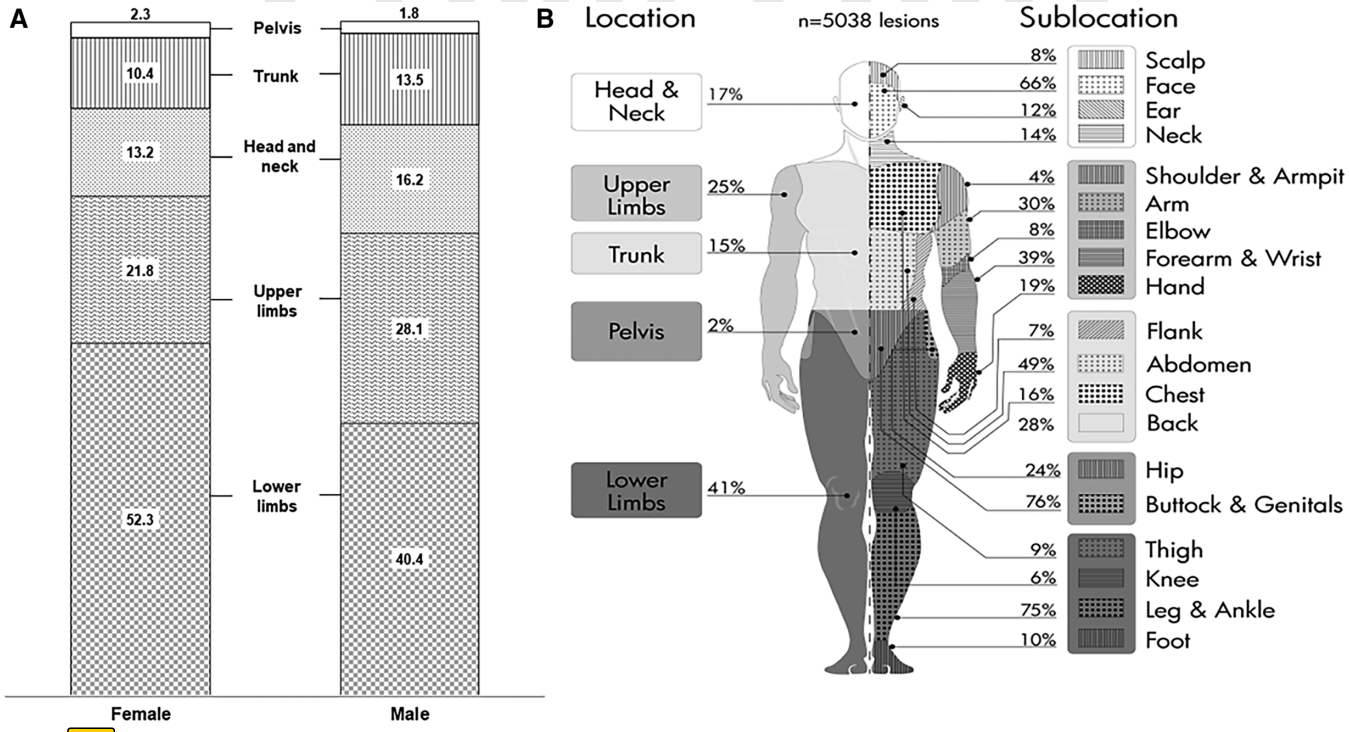

FIGURE 6. (**A**) Anatomical location of skin lesions (%) by sex. (**B**) Location and sublocation of skin lesions. This figure appears in color at www.ajtmh.org.

In summary, this study presents a clinical-demographic characterization of over 3,000 ATL cases from a referral center in northwestern Argentina. This is, to our knowledge, the largest case series in an endemic area for *L.* (*V.*) *braziliensis*. Information is provided and analyzed with a focus on clinical presentation, frequency, and distribution of cases. The mucosal form, the most characteristic aspects of infections by this species, is also characterized. Our results aim at being a baseline platform for further epidemiological, social, and entomological studies as well as clinical trials to identify programmatic interventions for the control of this endemic neglected tropical disease.

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The entire database was deidentified. The project was evaluated and approved by the Bioethics Committee of the Universidad Nacional de Salta as part of the research plan on entry to Consejo Nacional de Investigaciones Científicas y Técnicas of the senior author (A. J. K.).

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