

Study of cutaneous leishmaniasis in the State of Campeche (Yucatan Peninsula), Mexico, over a period of two years

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Abstract

Objective. To study cutaneous leishmaniasis (CL), in the Calakmul municipality of the Campeche State, during two years. **Materials and methods.** Individuals with skin lesions were evaluated. Aspirates taken from the lesions were cultured, PCR was performed to diagnose the *Leishmania* species. **Results.** The culture detected 42% of the samples. PCR diagnosed CL in 76% of the samples; of those 38% were from children and 62% from adults. 89% of the patients were infected with *L. mexicana*; 14.4% with Mexican strains of *L. mexicana*; 7% with *L. braziliensis*; 3.6% with *L. mexicana* and *L. braziliensis*. The most affected villages with CL were Dos Lagunas Sur with 12.3%, La Manicolona with 6.5% and La Guadalupe with 2.2% of prevalence, respectively. After the treatment with Glucantime, 96% of the patients were healed. **Conclusion.** CL is an important public health concern in Calakmul, and the parasite causing it belongs to *Leishmania mexicana* and *Leishmania braziliensis* complexes.

Key words: American cutaneous leishmaniasis; Campeche, Mexico

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Resumen

Objetivo. Estudiar la leishmaniasis cutánea en Calakmul, Campeche, México, durante dos años. **Material y métodos.** Se estudiaron individuos con lesiones cutáneas, se tomaron aspirados y se inocularon medios de cultivo; se realizó la técnica de PCR para identificar la especie de *Leishmania*. **Resultados.** Los cultivos detectaron 42% de las muestras. Con la PCR se amplificaron 76% de las muestras, 38% fueron tomadas de niños y 62% de adultos. En 89% de las muestras positivas se identificó *Leishmania mexicana*, en 14.4% cepas mexicanas de *L. mexicana*, en 7% *L. braziliensis* y en 3.6% *L. mexicana* y *L. braziliensis*. En Dos Lagunas Sur se encontró una prevalencia de 12.3%, en La Manicolona 6.5% y en La Virgen 2.2%. Del total de los pacientes, 96% se curó con Glucantime. **Conclusión.** La leishmaniasis cutánea es un problema de salud pública en Calakmul y las especies causantes pertenecen a los complejos *Leishmania mexicana* y *Leishmania braziliensis*.

Palabras clave: leishmaniasis cutánea americana; Campeche, México

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Leishmaniasis is caused by the protozoa *Leishmania* sp., which is transmitted to human beings and animal reservoirs by phlebotomine sand flies of the genus *Phlebotomus* (Old World) and *Lutzomyia* (New World). The World Health Organization (WHO) estimates the worldwide prevalence of leishmaniasis to be approximately 12 million cases, with annual mortality reaching 20 000-30 000. Leishmaniasis has a worldwide distribution and is present on four continents in 88 countries, where the size of the population at risk is about 310 million.^{1,2} More than 20 species of *Leishmania* are capable of causing a wide spectrum of clinical manifestations, ranging from the cutaneous (CL) to the visceral form (VL).^{2,3} VL is fatal if left untreated; it is highly endemic in the Indian subcontinent and in East Africa. An estimated 200 000 to 400 000 new cases of VL occur worldwide each year.¹

Cutaneous leishmaniasis (CL) is the most widespread clinical form of the disease. It is presented as a spectrum of clinical manifestations, such as primary localized skin lesions with frequent findings of amastigotes (LCL) that can self-heal usually in individuals with an effective immune response. However amastigotes parasites can disseminate from the skin lesions to the nasopharyngeal mucosa and cause secondary wounds with progressive destruction of mucosal and submucosal tissues, with rare findings of amastigotes typical lesions of mucocutaneous leishmaniasis (MCL), or disseminate to the entire body as nodular lesions usually full of parasites (DCL).⁴

About 95% of CL cases occur in the Americas, the Mediterranean basin, and the Middle East and Central Asia. An estimated 0.7 million to 1.3 million new cases occur worldwide annually.¹

American cutaneous leishmaniasis includes LCL and DCL caused by *Leishmania mexicana* complex members of subgenera *Leishmania* and MCL caused by members of the *Leishmania braziliensis* complex of the subgenera *Viannia*.² It is endemic to 21 countries where about 39 million individuals are considered to be at risk of acquiring the disease. In endemic regions, multiple species of *Leishmania* may co-exist.⁵ Natural *Leishmania* infections are found in humans and other mammal reservoir hosts (mainly marsupials, rodents, edentates, and carnivores).^{2,6}

