

American cutaneous leishmaniasis: clinical, epidemiological and laboratory studies conducted at a university teaching hospital in Campo Grande, Mato Grosso do Sul, Brazil *

Leishmaniose tegumentar americana: estudo clínico, epidemiológico e laboratorial realizado no Hospital Universitário de Campo Grande, Mato Grosso do Sul, Brasil

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Abstract: BACKGROUND: American cutaneous leishmaniasis is a disease with a wide variety of clinical manifestations that is expanding throughout Brazil, the state of Mato Grosso do Sul constituting a significant endemic area.

OBJECTIVES: To evaluate the clinical, epidemiological and laboratory characteristics of patients with American cutaneous leishmaniasis. Patients were recruited among those attending the Maria Aparecida Pedrossian Teaching Hospital of the Federal University of Mato Grosso do Sul, Brazil.

METHODS: This was a cross-sectional, observational study conducted using a descriptive and analytical approach. Data from patients suspected of having American cutaneous leishmaniasis who were receiving care at this institute between 1998 and 2008 and were referred to the institute's parasitology laboratory for confirmation of diagnosis were evaluated retrospectively. Clinical and laboratory criteria were taken into consideration for the inclusion of patients to the study.

RESULTS: Forty-seven patients were included in the study, the majority of whom were male and between 45 and 59 years of age. Most had the cutaneous form of the disease with a single, ulcerated lesion on exposed areas of the body, which had generally been present for periods of less than six months. Mucosal involvement increased with age and was highest in patients who had sought medical care at a later stage. The Montenegro skin test showed the highest sensitivity. Finding the parasite was more difficult in older lesions.

CONCLUSION: Suspicion of the disease at an early stage is of extreme importance for a precise diagnosis. A combination of parasitological and immunological tests renders laboratory diagnosis more reliable.

Keywords: Epidemiology; Leishmania; Leishmaniasis, cutaneous; Montenegro; Ulcer

Resumo: FUNDAMENTOS: Leishmaniose Tegumentar Americana é zoonose de manifestações clínicas variadas, em expansão no Brasil, sendo o estado de Mato Grosso do Sul importante área endêmica.

OBJETIVOS - Avaliar clínica, epidemiológica e laboratorialmente pacientes com Leishmaniose Tegumentar Americana, atendidos no Hospital Universitário Maria Aparecida Pedrossian, Universidade Federal de Mato Grosso do Sul, Brasil (HU/UFMS).

MÉTODOS - Trata-se de um estudo observacional do tipo transversal com abordagem descritiva e analítica. Foram avaliados, retrospectivamente, dados de pacientes suspeitos de Leishmaniose Tegumentar Americana, atendidos no HU/UFMS de 1998 a 2008, e encaminhados ao Laboratório de Parasitologia/UFMS para complementação diagnóstica. Para a inclusão neste estudo foram considerados critérios clínicos e laboratoriais.

RESULTADOS - Quarenta e sete pacientes foram incluídos no estudo. Houve predominância de homens de 45 a 59 anos, com a forma cutânea, lesão única, ulcerada, em áreas expostas do corpo e com duração menor que seis meses. O comprometimento de mucosas foi crescente com o aumento da idade e maior em pacientes que procuraram atendimento tardiamente. Intradermorreação de Montenegro foi o exame de maior sensibilidade e o encontro do parasito mostrou-se mais difícil em lesões antigas.

CONCLUSÃO - Suspeição diagnóstica precoce é de extrema importância para diagnóstico preciso. Associação de exame parasitológico e imunológico torna mais seguro o diagnóstico laboratorial.

Palavras-chave: Epidemiologia; Leishmania; Leishmaniose cutânea; Montenegro; Úlcera

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Introduction

American cutaneous leishmaniasis (ACL) is a zoonosis that affects humans and various species of wild and domestic animals. This infectious, non-contagious disease presents as a polymorphic manifestation of the skin and mucosae and is caused by various species of protozoa of the *Leishmania* genus, the most common in Brazil being *Leishmania (Viannia) guyanensis*, *L. (Viannia) braziliensis* and *L. (Leishmania) amazonensis*. The parasite life cycle is heteroxenic and its vectors are hematophagous insects of the Phlebotominae subfamily, *Lutzomyia* being the most common genus.^{1,3}

Between 1998 and 2008, approximately 282,000 cases of ACL were registered in Brazil, 2,240 of which were registered in the state of Mato Grosso do Sul.⁴

Over the years, ACL has behaved as a classic occupational disease, typically affecting adult males exposed to forested regions. Nevertheless, in recent decades, changes have occurred with respect to its epidemiological profile as a consequence of the extensive urbanization process, resulting in increasing numbers of women and children becoming infected.⁵⁻⁹

The incubation period of the cutaneous form of the disease may vary from a week to a month, with mucosal lesions generally appearing one to two years after the initial infection.¹⁰ The clinical manifestation of ACL may take various forms, ranging from self-limiting skin lesions to a disfiguring mucocutaneous forms of the disease. This variation has been associated with the patient's immunological status and with the species of *Leishmania*.^{3,11-13} This aspect of the infection makes clinical diagnosis difficult, and there needs to be a strong proof of suspicion of the disease.

