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Case Report

First Report of Autochthonous Visceral Leishmaniosis in Humans in foz Do Iguaçu, Paraná State, Southern Brazil

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Abstract

This is the first report of autochthonous visceral leishmaniosis in humans in Foz do Iguaçu city, Parana, Brazil, bordering Argentina and Paraguay. During his physical examination, a 28-year-old man from Foz do Iguaçu, showed good general health, a slightly bleached, discrete bilateral cervical lymphadenopathy, 38°C fever, confirmation of weight loss and no other symptoms About 15 days after the first visit, he presented with discrete hepatomegaly and splenomegaly palpation of the abdomen. Amyelogram, stained by May-Grunwald-Giemsa, showed normocellular bone marrow for age and presence of *Leishmania* amastigotes in macrophages and extracellularly. The parasite was isolated by bone marrow aspiration and cultured in Novy, Mac Neal-Nicolle medium. Serological exams by enzyme-linked immunosorbent assay and DPP were positive. Polymerase chain reaction (PCR) with primers RV1/RV2 was performed on DNA extracted from bone marrow aspirate and showed an identical pattern to the *Leishmania infantum* reference strain.The species of the parasite, isolated in culture, was confirmed by PCR using primers for the ITS-1 region followed by restriction fragment length polymorphism (RFLP) pattern analysis and sequencing. RFLP verified the parasite showed a very similar pattern to *L. infantum* and the forward and reverse sequences presented 99% identity with *L. infantum*. An epidemiological survey in dogs, in the house of the patient's home and neighborhood showed 13.3% positivity. *Lutzomyia longipalpis* was present in the perdomestic environment of the patient's home.

INTRODUCTION

Visceral leishmaniosis (VL) is responsible for over 50,000 deaths per year, with an estimated average of half a million new cases annually [1]. In Brazil, VL has an effect of close to 3000 cases and approximately 200 deaths per year [2]. Over the past 20 years, there has been a transition in the epidemiological profile of VL, with an increase in the proportion of cases in the south west region and movement from rural to urban areas. In the Southern Cone, the triple borders of Argentina, Brazil and Paraguay, work has been conducted under the coordination of the Pan American Health Organization for research vectors and reservoirs of visceral leishmaniosis since 2012. In 2012 *Lutzomyia longipalpis* was confirmed in Foz do Iguaçu [3], and from 2012-2013, Thomaz-Soccol et al., [4] showed dog samples positive by serological tests and the parasites isolated were identified as *L. infantum* [5].

Here we report the first case of a patient diagnosed with visceral leishmaniosis with autochthonous transmission in Foz do Iguaçu city, Paraná State, in southern Brazil.

First Report of Vl in a man from Foz Do Iguaçu

The first report of VL in Foz do Iguaçu, Paraná, Brazil 25°32'49"S, 54°35'18"W was a 28-year-old, brown man who worked for the military police. He attended outpatient consultation with a complaint of 30 days of daily fever of 38-39ºC and night sweats accompanied by weight loss of 3 kg. He denied travelling to any other locations in the preceding 12 months and reported that he had been tested for tuberculosis two years previously, and the results were negative after extensive research

with a pulmonologist.

A physical examination showed good general health, a slightly bleached, discrete bilateral cervical lymphadenopathy, and fever of 38°C, the confirmation of weight loss, and no other signs or symptoms. An initial blood examination showed data compatible with acute infection or inflammatory disease (Table 1). The patient showed negative blood and urine cultures, an X-ray chest indicated injury, with a scar on the right apex, and an abdominal ultrasound was normal. About 15 days after the first visit, he presented discrete hepatomegaly and splenomegaly palpation of the abdomen, which were absent in the initial physical examination. The patient was referred to a hematologist to assess hematologic disease in the bone marrow. The results of samples stained by the May-Grunwald-Giemsa method and data are shown in Table (1). The myelogram showed normocellular bone marrow for the patient's age. The myeloid series showed elements at all stages of maturation. The erythroid component presented numerous hyperplasic cells (42%) without gross changes in shape. The megakaryocytic component presented amastigotes of *leishmania* sp. in the macrophages and extracellularly (Table 1, Figure 1). ELISA and DPP® serology using *L. infantum* antigen were reactive. The parasite was isolated by bone marrow aspiration and was cultured in Novy, Mac Neal-Nicolle medium. Species identification was performed by PCR using primers RV1 and RV2 [5]. In addition, amplification of the internal transcribed spacer 1 (ITS-1) was performed, followed by RFLP, as proposed by Schönian et al. (2003) [6]. RFLP showed an identical pattern to the reference strain of *L. infantum* (Figure 1). The forward and reverse sequences showed 99% identity with *L. infantum* (MHOM/BR/74/PP75; Gene Bank access number AF103739).