In Mexico, CL is the most wide spread form of leishmaniasis. It is distributed in several states, with the main endemic areas located in the southeast of the country. LCL, also known as "chiclero's ulcer" in south-eastern Mexico, was described by Seidelin in the sylvatic region of the Yucatan peninsula.⁷ In this area, the state of Campeche is affected by the LCL and MCL clinical

forms; the *Leishmania* species involved are members of the *L. mexicana* and *L. braziliensis* complexes.^{5, 8-10}

Therefore, early differential diagnosis of CL and the identification of the *Leishmania* strains are important for monitoring clinical outcome and adequately targeting treatment. Furthermore, diseases with other causes but with a clinical manifestation similar to that of leishmaniasis (e.g., leprosy, skin cancers, tuberculosis, cutaneous mycoses) are common in these leishmaniasis endemic areas. Molecular diagnosis for cutaneous leishmaniasis has been extensively developed, essentially by PCR-based methods. Kumar¹¹ reported that kDNA PCR had 96.6% of sensitivity for detecting *L. tropica* as the causative organism in India and El-Beshbishy's¹² studies showed that kDNA PCR had 90.7% sensitivity to identified *L. tropica* in CL patients from western Saudi Arabia. The kDNA PCR assay is very useful in lesions with low parasite load (e.g., mucocutaneous leishmaniasis). PCR also has proven to be a very successful tool in molecular diagnostics in the field (e.g. detection of parasite DNA in blood, tissue smears, in samples taken on filter paper, etc.) without parasite isolating and enabling determination of the infecting species of New World *Leishmania* in a relatively short time.^{5,13}

In this study parasitological diagnosis procedures, such as medium cultures as well as DNA analysis using PCR, were used to establish the accurate diagnosis of CL and identification of *Leishmania* species over a period of two years (2002-2004) in Calakmul municipality, Campeche state, Mexico.

Materials and methods

Study area. The study area was the forest of the state of Campeche, Mexico. LC is endemic to this area. Calakmul, the largest municipality located in the state of Campeche, is situated in the most southern part of the Yucatan Peninsula, bordering with Guatemala and Belize. Calakmul is located within the Peten biogeographic province, which also comprises northern Guatemala and Belize.¹⁴ Weather in the region is classified as hot and sub-humid, with a marked period of drought (March-June), followed by a rainy season.¹⁵ The mean annual temperature is >22°C and an average annual rainfall in the range of 1439-1561 mm. Vegetation in the region is described as medium sub-perennial forest, which is characterized by three strata consisting of trees of 4-12 m, 12-22 m and 22-35 m in height.¹⁶

Ethical considerations. Informed consent was obtained from all the adults who participated in the study. Consent for inclusion of young children was obtained from

parents or guardians. This study was reviewed and approved by the Ethics Committee of the Health Authorities of Calakmul Municipality, Campeche, Mexico, in agreement with the International Ethic Guidelines for Biomedical Research Involving Human Subjects and the Norma Oficial Mexicana de Salud: NOM-003-SSA 2-1993, for sampling blood from human beings for diagnosis and therapeutics. The study was registered in the "Libro de Actas del Cabildo" with date May 24, 2002.

Patient population. One hundred and forty-six individuals with skin lesions suggestive of *Leishmania* infection were studied. Patients from sixteen villages of the municipality Calakmul of Campeche state in the Yucatan Peninsula, Mexico (table I and table II), were evaluated during a 2-year period (2002–2004).

Characterization of parasites. Needle aspirates were taken from the edge of cutaneous lesions, 100 µL of each aspirate was inoculated in Senekje's medium and stored at 24°C for isolation of *Leishmania* promastigotes.

***Leishmania* reference strains and culture conditions.** The following strains were used as positive controls: *L. (Viannia) panamensis* LS94 (MHOM/PA/71/LS94), *L. (Leishmania) mexicana* M379 (MHOM/BZ/62/M379), *L. (Leishmania) mexicana* Bel 21 (MHOM/BZ/82/BEL21), and *L. (Leishmania) mexicana* MC (MHOM/MX/88 HRCMC). The *Leishmania* strains were cultured in RPMI medium supplemented with 10% fetal calf serum at 24°C.