The localized cutaneous form presents as a typical, painless ulcer with a raised border and granulomatous base. A single lesion or multiple lesions may be present. The disseminated cutaneous form presents itself as multiple small ulcers resulting from hematogenous or lymphatic dissemination and is generally found in immunodepressed patients. These forms of diseases are caused principally by the following species *L. (Viannia) braziliensis*, *L. (Viannia) guyanensis*, *L. (Viannia) lainsoni* and *L. (Leishmania) amazonensis*.^{1,2}

The mucocutaneous form is characterized by aggressive lesions that affect the mucosa of the nasopharyngeal region and are a direct extension or hematogenous metastasis of the primary skin lesion. The principal etiologic agent is *L. (Viannia) braziliensis*.¹

The diffused cutaneous form, caused in Brazil by *L. (Leishmania) amazonensis*, is characterized by nonulcerated nodular lesions, preceded by a single ulcer that evolves with lymphatic dissemination of the parasite. This form is associated with a depressed

immune cell response that leads the patient to a state of immunological anergy.^{1,13}

Although in some regions the diagnosis is only possible on the basis of a clinical and epidemiological criterion, laboratory diagnosis is extremely important in view of the numerous differential diagnoses that must be made with other granulomatous dermatoses and also because of the severe side effects of the drugs used for the treatment of ACL.^{1,2}

Diagnostic confirmation may be possible by detection of the parasite on a direct microscopic examination of the tissue impression smears, and by isolating the parasite in culture with specific medium and/or performing hamster inoculation. In addition, confirmation may also be obtained by histopathology or using polymerase chain reaction (PCR). Immunological tests such as the Montenegro skin test and indirect immunofluorescence assay are indirect methods that also help define diagnosis.^{1,13}

The objective of this study was to descriptively and analytically evaluate the clinical, epidemiological and laboratory characteristics of a series of ACL cases seen at the *Maria Aparecida Pedrossian* Teaching Hospital of the Federal University of Mato Grosso do Sul between 1998 and 2008.

Material and Methods

A descriptive, cross-sectional, observational study was conducted to evaluate clinical and epidemiological data, as well as the results of laboratory tests of 168 patients attending the *Maria Aparecida Pedrossian* Teaching Hospital of the Federal University of Mato Grosso do Sul between 1998 and 2008. The patients had lesions of the skin and/or mucosa that were suspected of being ACL and had been referred to the Parasitology Laboratory of the Center for Biological and Health Sciences at the Federal University of Mato Grosso do Sul for the confirmation of diagnosis.

Clinical and epidemiological data, as well as serologic findings (indirect immunofluorescence), Montenegro skin test results up to 2001 and histopathology reports were obtained from a retrospective review of the patients' records. Parasitological test results (direct microscopic examination, culture and hamster inoculation) and findings of the Montenegro skin test from 2002 onwards were obtained from the records of the parasitology laboratory.

Case report forms were completed with the patient's identification data (age, gender and place of origin), year of first consultation, time between onset of the lesions and initial consultation, clinical characteristics, number and site of lesions, and the diagnostic tests performed.

The patients with skin lesions suspected of being ACL were submitted to the following tests:

- Parasitological tests consisting of: direct microscopic examination, culture and hamster inoculation;
- Immunological tests: Montenegro skin test and indirect immunofluorescence;
- Histopathology.

The material for parasitological diagnosis was obtained by performing a biopsy of the border of the lesion. Part of this material was used to perform tissue impression smears to detect the amastigotes, and the remainder to isolate the parasite in culture and/or hamster inoculation. For a direct microscopic examination, the smear was fixed in methanol and stained using the Giemsa technique. For in vitro and in vivo isolation of the parasite, the specimen was washed in saline solution containing antibiotics (2000 IU penicillin G potassium and 200 mcg/ml of streptomycin sulphate) and then macerated. Next, it was seeded in 3N medium (Nicolle, Novy and McNeal) with the liquid phase constituted of Schneider medium to which 20% of fetal bovine serum, 1000 IU of penicillin and 100 mcg of streptomycin sulphate had been added per ml. Part of this material (0.5 ml) was inoculated intradermally into the anterior paws of a hamster.

