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Preliminary epidemiological findings of *Leishmania* infection in the municipality of Tinum, Yucatan State, Mexico

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ABSTRACT

Localized cutaneous leishmaniasis (LCL) is endemic in Mexico, mainly in the states of Campeche and Quintana Roo, hyperendemic areas of *Leishmania (Leishmania) mexicana* transmission. In this report, epidemiological features of *Leishmania* infections in the municipality of Tinum, Yucatan State, Mexico are presented. Nine cases of LCL were diagnosed in 2015. Patients were men between 30 and 74 years of age, without a history of living or traveling to endemic areas (Quintana Roo or Campeche). Due to asymptomatic infection is the most common outcome after *Leishmania* inoculation, between November 2017 to June 2018, 47 men working in the forest were tested by Montenegro skin test (MST). Thirteen of them (27.6%) were identified MST positive, in absence of either lesion or typical scar, and evidence of exposure to vector. Findings in Tinum, Yucatan, supported the presence of specific environmental conditions that seem to favor *Leishmania* transmission in this region. Thus, active surveillance for the detection of new cases in the municipality of Tinum as well as the eco-epidemiological characterization to identify all the transmission components (parasite, vector, and reservoir species) are urgently needed.

1. Introduction

Localized cutaneous leishmaniasis (LCL) caused by *Leishmania* (*Leishmania*) *mexicana*, is a zoonotic disease endemic in the Yucatan Peninsula, Mexico (Chable-Santos et al., 1995; Canto-Lara et al., 1999). The population at risk is mainly located in the sylvatic region from the states of Campeche and Quintana Roo where most cases are reported (Mexican Secretariat of Health Services, 2017). Most of the studies have been done in Campeche characterizing clinical and histopathological pictures (Andrade-Narváez et al., 2001; Andrade-Narvaez et al., 2005), incidence of clinical infection (Andrade-Narváez et al., 1990), risk factors (Andrade-Narváez et al., 1992), response to treatment - meglumine antimoniate - (Vargas-Gonzalez et al., 1999), prevalence of asymptomatic infection (Arjona Villicaña, 2002), and identification of *Leishmania* sp. (Canto-Lara et al., 1999; Canto-Lara et al., 1998), vectors (Rebollar-Téllez et al., 1996; Rebollar-Téllez et al., 2005), and reservoirs (Chable-Santos et al., 1995; Van Wynsberghe et al., 2009). In the Yucatan Peninsula, the transmission occurs between November and March, when both

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high humidity and lower temperature result in an increased abundance of female sand flies concurrently with the multiplication of *L. (L.) mexicana* in the base of the tail of rodent reservoirs, resulting in a high rate of human infection acquired in the forest (Andrade-Narvaez et al., 2003).

In the Yucatan State, all cases of LCL had been restricted to villages located in the Southern cone of the state, close to the well-known endemic area of Campeche and Quintana Roo. In 2015, nine cases of autochthonous LCL were reported in the municipality of Tinum, by personnel of the *Servicios de Salud de Yucatan* (SSY). Surprisingly, none was reported in 2016 and 2017. Since asymptomatic infection is normally more common than clinical one and often underestimated, we considered important to document the magnitude of leishmanial infection using the Montenegro skin test (MST).

In this brief report, we presented the main characteristics of the LCL cases identified in 2015, and the result of an active epidemiological survey of asymptomatic cases in three villages of the Tinum Municipality, Yucatan.

2. Methods

2.1. Studied area

The municipality of Tinum is localized in Eastern Yucatan State between 20°40′ and 20°53′ latitudes North and 88°21′ and 88°33′ longitudes West. The area is 22 m above sea level and encompasses 471.84 km^2. The mean annual rainfall reaches 85.9 mm mostly during the summer, the average temperature is 26.3 °C and vegetation corresponds to the dry medium-height semi-deciduous forest. In 2010, the municipality of Tinum counted 11,421 inhabitants and the main economic activities are agriculture and tourism (Mexican Ministry of Social Development, 2018; National Institute of Statistic and Geography, 2009).

2.2. Studied population

As it has been mentioned, subjects with a suggestive clinical picture of LCL were evaluated by personnel from the SSY and diagnosed by the visualization of the parasite in either smears or biopsies, and if positive treated by meglumine antimoniate. At that time, no MST was applied.