DNA extraction. DNA from 100 µL of sample aspirates was extracted by adding NET 100 (100mM Tris-HCl pH8, 100mM EDTA pH8, 100mM NaCl) and 1% SDS, centrifuged at 200g for 10 min at 4°C. DNA from *Leishmania* cultures was prepared by centrifuging 10⁸ parasites of exponential phase of growth at 2000g for 10 min at 4°C. The DNA was extracted from the pellet using the High Pure PCR template preparation kit (Roche Diagnostics), following the manufacturer's instructions. The DNA was stored at –20°C until used.

Table I
LOCATION OF LESIONS IN PATIENTS WITH CUTANEOUS LEISHMANIASIS AND LEISHMANIA SPECIES IDENTIFIED BY POLYMERASE CHAIN REACTION, CALAKMUL, MEXICO

Village	Site of lesion (N)*	<i>Leishmania</i> species‡ (N)§
La Mancolona	Face (6), arms (8), legs (10)	<i>L. mexicana</i> (16)
		<i>L. mex</i> + <i>Mx L. mex</i> (5)
		<i>L. braziliensis</i> (2)
		<i>L. b</i> + <i>L. mex</i> (1)
Ricardo Flores Magon	Right arm (1)	<i>Mx L. mexicana</i> (1)
La Virgen	Face (7), arms (6), legs (2)	<i>L. mexicana</i> (12)
		<i>L. mex</i> + <i>Mx L. mex</i> (3)
El Carmen	Face (3), arms (3), legs (3)	<i>L. mexicana</i> (7)
Narciso Mendoza	Face (2), arms (1), legs (2)	<i>L. mexicana</i> (5)
Nuevo Campanario	Face (1), arms (1), legs (2)	<i>L. mexicana</i> (3)
		<i>L. mex</i> + <i>Mx L. mex</i> (1)
La Lucha	Hand (1)	<i>L. mex</i> + <i>Mx L. mex</i> (1)
Ricardo Payro	Face (4), legs (2)	<i>L. mexicana</i> (4)
		<i>L. mex</i> + <i>Mx L. mex</i> (2)
Castilla Brito	Face (1), legs (1)	<i>L. mexicana</i> (2)
La Guadalupe	Face (4), arms (2)	<i>L. mexicana</i> (5)
		<i>L. mex</i> + <i>Mx L. mex</i> (1)
Crisobal Colon	Leg (1)	<i>L. mexicana</i> (1)
Niños Heroes	Face (2), arms (1), legs (1)	<i>L. mexicana</i> (2)
		<i>L. mex</i> + <i>Mx L. mex</i> (2)
Tres Huastecas	Arms (2)	<i>L. mexicana</i> (1)
		<i>L. braziliensis</i> (1)
Sacrificio	Face (1)	<i>L. mexicana</i> (1)
Dos Naciones	Face (3), arms (2), legs (1)	<i>L. mexicana</i> (5)
		<i>L. braziliensis</i> (1)
Dos Lagunas Sur	Face (9), arms (14), legs (3)	<i>L. mexicana</i> (19)
		<i>L. braziliensis</i> (4)
		<i>L. b</i> + <i>L. mex</i> (3)

* (N) Number of cases

‡ *L. mex* + *Mx L. mex*: Mix infection of *L. mexicana* + *Mx L. mexicana*; *L. b.* + *L. mexicana*: Mix infection of *L. mexicana* + *L. braziliensis*

§ (N) Number of cases which presented this *Leishmania* species

Table II
PREVALENCE OF CUTANEOUS LEISHMANIASIS IN THE VILLAGES OF CALAKMUL, MEXICO, STUDIED OVER
A PERIOD OF TWO YEARS (2004–2006) IN THE PRESENT WORK