The cultures were incubated at 24°C in a biochemical oxygen demand (BOD) incubator (FANEM, model 347) and checked between the 7th and the 30th days. The hamsters were examined weekly for skin lesions over a period of at least one year. After this time, or whenever the lesions appeared, necropsy was performed on the animals and the skin, the spleen and the liver fragments were processed to investigate for amastigotes and to isolate the parasite in 3N-Schneider medium, as previously described.

The Montenegro skin test was performed at the parasitology laboratory by intradermal inoculation of 0.1 ml of standardized antigen in 40 mcg of protein nitrogen per ml into the left forearm, results being read 48 and 72 hours after the inoculation. The limits of the indurated area were marked and printed onto filter paper moistened with alcohol. Tests in which the indurated area was ≥ 5 mm in diameter were considered positive.

Indirect immunofluorescence titers $\geq 1/40$ according to the data on the patients' charts were considered positive.

According to the histopathological results, three possible diagnoses were taken into consideration: 1) confirmatory, if amastigote forms of *Leishmania* were found; 2) suggestive, if granulomatous inflammatory infiltrate composed of lymphocytes, histiocytes and plasma cells suggestive of a tissue reaction compatible with ACL was found; and 3)

negative, in all other cases.

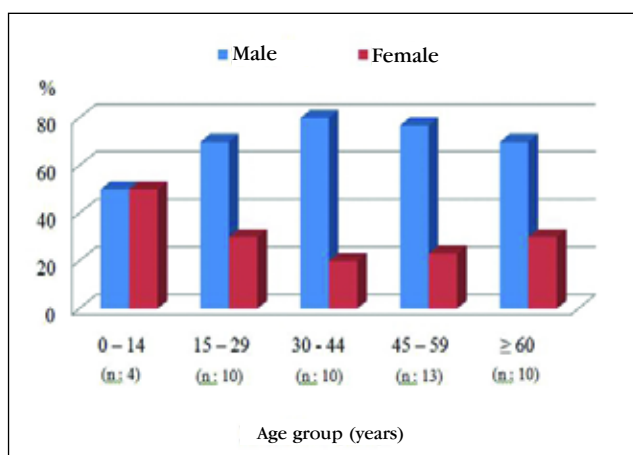
To reach a diagnosis of ACL in a patient, the following criteria should be present: 1) at least one positive parasitological test (direct microscopic examination and/or culture and/or hamster inoculation); or 2) confirmatory histopathological findings; or 3) anatomopathological results suggestive of ACL and at least one positive immunological test (indirect immunofluorescence and/or Montenegro skin test). Of the 168 patients initially suspected of having the infection, 47 were considered to have ACL and they were included in the study.

Descriptive and analytical statistical analyses were performed using the Epi Info software program, version 3.4.3 (November, 2007) and the BioEstat program, version 5.0. To verify possible associations between the study variables, the following tests were used: the chi-square test for trend and Fisher's exact test. Significance level was defined as 5%.

Results

Forty-seven patients, 72.3% male and 27.7% female, who attended the teaching hospital of the Federal University of Mato Grosso do Sul between 1998 and 2008, were diagnosed with ACL. Patients' age ranged from 6 to 85 years old (average 44 years). Of the 47 patients, 8.5% were between 0-14 years old, 21.3% were between 15-29 years old, 21.3% were between 30-44 years old group, 27.7% were between 45 and 59 years old and 21.3% were between ≥ 60 years old. In the 0-14 years old group, both sexes were equally affected, whereas in all the other age groups there was a predomination of males (Graph 1).

With respect to the year of the first consultation, 27.7% of the patients were seen in 2008, 10.6% in 2007, 21.3% in 2006, 6.4% in 2005, 8.5% in 2004, 8.5% in 2003, 6.4% in 2002, 6.4% in 2001, 0.0% in



GRAPH 1: Percentage of patients diagnosed with ACL according to the age group and gender. Teaching Hospital, Federal University of Mato Grosso do Sul, 1998-2008

2000, 2.1% in 1999 and 2.1% in 1998. The majority of the patients were from Campo Grande, the capital city of the state of Mato Grosso do Sul (29.8%), followed by the town of Bodoquena in the same state (8.5%). A further 44.6% of patients came from other towns in the state of Mato Grosso do Sul, while 17.1% were from other states (Figure 1).

The cutaneous form of the disease was found in 68.1% of cases, the mucosal form in 27.7% and the mucocutaneous type in 4.3%. With respect to the number of lesions, 61.7% of patients had a single lesion, 34.0% had 2-5 lesions and 4.3% had more than 5 lesions. In 34.0% of cases, the lesions were localized on the lower limbs, in 25.5% on the face, in 19.1% on the upper limbs, 19.1% in the nasal mucosa, 19.1% in the oral mucosa, 6.4% on the abdomen, 4.3% on the back, 2.1% on the ear and 2.1% on the gluteal region, with no lesions being found on the chest (Figure 2).