An active epidemiological survey for the detection of asymptomatic cases was carried in three villages of Tinum, namely Pisté, X'Calacoop, and Tohopkú in 2017. The participants in this preliminary survey were selected by convenience sampling accordingly to known risk-factors for LCL in the Yucatan Peninsula, that is men who carry out activities such as agriculture, lumberjack, and hunting in which they are exposed to the infected *Phlebotomine* bites (Andrade-Narváez et al., 1992). Interviews were completed by a house-to-house visit, during November 2017 to June 2018, thus collecting epidemiological information such as age, occupation, time of exposure, places where they are lived or traveled and presence of suggestive scars of leishmanial infection. The acquisition of asymptomatic infection was determined in persons performing risk activities, by a positive MST in the absence of signs of LCL and a history of exposure (Andrade-Narvaez et al., 2016). The leishmanine was prepared using 10^6 promastigotes of *L. (L.) mexicana* in 0.05% phenol saline, and participants were inoculated with 0.04 ml of the solution. The test results were read after 48 h and an induration of ≥5 mm of diameter was considered positive (Andrade-Narváez et al., 1990).

This study was reviewed and approved by the Ethical Committee of the National Institute of Public Health (Identification number: T-49) in agreement with international ethical guidelines for biomedical research involving human subjects. Informed written consent was signed by each participant or by parents of minor-aged children. Data were presented as mean and standard deviation (SD). Frequencies and association of variables (asymptomatic infection, age, and time of exposure) were assessed by Chi-square test using the software Graph Pad Prism 5 (Graph Pad Software Inc., San Diego, CA, USA).

3. Results

In 2015, nine autochthonous LCL cases were recorded in the municipality of Tinum. An autochthonous case is considered when the patient knew the time, the activity he was performing while bitten by the vector, as well as the place where he became infected. The most common lesions were single ulcers (66.6%) located predominantly on the ear or head (Table 1). Time of evolution ranged from 15 days to 3.6 months. Five individuals (55.6%) acquired the infection in the village of X'Calacoop, three (33.3%)

Table 1
Clinical features of nine LCL patients diagnosed in 2015 in Tinum, Yucatan.

Case	Age (years)	Type/number of lesions	Location	Time of evolution (months)
1	62	Ulcerated/1	Ear	1.2
2	64	Ulcerated/1	Head	3.6
3	74	Ulcerated/1	Head	1.9
4	56	Ulcerated/1	Head	1.5
5	30	Ulcerated/6	Head and ears	2.1
6	50	Ulcerated/2	Ear	1.8
7	42	Nodular/3	Arm	0.9
8	50	Ulcerated/1	Ear	0.7
9	48	Ulcerated/1	Trunk	0.4

in Pisté and one (11.1%) in Tohopkú. All patients were males between 30 and 74 years of age (mean 52.8, SD: 12.9), from which 6 of 9 were ≥50 years old. They were working mainly in agriculture (44.4%) and wood collecting for either domestic or artisanal purposes (55.6%). The meglumine antimoniate treatment consisted in ampule containing 425 mg of Sb^{+5}, administered daily either intralesional or intramuscular according to the size, location, and time of evolution of the lesion. The total of ampules ranged from 1.2 to 30 per patient (mean 22.1, SD: 11.54).

Interviews and the MST were applied to 47 men. Thirteen of them (27.6%) were identified as asymptomatically infected (Table 2). The positive induration diameter ranged from 5 to 12 mm (mean 8.30, SD: 2.21). None of those asymptomatic persons referred to have lived or traveled to known endemic area of leishmaniasis. Their age ranged from 17 to 78 years old (mean 58.62, SD: 18.32), interestingly 76.9% were 50 years old or more. Their main occupations were agriculture, wood collecting, and hunting. Subjects referred to spend between 2 and 10 h working in the forest (Table 2). However, no associations were found between asymptomatic infection and either age or time of exposure.

4. Discussion

In 1912, Seidelin described LCL as "chiclero's ulcer" because the main affected population were collectors of chewing gum (chicle) working in the sylvatic region of the Yucatan Peninsula (Seidelin, 1912). Since then, mainly the states of Campeche and Quintana Roo have been considered endemic areas of the disease (Andrade-Narvaez et al., 2017).