Village	Infected /Total	Age years	% Infected children	% Infected adult	CL %Prevalence
	Northern	Calakmul			
La Mancolona	24/368	6-63	37	63	6.5
Ricardo Flores Magon	1/250	27	0	100	0.4
La Virgen	15/405	9-65	47	53	3.7
El Carmen	7/314	10-54	14	86	2.2
Narciso Mendoza	5/368	10-52	40	60	1.3
Nuevo Campanario	4/318	21-49	0	100	1.3
	Central	Calakmul			
La Lucha	1/265	47	0	100	0.4
Ricardo Payro	6/616	13-50	17	83	1
Castilla Brito	2/496	29-31	0	100	0.4
La Guadalupe	6/273	12-40	67	33	2.2
Cristobal Colon	1/371	34	0	100	0.2 6
Niños Heroes	4/217	12-44	25	75	1.8
Las Tres Huastecas	2/25	26-37	0	100	8
	Southern	Calakmul			
Sacrificio	1/525	30	0	100	0.19
Dos Naciones	6/252	10-30	67	33	2.4
Dos Lagunas Sur	26/211	8-64	50	50	12.3

Polymerase chain reaction. The primers AJS1 (GGGGTTG-GTGTAATAAG) and DeB8 (CCAGTTTCCCGCCCCG), specific for kDNA minicircles of the *Leishmania* subgenus,¹⁷ the LMO1 (TTGGTGTAATAAGGATGGG) and LMO2 (TTGGGGAAATTATGAACGG) primers, specific for minicircles of Mexican *L. (L.) mexicana* strains (Mx *L. mexicana*),¹⁸ the B1 (GGG GTT GGT GTA ATA TAG TGG and B2 (CTA ATT GTG CAC GGG GAG G) specific for species of *L. braziliensis* complex¹⁹ were used to amplify kDNA from reference strains and from Mexican aspirates. PCR amplifications were performed in a solution of 0.2 mM of each deoxyribonucleotide (Invitrogen Life Technologies, Carlsbad, CA, USA), 50 pmol of each specific primer, 2.5 units of Taq DNA polymerase (Perkin Elmer Cetus), 100 ng of DNA template, 1.5 mM of MgCl₂ in a final volume of 100 µL. Samples were denatured at 96°C for 6 min. PCR (35 cycles) consisted of annealing at 60°C for the AJS1 and DeB8 primers, 64°C for the LMO1 and LMO2 primers, and 63°C for the B1 and B2 primers, with a 1 min extension at 72°C and a final extension at 72°C for 10 min on a GeneAmp PCR System Model 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products (10 µL) were fractionated by

electrophoresis in 2% agarose gels in TBE (90 mM Tris-HCl pH 8.3, 90 mM boric acid, and 2mM EDTA), stained with ethidium bromide (10mg/mL), and were observed under a transilluminator (SIGMA Chemical Co., St. Louis, MO, USA).

Administration of meglumine antimoniate (Glucantime®). A total of 111 CL diagnosed patients accepted treatment with meglumine antimoniate (Glucantime). Glucantime is marketed in 5-ml ampules containing 1.5 grams of N-methyl-glucamine antimoniate, which corresponds to 425 mg of Sb51. Treatment consisted in one ampule by intramuscular injection per day until healing.²⁰

Results

Parasite culture detected sixty-two of the positive samples giving 42% of sensitivity (data not shown).

From the total group of patients with skin lesions suggestive of CL analyzed by kDNA PCR, 111 / 146 (76%) samples were positive for CL diagnosis, from these 99 (89%) were amplified with the AJS1 and DeB8 primers specific for the *Leishmania* subgenus, giving an amplification band typical of *L. (L.) mexicana* (not shown); 16

(14.4%) of these samples were also amplified with the primers specific for minicircles of Mexican strains of *L. (L.) mexicana* (table I).

DNA from eight (7.2%) samples of infected individuals was amplified with primers specific for the *L. braziliensis* complex and four (3.6%) were amplified with both primers specific for the *Leishmania* subgenus, giving an amplification band typical of *L. (L.) mexicana* and primers specific for *L. braziliensis* complex members (table I).

A complete response to treatment was defined as a complete re-epithelization of all lesions with no residual erythema and no relapses during the monthly follow-ups for a period of two years. Of the patients diagnosed with CL and treated with meglumine antimoniate 105/109 (96%) were cured in response to treatment.

Discussion

Leishmaniasis is considered by many experts to be an emergent disease in some areas and in others as a re-emergent disease because over the last 20 years the number of cases of all forms of leishmaniasis throughout the world has soared.^{2,6,21} In the present study conducted over two years in 146 patients with cutaneous lesions from several villages in Calakmul, 76% of them were diagnosed as CL, of those 38% were children and 62% adults (table I and II) (figures 1a, 1b and 1c).