Concerning the clinical characteristics of the lesions, ulcerated lesions were found in 72.3% of cases, infiltrative lesions in 8.5%, sarcoid lesions in 8.5%, vegetative lesions in 6.4%, papular lesions in 4.3%, verrucous lesions in 2.1% and subcutaneous nodules in 2.1% of cases (Figure 3). Other forms such

as plaques and exulceration were found in 14.9% of cases, while in 2.1% of the cases the data on the characteristics of the lesion were missing.

The time between the onset of the lesions and the first medical consultation was less than six months representing 53.2% of cases, between six months and one year it was 17.0% and over a year it was 27.7% of patients. In 2.1% of cases, this information was missing.

Of the 47 patients included in the study, 34 have had their biopsied skin submitted for a direct microscopic examination, with 58.8% of these patients testing positive. Culture was carried out for 33 patients, and 30.3% of them tested positive. Hamster inoculation was performed for four patients, and three of them tested positive.

Of the twenty four patients, 91.7% reacted positively to the Montenegro skin test. Twenty-eight patients underwent indirect immunofluorescence, 46.4% of them tested positive. Histopathology as carried out in 46 patients, results being positive in 26.1% of cases, suggestive of ACL in 69.6% and negative in 4.3% of cases.

In the 25 cases in which the time between onset of the lesion and the patient's first consultation was less than six months, the direct microscopic examination was positive in 68.0% of patients and confirmatory histopathology in 32%. Of the 8 patients for whom the progression of the lesions was between 6 months and one year, direct examination was positive in 25% with histopathology confirming this result in 12.5%. In those cases in which the course of the lesion exceeded one year (n=13), direct examination was positive in 7.8% and confirmatory histopathology in 15.4% (p<0.001 for direct examination and p<0.256 for histopathology, Fisher's exact test) (Graphs 2 and 3).

Table 1 shows that of the patients with a positive parasitological test (n=26), 6 were submitted to

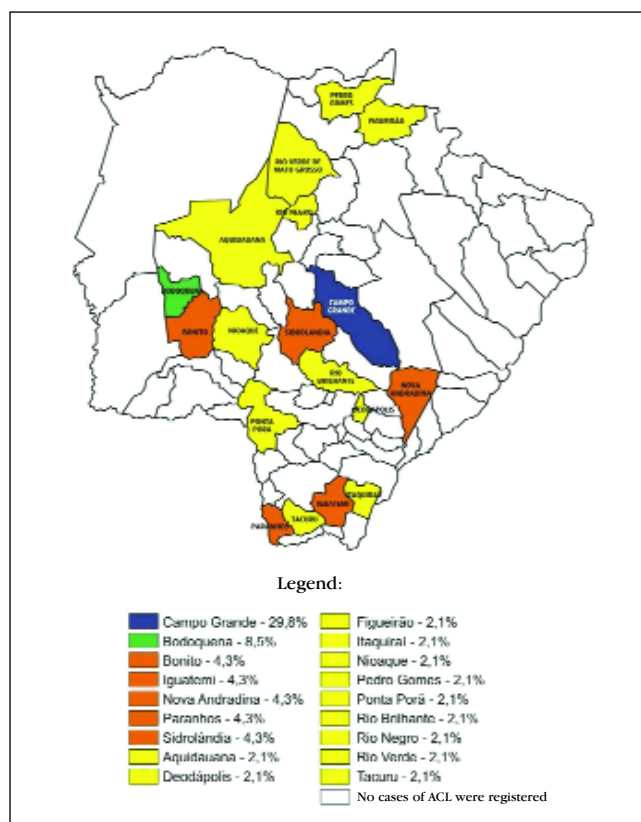
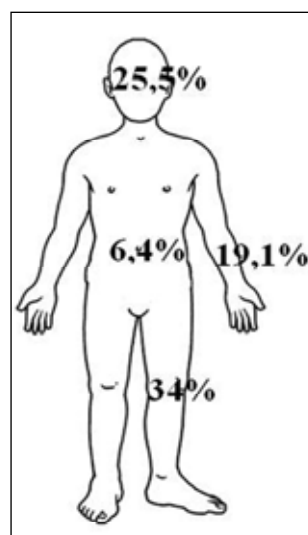


FIGURE 1: Map of the state of Mato Grosso do Sul and the percentage of the patients with a diagnosis of ACL according to their city of origin. Teaching Hospital, Federal University of Mato Grosso do Sul, 1998-2008



25.5%: face;
6.4%: abdomen;
19.1%: upper limbs;
34.0%: lower limbs;

FIGURE 2: Percentage of patients with a diagnosis of ACL according to the most common sites of skin lesions. Teaching Hospital, Federal University of Mato Grosso do Sul, 1998-2008