The patient was treated with deoxycholate of amphotericin B at a dose of 50 mg daily for a total period of 15 days, non-sequentially. He became asymptomatic, and blood count recovery was achieved after the seventh dose of medication. He presented complications of lung disease four days after the use of amphotericin, but showed excellent clinical response with the use of trimethoprim-sulfamethoxazole. After being attended at our service for six months, he has undergone complete clinical and blood count recovery. No marrow aspiration was performed for control of cure.

Here, we report the first patient with clinical and laboratorial confirmation of VL; in the Foz do Iguaçu city, Paraná State.

Epidemiological Research in the Address of the Patient

Serological evaluation in dogs: A serological study was performed, using TR-DPP® as screening and the ELISA test for confirmation, on 165 dogs in the patient's house and the surrounding neighborhood. Twenty-two of these samples were positive (13.3%), 142 were negative (85%) and one had an indeterminate ELISA result (1.6%). The two dogs owned by the patient had negative results. Among the positive animals, 18 were euthanatized with the owner's consent. Four owners, who did not agree to the removal of their positive dogs, signed a term of responsibility for rejecting the euthanasia of a positive dog.

Investigating the presence of Phlebotominae: Sand flies were captured with automatic CDC light traps, used for sampling the Phlebotominae in peridomestic environments and inside the patient's home. The traps were active from approximately 6:30 p.m. to 7:30 a.m., for three consecutive rainless nights. They were placed 1.5 m above the ground. The study was conducted from July 16th to 18th 2015. One female of *Lu. longipalpis* was found

Table 1: Results of Hematological Parameters.

First Exam	Hemogram			
	Hemoglobin (g/dL)	10.9		
	Hematocrit (%)	34.9		
	Leukopenia (cells/mcL)	2,600		
	Platelets/mcL	107,000		
	Enzymes AST and ALT (U/L of serum)	50.6 and 71.5		
	LDH	-283		
	PCR	13*	Shows acute disease or inflammatory syndrome	
Second Exam	**Hemogram**	**Myelogram**		
	Hemoglobin (g/dL) -11.8	*Myeloid*	*Erythroid*	*Megakaryocytic*
	Hematocrit (%) - 36.1	4% -promyelocytes	Present and numerous hyperplastic cells (42%) without gross changes	7%- lymphocytes
	RBCs (/mm^3) - 4.62	16% - myelocytes		1% - monocytes
	Mean corpuscular volume - 78.1 fl	20% - metamyelocytes		4% - blasts
	WBC (/mm^3) - 2,400	4% - segmented rods		2% - plasma cells
	43% neutrophils/ 8% lymphocytes (1% eosinophils/ 8% monocytes)			
	Platelets(/mm^3)- 109,000			

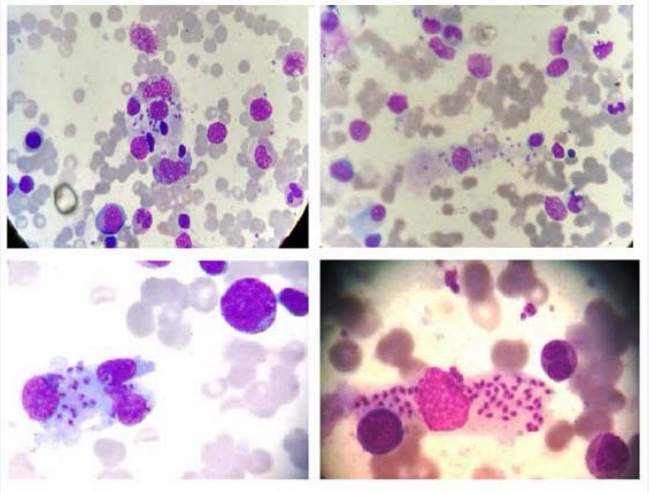

Figure 1 Bone marrow showing intra and extracellular amastigotes of *Leishmania* by May-Grunwald-Giemsa stain.