The parasite culture detected only 42% of the CL positive samples. This result agrees with those found for Kumar¹¹ in India where 48.2% of positive samples with *Leishmania tropica* were detected, and Beshbishy's¹² studies in Western Saudi Arabia where 39.2% of the positive culture samples as well as *Leishmania tropica* were found. This result could stem from several factors: 1) contamination with associated bacterial infection

in the aspirate sample from the lesion; 2) the ambient temperature in the field is high and if an incubator is not available the *Leishmania* parasite can die; and 3) the primary cultivation of *Leishmania braziliensis* complex members is not easy to perform.

The diagnosis and identification of *Leishmania* species as provided by kDNA-PCR amplification detected in the villages of Northern Calakmul was the following: 43% of cases infected with *L. mexicana*, 25% of cases with *L. braziliensis* complex members, 62% of mixed infection of Mx *L. mexicana* + *L. (L.) mexicana* and 25% of cases infected with *L. braziliensis* complex + *L. (L.) mexicana*. From this part of Calakmul in Union 20 de Junio (locally referred to as La Mancolona) cases infected with each of these strains of *Leishmania* were found (table I). In central Calakmul kDNA PCR assay detected 15% of the cases infected with *L. (L.) mexicana*, 25% of the cases with *L. braziliensis* complex members, 37% of the cases infected with Mx *L. mexicana* + *L. (L.) mexicana* but cases with mix of *L. braziliensis* complex + *L. (L.) mexicana* were not detected (table I).

In southern Calakmul 25.3% of the cases infected with *L. (L.) mexicana* were detected, 62% of the total infected with *L. braziliensis* complex members were found, 25.3% of the cases infected with *L. (L.) mexicana* were detected also in the same region, 75% of cases with *L. (L.) mexicana* + *L. braziliensis* complex members, but cases infected with Mx *L. mexicana* + *L. (L.) mexicana* were not found (table I).

Regarding skin lesions, in most of the patients they were rounded and ulcerated, with elevated borders and necrotic center. There were no cutaneous metastases and neither lymphatic involvement nor mucosal involvement was observed in any of the 111 cases (figures 1a, 1b and 1c). There was no a special pattern in the site nor in the number of lesions in relation to the *Leishmania* species and patients' age, as in 15% of cases lesion was



FIGURE 1. PATIENTS FROM CALAKMUL, CAMPECHE, WITH SKIN LESIONS SUFFERING FROM CUTANEOUS LEISHMANIASIS

on the face, on the ear in 30%, on the arms in 36.6% and on the legs in 18.2%, this results agree with those of Andrade-Narvaez²² (table I).

The village most severely affected with CL was Dos Lagunas Sur, located in the southern part of Calakmul, close to the border of Belize, with 12.3% prevalence, (figure 2c), where most of the cases of CL caused by *L. braziliensis* and mixed infection with *L. braziliensis* and *L. mexicana* were found. People in this village farm chili crops around their houses, which are located very close to the forest, and the population affected includes all of its sectors: children (50%), women, men and the elderly (50%) (tables I and II).

In central Calakmul, La Guadalupe village had the highest prevalence rate (2.2%) and children were the most affected (67%) (table II).

In northern Calakmul the most affected communities were La Mancolona with a 6.5% prevalence, and other nearby cities: La Virgen, with 3.7% and El Carmen, with 2.2% of prevalence, respectively (table II). These villages are located 3–4 km away from the crops and are more urbanized due to deforestation (figure 2a). The most severely affected population for CL in these villages were mainly adult males (66%) who traveled every day to work the crops near the forest (table II).

On the other hand, treatment of CL patients with Glucantime was successful in 96% of those who accepted the treatment, regardless of the number and location of lesions, and number of doses taken. The doses needed to obtain complete healing of lesions varied in children from 2 to 20 and in adults from 2 to 67 ampules (table III). Two patients, one child from Narciso Mendoza (14 years old) and one adult from Dos Naciones (30 years old) were cured spontaneously. No statistically significant difference was observed among patients

infected with *L. (L.) mexicana* and those infected with *L. (V.) braziliensis*, mix infection with *L. (L.) mexicana* + *L. braziliensis* complex or Mx *L. mexicana* + *L. (L.) mexicana* (data no shown). The daily dose of 425 mg of Sb51/day was well tolerated in all cases (data no shown), these results agree with those found in Vargas-Gonzalez.²⁰