FIGURA 3: American cutaneous leishmaniasis. Dermatology Department, Teaching Hospital, Federal University of Mato Grosso do Sul, 1998-2008. **A.** Cutaneous form: papules, nodules and infiltrated, ulcerated plaques with a granulomatous core in the left lower limb; **B** Mucosal form: erythema and mid-facial infiltration with superficial ulceration of the labial and nasal mucosa

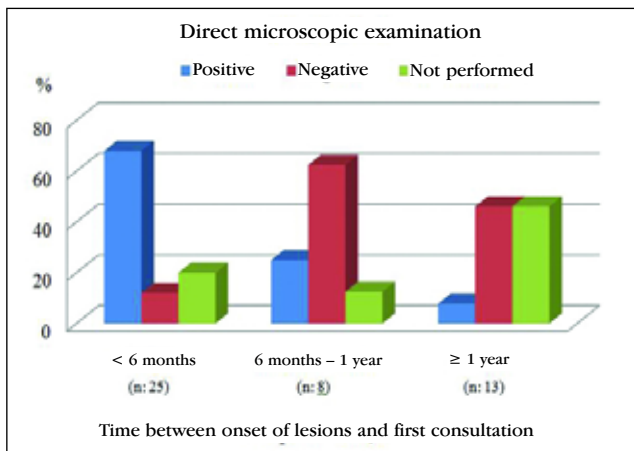
the Montenegro skin test, with four testing positive (66.7%). Among the patients whose parasitological test was negative (n=8), 5 were submitted to the Montenegro skin test, which was positive in 100.0% of these patients. Among the patients with a positive Montenegro skin test (n=22), parasitological testing was performed in 9 patients, 4 of them (44.4%) tested positive. Among the two patients with a negative Montenegro skin test, the parasitological test was positive in both cases.

In the group of patients with a positive parasitological test, indirect immunofluorescence was performed in 10 and results were positive in 6 cases (60.0%). Among the patients with negative parasitological test, 5 were submitted to indirect immunofluorescence, with positive results in two cases (40%). Among the patients with positive indirect immunoflu-

orescence (n=13), a parasitological test was performed in eight, six of them tested positive (75.0%). Finally, among the 15 patients whose indirect immunofluorescence test was negative, a parasitological test was carried out in 7, with positive findings in 4 (57.1%) (Table 1).

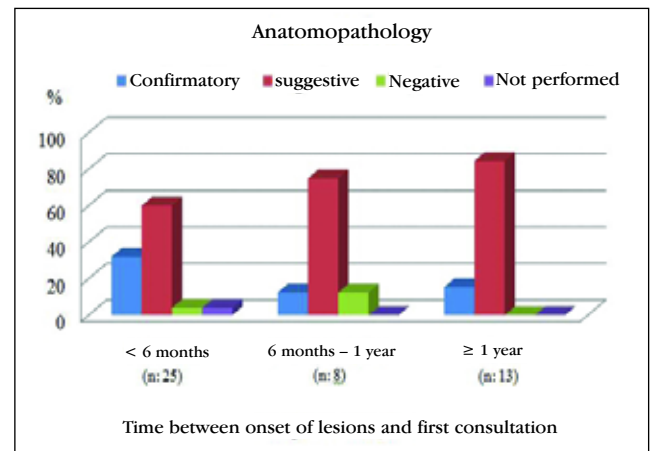
When a parasitological test was associated with the Montenegro skin test, at least one of these tests was positive in 100.0% of cases (11/11). When the Montenegro skin test was associated with indirect immunofluorescence, positivity was found in 94.7% of cases (18/19) and when a parasitological test was associated with indirect immunofluorescence, results were positive in 80.0% (12/15) (Table 1).

Among the patients with mucosal involvement who were submitted to indirect immunofluorescence (n=12), 8 (66.7%) tested positive. On the other hand,



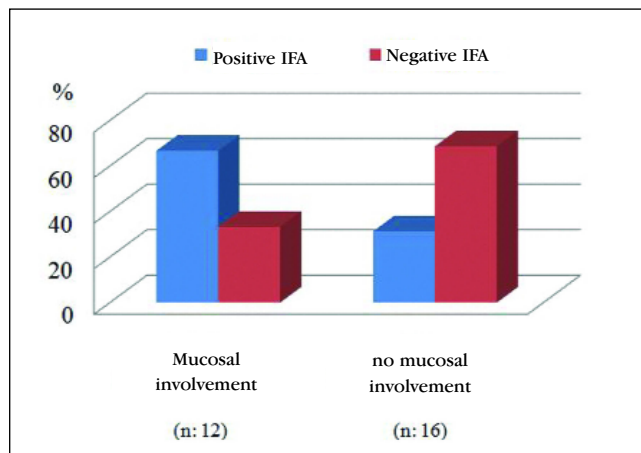
GRAPH 2: Percentage of patients diagnosed with ACL according to the time since onset of the lesions and direct microscopic examination. Teaching Hospital, Federal University of Mato Grosso do Sul, 1998-2008

NB: In the case of one patient, data on the time since the onset of the lesion is missing.