In this paper, we reported for the first time both autochthonous LCL cases and asymptomatic infections by *Leishmania* in the municipality of Tinum, Yucatan, Mexico. The clinical picture of these autochthonous cases was similar to that described in Campeche, a single ulcer with indurated border mostly located on the ear or head (Andrade-Narváez et al., 2001). The most important feature is that affected people seemed to have acquired the infection in the tropical dry forest surrounding their villages. This finding supports the existence of the environmental conditions for a local *Leishmania* transmission in this area where no autochthonous cases were ever reported.

Tinum Municipality is located in the North-East of the Yucatan Peninsula between Chichen-Itza and Valladolid, two very important tourist attractions. Since mainly young people perform tourism-related activities, they were not affected by leishmaniasis. Thus, this might explain why in both groups (clinical and asymptomatic) most infected people were over 50 years old working in high-risk activities like agriculture, wood collecting, and hunting. Considering the primordial necessity of these rural activities, we can expect new cases of LCL in the future.

Montenegro (1926) developed a skin test through intradermal application of phenolized cultured promastigotes of *Leishmania* as antigens to facilitate the diagnosis of LCL in endemic areas of South America where parasite visualization was difficult (Montenegro, 1926). Since then, the MST has been widely used to support the diagnosis of LCL in the New World. Since the MST reactivity is induced by either asymptomatic or clinical infection and is typically long-lasting, this test does not discriminate between present or past infection. However, the MST is a very useful epidemiological tool that we used as a marker of exposure in individuals without clinical evidence of *Leishmania* infection (Andrade-Narvaez et al., 2016; Biagi, 1953; Córdova-Uscanga et al., 1993).

Our finding of men performing risk activities, having positive MST in the absence of signs of LCL - asymptomatic infection - supported the presence of leishmanial transmission in the municipality of Tinum, Yucatan. In most endemic areas of leishmaniasis, the asymptomatic infection is by far the most common outcome of *Leishmania* infection (Andrade-Narvaez et al., 2016). In Campeche, the asymptomatic infection is much higher (18.9%) than the incidence of LCL (0.05%) (Andrade-Narváez et al., 1990; Arjona Villicaña, 2002). Similarly, in Tinum, we found 27.6% of MST positivity among men who either work all day or at least spend a couple of hours daily in the forest. Long-term studies should be carried out to determine both the incidence and the prevalence of asymptomatic infection in the Yucatan State.

Table 2
Epidemiological characteristics of 13 positive Montenegro skin test from the Municipality of Tinum, Yucatan.

Characteristic	Number	%
Age (years)		
15–29	1	7.7
30–59	3	23.1
60–78	9	69.2
Size of induration (mm)		
5–7	5	38.5
8–10	7	53.8
Eleven or more	1	7.7
Time of exposure (hrs./day)[a]		
1–5	6	46.2
6–10	7	53.8
Rural community		
X'Calacoop	6	46.2
Pisté	5	38.5
Tohopkú	2	15.3

[a] Duration of working in a forested area.

Asymptomatic infection studies have been done mainly in areas of visceral leishmaniasis, while asymptomatic infection with American cutaneous leishmaniasis has been neglected. It has been suggested that the absence of pathology could be due to an immune response able to control parasite replication without tissue damage (Andrade-Narvaez et al., 2016). Moreover, the persistence of viable parasites in asymptomatic subjects has been demonstrated in endemic areas of *L. (Viannia)* transmission, suggesting that these hosts could contribute to the endemicity of LCL (Rosales-Chilama et al., 2015). Thus, the possible role of asymptomatic hosts in transmission needs to be studied. Giving these findings, including asymptomatic infection in epidemiological surveys is very important to evaluate the real incidence of *Leishmania* infection and establish effective programs of both prevention and control.

The new detection of autochthonous clinical and asymptomatic *Leishmania* infections in the municipality of Tinum, revealed the presence of specific environmental conditions that seems to favor the establishment of *Leishmania* transmission. Thus, active surveillance for the detection of new cases in the municipality of Tinum as well as the eco-epidemiological characterization to identify all the transmission components (parasite, vector, and reservoir species) are urgently needed to understand the dynamic of the transmission and contribute to the development of an appropriate local prevention program.

Declaration of interest

None.

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