Among the conditions that have been reported as risk factors by several authors, that explain the permanent presence, spread, and increase of CL cases in Calakmul, Campeche and the presence of *L. braziliensis* complex, which is restricted mainly to the primary forest, as well as the mixed infections, the present study and other authors found mainly: 1) human colonization of large areas of previously untouched forests, for example migration to Calakmul of people from other states of Mexico and mainly people from Guatemala, where CL caused by *L. braziliensis* complex members is endemic,^{23,24} as well as urbanization and deforestation, by which transmission cycles are adapting to peridomestic environments and are spreading to previously no endemic areas with domestic animals as potential reservoirs (figures 2a, 2b and 2c);²⁵ 2) spending nocturnal periods in the forest for cultivation of agricultural crops (e.g., chilli and coffee);^{26,27} and 3) with the increase of military troop maneuvers, soldiers have become another high-risk group.²⁸ All this ecological, social, and educational conditions are still occurring at the present time in Calakmul.

Furthermore Canto-Lara²⁹ identified *L. (L.) mexicana* in four species of wild rodents: the black-eared rice rat, *Oryzomys melanotis*; the hispid cotton-rat, *Sigmodon hispidus*; the big-eared climbing rat, *Oryzomys phyllotis*; and the Yucatan deer-mouse, *Peromyscus yucatanicus*. Moreover it has been demonstrated that reservoir mammals of several orders can be infected with the same



FIGURE 2. A: VILLAGE EL CARMEN; B: DOS NACIONES, PEOPLE IN THIS VILLAGE FARM CHILI CROPS AROUND THEIR HOUSES, LOCATED VERY NEAR THE FOREST; C: DOS LAGUNAS SUR; LOCATED IN THE SOUTHERN PART OF CALAKMUL, MEXICO, CLOSE TO THE BORDER OF BELIZE AND GUATEMALA, THIS VILLAGE WAS THE MOST SEVERELY AFFECTED WITH CUTANEOUS LEISHMANIASIS (12.3% PREVALENCE)

Table III
RESPONSE OF PATIENTS WITH CUTANEOUS LEISHMANIASIS FROM CALAKMUL, MEXICO, TREATED WITH MEGGLUMINE ANTIMONIATE (GLUCANTIME)

Village	Children		Adults	
	Age years	Dose*	Age years	Dose*
	Northern	Calakmul		
La Mancolona	6-14	5-18	18-63	5-45
Ricardo Flores Magon	0	0	27	5
La Virgen	12-17	8-16	22-65	10-65
El Carmen	10	6	18-54	5-20
Narciso Mendoza	10-14	10	20-52	10-22
Nuevo Campanario	0	0	21-49	15-39
	Central	Calakmul		
La Lucha	0	0	47	10
Ricardo Payro	13	10	28-50	37-67
Castilla Brito	0	0	29-31	25-50
La Guadalupe	12-16	3-10	40	25
Cristobal Colon	0	0	34	5
Niños Heroes	0	0	34	5
Las Tres Huastecas	0	0	26-28	5-10
	Southern	Calakmul		
Sacrificio	0	0	30	20
Dos Naciones	10-14	10-20	22-30	30
Dos Lagunas Sur	8-16	2-20	21-64	2-30

*Glucantime: one dose= 25 mg/day

Leishmania species.² Regarding the vectors Pech-May,³⁰ they found in Dos Lagunas Sur *L. mexicana* infections in two sandfly species, *Lu. shannoni* and *Lutzomyia ylephiletor*, whereas in La Mancolona they found infections in *Lu. shannoni*, *Lu. cruciata*, *Lu. o. olmeca* and *Lu. panamensis*.

In conclusion, zoonotic cutaneous leishmaniasis (ZCL) caused by *L. (mexicana)* and *L. braziliensis* complexes, in foci located in Central and South America including Mexico, is disseminating due to urbanization and new settlements, as it has occurred in Calakmul. As it was established by Desjeux³¹ these factors are making ZCL a growing public health concern for many countries of the American continent. The findings in the present study showed that CL is still an important public health concern in the Municipality of Calakmul, Campeche, Mexico. This is due maybe to ecological, social and educational conditions favorable to the permanence and transmission of LC.

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Declaration of conflict of interests. The authors declare that they have no conflict of interests.

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