GRAPH 3: Percentage of patients diagnosed with ACL according to the time since onset of the lesions and anatomopathology. Teaching Hospital, Federal University of Mato Grosso do Sul, 1998-2008

NB: In the case of one patient, data on the time since the onset of the lesion is missing.



GRAPH 4: Porcentagem de pacientes com diagnóstico de LTA, segundo presença de comprometimento mucoso e IFI, Núcleo do Hospital Universitário/ UFMS - 1998 a 2008

Nota: Dos 47 pacientes incluídos no estudo, a IFI foi realizada em 28.

in those that mucosa was not affected and who were submitted to indirect immunofluorescence (n=16), results were positive in only 5 cases (31.3%) (Graph 4).

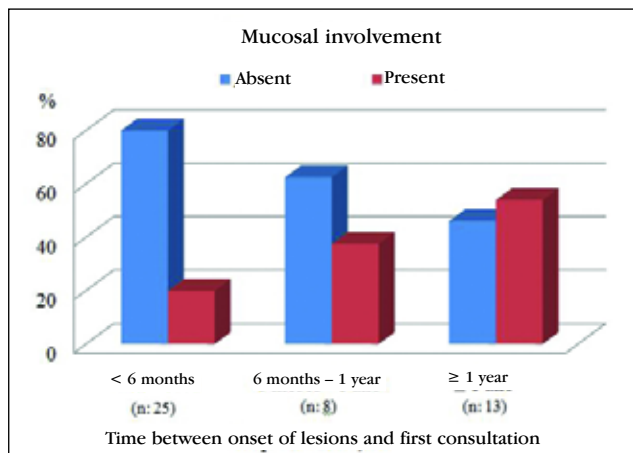
Graph 5 shows that in cases in which progression of the lesion was less than six months (n=25), the mucosa was affected in 20% of the patients, whereas in those for whom this time was 6-12 months (n=8), the mucosa was affected in 37.5% of patients and in those cases which were more than one year old, there was mucosal involvement in 53.9% of cases. With respect to age-group, no patients in the 0-14 years age group had mucosal involvement, whereas the mucosa was affected in 10% of patients in the 15-29 years age group, 30% of the 30-44 years age group, 30.8% of the 45-59 years age group and 70% of the ≥ 60 years age group ($p < 0.002$; chi-square test for trend) (Graph 6).

Among the 9 patients with lesions at the nasal mucosa, 7 (77.8%) suffered collapse and/or perforation of the nasal septum and of the 9 patients with oral mucosa lesions, 3 (33.3%) developed further perforation of the palate.

Discussion

In Brazil, ACL is expanding geographically, with peak incidence rates being recorded every five years. Incidence of the disease is distributed throughout the country; however, the Midwest currently occupies third place in the ranking.¹⁴ According to the Ministry of Health, in the state of Mato Grosso do Sul, the case detection rate underwent variations between 1998 and 2008, ranging from a maximum of 17.6 cases per 100,000 inhabitants in 2001 to a minimum of 5.0 cases per 100,000 inhabitants in 2006.¹⁴

In the present study, the group most affected



GRAPH 5: Porcentagem de pacientes com diagnóstico de LTA, segundo tempo de evolução e presença de comprometimento mucoso, Núcleo do Hospital Universitário/ UFMS - 1998 a 2008

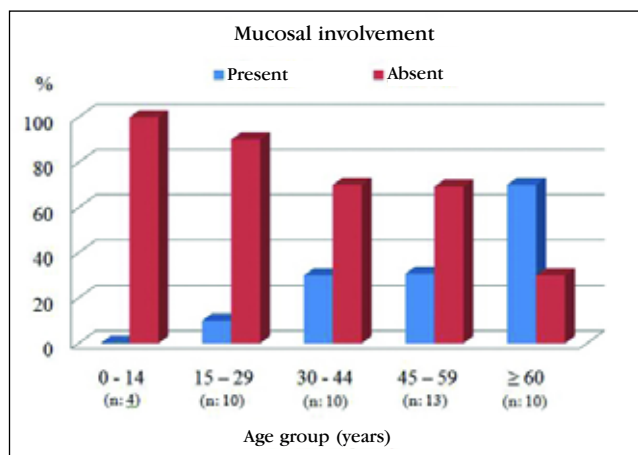
Nota: Para um paciente o tempo de evolução da lesão não foi determinado.

consisted of adult males and, to a lesser extent, women and children, suggesting changes in relation to the epidemiological profile of the disease as a result of its expansion to areas in and around houses. This finding has also been observed in various other regions of the country.^{5,8,9,15}