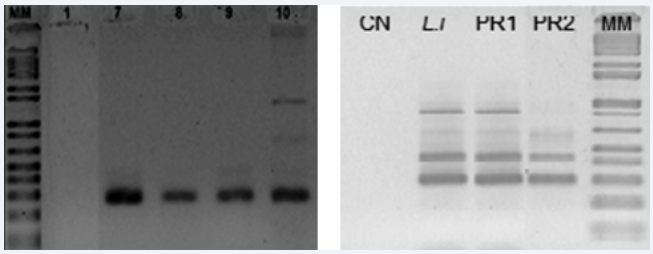

Figure 2 Molecular identification of the parasite isolated from the patient by Polymerase chain reaction, amplified with ITS1/ITS2 primers followed by cut with *Hae*III restriction enzyme (RFLP)
Legend (A): MM, 1Kb DNA ladder; 1, Negative control; 7, *Leishmania infantum* reference strain; 8, DNA extracted from bone marrow; 9, DNA extracted from bone marrow aspirate; and 10, DNA extracted from strain isolated from the patient. (B). CN, Negative control; *Li*, *Leishmania infantum* reference strain; PR1, DNA extracted from strain of the parasite isolated from the patient; PR2, DNA extracted from bone marrow aspirate, and MM, 100 BP DNA ladder.

in the patient's residence. Two males of the same species were captured at another residential address in the vicinity.

Using the same methodology, 12 traps were installed where the patient worked, from August 3rd o 5th 2015. In this environment, one female *Lutzomyia* sp. was captured.

DISCUSSION

The southern regions of Brazil were not considered an important area for VL until the beginning of 2008, when a dog was diagnosed with the disease in São Borja County, in the State of Rio Grande do Sul. In the same year, *Lu. longipalpis* were captured and autochthonous canine cases were confirmed [7,8] followed by the first human case registered in 2009 [9]. In Santa Catarina, Steindel et al., (2013) [10] described an outbreak of autochthonous canine VL (CVL), and in 2014, Maziero et al., [11] reported dogs with serology positive for VL in São Miguel do Oeste. Neighbouring countries Paraguay and Argentina have also recorded VL in recent years [12,13]. This prompted further research and the vector *Lu. longipalpis* was confirmed in areas of Foz do Iguaçu near Argentina [3]. However, the real distribution of the vector, in Foz do Iguaçu city remains unknown. Autochthonous transmission of *L. infantum* in dogs has been verified in regions near Argentina and Paraguay [14]. Again, the real CVL distribution in Foz do Iguaçu city (is not known) has yet to be determined. These data suggest a time sequence in the establishment of the disease in a given geographic area. Thus, the stage is set for the development of the disease in humans. In anticipation of diagnosing human cases, anyone who has had a fever for over a week or who reports rapid weight loss is being including in the differential diagnosis of VL.

With this first case of human VL, it was essential to determine whether this case was autochthonous or imported, because nowadays people travel within and to different regions very quickly and easily. The serological survey showed sero reactive dogs and the presence of the vector confirmed local transmission.

From a clinical perspective, this case was a typical manifestation of visceral leishmaniosis in the initial phase. We chose to prescribe amphotericin B, using a cumulative dose for a total period of 15 non-sequential days. The increased creatinine levels and hypokalemia were controlled during hospitalization. At the end of the therapy, the patient's splenomegaly was reduced by approximately 60%, the fever had ceased, and the pancytopenia was completely reversed.

Diagnostic tests for VL have certain limitations, for example, the serological tests can remain positive for a long time after patient treatment, thus they do not allow health professionals to evaluate the effect of the therapy and they have been known to cross-react with other diseases. Since subclinical infections occur, a positive test does not necessarily indicate active disease. Clinicians and epidemiologists always request serological tests to confirm the diagnoses, because parasitological methods are invasive. They expect the results to be reliable, with high specificity and sensitivity and as fast as possible, to enable them to treat preventable disease. However, no method provides 100% of sensitivity and the clinical features of this disease render the diagnosis difficult and complex. It is therefore important that the different specialties talk to each other and the laboratory to make decisions on patient referrals.

From now on, in Paraná state, physicians need consider a potential diagnosis of visceral leishmaniosis when a patient has fever for more than 10 days, and people traveling to Foz do Iguaçu need to know that this region is now endemic for visceral leishmaniosis.

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