The largest concentration of patients was in the 45 to 59 age group, an older group than those reported in other studies, albeit still within the susceptible age range. 9,15-17 The results of the present study also show that the disease affected individuals in the 15-29, 30-44 and ≥ 60 years age groups to a similar degree. In a study conducted in 1990 in the municipality of Nioaque, Mato Grosso do Sul, Fernandes reported no difference between the sexes and higher rates in individuals under 20 years old, results that differ from the findings of the present study.¹⁸

No difference was found between males and females in patients of 0-14 years old; however, there was a predominance of males in all the other age groups. This suggests that in children the transmission of ACL occurs around the home, affecting girls and boys equally. After reaching adolescence (15 years old), men tend to leave the home environment looking for a job and are even more likely to be exposed to forested areas, resulting in a greater proportion of males being affected by this disease compared to females.

With respect to the year of first consultation, the majority of cases were seen for the first time in 2008 (27.7%) and 2006 (21.3%), whereas no cases were registered in 2000. Regarding the geographical distribution of the cases of ACL registered in the state of Mato Grosso do Sul, a higher concentration was found in the Midwestern region (Campo Grande), fol-



GRAPH 6: Porcentagem de pacientes com diagnóstico de LTA, segundo faixa etária e presença de comprometimento mucoso, Núcleo do Hospital Universitário/ UFMS - 1998 a 2008

lowed by the south of the state. No cases were recorded in the east and there were few cases in the north of the state. This profile may be explained by the fact that Campo Grande represents the closest referral center to the areas with the greatest number of cases, whereas patients from the east of the state are seen at other state referral centers such as Três Lagoas and Dourados, which are closer.

The most commonly found clinical form of the disease was the cutaneous form (68.1%), although the

TABLE 1: Number of patients with a diagnosis of ACL according to the parasitological tests, the Montenegro skin test, the indirect immunofluorescence and a combination of these techniques. Teaching Hospital, Federal University of Mato Grosso do Sul, 1998-2008

Parasitological test	Montenegro Skin Test		Total
	Positive	Negative	
Positive	4	2	6
Negative	5	0	5
Total	9	2	11

Parasitological test	Indirect Immunofluorescence		Total
	Positive	Negative	
Positive	6	4	10
Negative	2	3	5
Total	8	7	15

Montenegro skin test	Indirect Immunofluorescence		Total
	Positive	Negative	
Positive	6	11	17
Negative	1	1	2
Total	7	12	19

NB: Positive parasitological test means that at least one test (direct microscopic examination, culture or hamster inoculation) was positive.

percentage of patients with the mucosal form was also high (27.7%), which may be due to the fact that the study was conducted in a state referral center where more severe cases and those refractory to treatment, a characteristic of mucosal lesions, are more common.^{5,8,19} A trend towards fewer lesions per patient was also found, with a high rate of single lesions (61.7%) and few cases with more than five lesions (4.3%), a finding that is in agreement with data published by several other investigators.^{16,20} The areas most affected were those that are most exposed to bites from the phlebotome, particularly the lower limbs, the face and the upper limbs.^{15,17} With respect to the clinical characteristics of the lesions, ulcers were found in the great majority of cases (72.3%), this being the lesion most commonly reported in the literature.^{8,15,17,21} In a retrospective study conducted in a teaching hospital of the Federal University of Mato Grosso do Sul between 1976 and 1999, Noguchi also found that the majority of patients had one single skin ulcer and that the infection affected predominantly males.²²

The patients with the cutaneous form of the disease sought medical help earlier than those with the mucosal form, probably due to esthetic reasons and because the patients may have attributed the mucosal symptoms to other diseases such as rhinitis, sinusitis or respiratory infections.¹⁶ Furthermore, it was found that the proportion of patients with mucosal involvement increased with age, this correlation being significant. These data suggest that debilitation due to age, to the presence of comorbidities and to the fact that the etiologic agent had remained in the body for a longer period of time may contribute towards mucosal activation and/or dissemination of *Leishmania*.²³

In the present study, similar rates of nasal and oral mucosal involvement were found. These results differ from the majority of published studies, which show nasal mucosa to be the most commonly affected.^{16,21} The majority of patients with nasal mucosa involvement had some form of complication (perforation and/or collapse of the nasal septum). On the other hand, in the patients in whom the oral mucosa was affected, fewer had complications (perforation of the palate).

In those patients who were submitted to some form of parasitological test, 76.5% tested positive in at least one of these tests. The rate of positivity with direct microscopic examination (58.8%) was close to the rates reported in various other studies.^{19,24} However, culture was negative in the majority of cases (69.7%). There is much discrepancy with respect to the success of culture isolation, positivity ranging from 13 to 90%. This discrepancy may be explained by the differences in the techniques used to obtain the samples, possible material contamination and differ-

ences between patient populations and between the species of *Leishmania* that circulate in the different geographical areas.²⁴

A statistically significant reduction was found in the sensitivity of direct examination as the time since infection increased.²³ In recent cases, this test is positive in practically 100% of patients but becomes progressively negative as time goes on and is rarely positive in mucosal lesions.²⁵ A higher percentage of confirmatory histopathology was also found in recent lesions. These data confirm that detection of the parasite becomes more difficult as the infection progresses. As the chronic granulomatous process becomes installed, the number of parasites decreases and it becomes difficult to detect them in older lesions, thus increasing the proportion of tests suggestive of ACL, as found in the present study. The percentage of confirmatory tests varies according to the authors^{5,8,19,21} and was found in 26.1% of cases in this particular study.

In the present study, patients were not included solely on the basis of a positive immunological test (Montenegro skin test and/or indirect immunofluorescence). In highly endemic areas, reactivity to the Montenegro skin test is very common in individuals with no active or cicatricial lesion, which may mean that a subclinical infection or other co-existent endemic infections are present.²⁴ Likewise, a positive indirect immunofluorescence test may be indicative of a recent infection in the absence of lesions or subclinical reinfections,^{6,26,27} merely indicating contact with the parasite and not necessarily an active lesion.²⁴ Furthermore, the possibility of cross-reactions with other species of *Leishmania* should also be considered, and it is important to emphasize that visceral leishmaniasis and ACL are both present in the state. Nevertheless, both Montenegro skin test and the indirect immunofluorescence assay can be useful when there are few parasites in the lesions and in diagnosing individuals who have visited endemic areas.²⁴

The Montenegro skin test, a late hypersensitive reaction, is positive in around 90% of individuals with the active disease or a past infection, as found in the present sample in which, of all the diagnostic methods used, the Montenegro skin test achieved the highest percentages of positivity (91.7%).^{16,21,28} The same was not true for the indirect immunofluorescence, with negative results being found in 53.6% of cases. The efficacy of the indirect immunofluorescence for the diagnosis of ACL is poor because of the low levels of circulating antibodies.²⁴ Patients with mucosal involvement were more likely to test positive for the indirect immunofluorescence, whereas in those that the mucosa was unaffected, the indirect immunofluorescence was more likely to be negative (68.7%). In a review paper published in 1980, Furtado reported

that some investigators had found higher rates of positivity in later forms of the disease with mucosal lesions compared to more recent cutaneous forms of the disease; however, other investigators failed to confirm this association.²⁹

Sensitivity was higher with the Montenegro skin test than with indirect immunofluorescence, in the group of patients with a positive parasitological test and in those with negative findings. With respect to the combination of techniques, parasitological tests associated with the Montenegro skin test were found to result in greater sensitivity, followed by the Montenegro skin test associated with indirect immunofluorescence and, lastly, parasitological tests associated with indirect immunofluorescence. These findings vary in part from those reported by Silveira et al. in a study conducted in the state of Paraná in 1999, which found higher sensitivity when the Montenegro skin test was associated with indirect immunofluorescence and lower positivity for the association of parasitological tests with indirect immunofluorescence.²⁸

Conclusion

The majority of patients in the present study came from the Midwestern region of the state. As reported, the disease continues to be associated with forested areas, with higher rates of infection in males of working age; however, a considerable percentage of women and children were affected, suggesting that the vector is present in areas in and around residences. The cutaneous form of the disease was the most common, with the majority of patients having a single, ulcerated lesion of less than six months' duration. The uncovered areas of the body were those most affected, principally the lower limbs. The finding of mucosal involvement increased with age and was more common in patients who had sought medical help at a later stage. Nasal and oral mucosa was equally affected.

Of all the laboratory tests, the Montenegro skin test achieved the highest rates of positivity. Direct microscopic examination became less sensitive as the time of progression of the infection increased, while finding the parasite at histopathology is more likely in recent lesions, highlighting the fact that a suspicion of ACL at an early stage of the infection makes diagnosis simpler and more reliable. Although indirect immunofluorescence had the highest percentage of negativity in the patients in general, it achieved the highest rates of positivity in patients with mucosal lesions, hence emphasizing the importance of this test. The combination of parasitological and immunological tests improves the efficacy of laboratory diagnosis. Sensitivity was high when a parasitological test was associated with the Montenegro skin test. This shows

the importance of performing the Montenegro skin test, a simple and readily accessible test, together with parasitological tests, the most important of which is direct microscopic examination, to establish diagnosis.

Therefore, diagnosis of ACL should be made by taking the clinical status of the patient and epidemiological data into consideration and by using a combination of laboratory techniques. □

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