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## NATURAL INFECTION BY *Trypanosoma cruzi* IN ONE DOG IN CENTRAL WESTERN BRAZIL: A CASE REPORT

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### SUMMARY

It is estimated that about 10 million people are infected with *Trypanosoma cruzi* worldwide, mostly in Latin America and more than 25 million are at risk of acquiring this infection in endemic areas. Dogs are an important reservoir for this pathogen and thus, considered a risk factor for human populations. This report describes one case of Chagas disease in a dog from Cuiabá, Mato Grosso State, Brazil. The diagnosis was obtained by direct examination of trypomastigote forms in blood smears. Amastigotes forms were visualized in microscopy of the bone marrow, lymph nodes, kidneys, liver and brain. The *T. cruzi* (ZIII) infection was confirmed by Polymerase Chain Reaction, and sequencing. The animal presented multisystemic failure and died. Although acute Chagas disease in humans is not reported in Cuiabá, this is the first report of a canine case in this region. This case represents a warning, to health professionals and authorities, to the possibility of transmission of this zoonosis in Cuiabá.

**KEYWORDS:** Chagas disease; *Trypanosoma cruzi*; Dog; Acute infection; Cuiabá.

### INTRODUCTION

It is estimated that 10 million people are infected worldwide by the protozoan *Trypanosoma cruzi*, the etiological agent of Chagas disease. Chagas disease is endemic in Latin America where more than 25 million people are at risk of acquiring this infection<sup>21</sup>. Chagas disease can be transmitted through excretion from infected insect vectors, blood transfusion, organ transplants, oral transmission, and laboratory accidents<sup>8</sup>. Four cases of human *T. cruzi* acute infection have been so far reported in the state of Mato Grosso - Midwest Brazil and only infected vectors are found in Cuiabá where no notification of human or canine cases have been described<sup>15</sup>.

Dogs are considered an important parasite reservoir of *T. cruzi* in the domestic cycle, and because their proximity to human beings, they represent a risk factor for the human population<sup>6,11</sup>. Clinically infected dogs can develop both the acute and chronic phases of the disease, similar to the human forms; dogs are indicated as the experimental model for chagasic infection in humans<sup>3,7</sup>.

Dogs frequently present nonspecific clinical signs in the acute phase of this disease; however, the chronic phase is characterized by alterations indicative of dilated cardiomyopathy and right cardiac insufficiency, observed in electrocardiographic (EKG) and echocardiographic (ECG) examinations<sup>14</sup>. According to BAHIA *et al.*<sup>2</sup> and GONZÁLEZ-VIEYRA

*et al.*<sup>10</sup>, this clinical presentation is highly variable and depends on the type of strain involved in the infection, route of infection, and parasite burden. The diagnosis is based on serology, direct or indirect parasitological tests, and molecular tests<sup>1</sup>.

The present report describes the clinical and pathological analyses of a dog naturally infected with *T. cruzi* in Cuiabá, Mato Grosso, Brazil.

### CASE REPORT

A Brazilian Mastiff female dog, five years old, was brought to the Veterinary Hospital with a history of hind limbs paresis progressing to quadriparesis, apathy, and suspected pregnancy. The animal lived on a farm and was born in the area. The animal had probably gone hunting close to the home, yet had no history of travel to other municipalities. The physical examination indicated normal physiological parameters, abdominal distension, limbs edema, and generalized flaccid paralysis.

The ultrasonography showed three viable fetuses at 47 days of age; the chest radiography showed a bilateral ventricular cardiac enlargement. The hematological results included hypochromic normocytic anemia (RBC 27), leukocytosis (25,500 cells/mm<sup>3</sup>), neutrophilia, lymphopenia, and the presence of trypomastigote forms of *Trypanosoma* sp. The biochemical tests showed renal profile, alanine aminotransferase and globulin within the normal range, increased serum activities of aspartate aminotransferase

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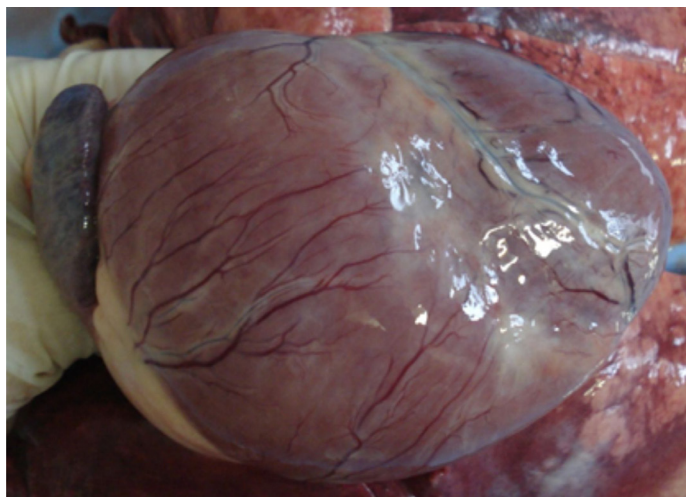
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(80 µ/mL), and decreased albumin (1.0 g/L). Bilirubinuria, occult blood, leukocytes, bilirubin crystals, rare squamous epithelial cells, acidic pH, and normal density were observed in the urinalysis.

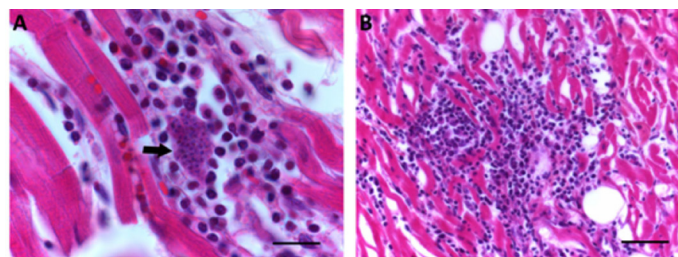
The analysis of blood samples in light microscopy showed high amounts of flagellar structures consistent with *Trypanosoma* (1000x magnification); blood aliquots were separated and stored at -20 °C for DNA extraction and subsequent polymerase chain reaction (PCR). The cytology of the bone marrow (sternum) and popliteal lymph node revealed the presence of amastigotes forms.

The treatment was established on the second day with diminazene diaceturate at 3.5 mg/kg/day, fluid, and multivitamin support. On the third day a deterioration in clinical signals was detected including hypothermia, anasarca, anorexia, abdominal contractions, vaginal discharge, and vocalization. A second round of hematological analysis and abdominal ultrasonography were performed and revealed increased leukocytosis with neutrophilia, thrombocytopenia, reactive lymphocytes, monocytes, fewer trypomastigotes, and nonviable fetuses. The animal died on the fourth day after presenting dementia, abortion of the fetuses, and cardiac arrest.

Mild hydrothorax and hydropericardium were the main postmortem findings characterized at necropsy. The heart was enlarged and remarkably globoid, with multiple white bands and foci on the epicardium and myocardium, especially in the right ventricle (Fig. 1). The right heart chamber was also dilated. Hepatic and pulmonary congestions and enlarged lymph nodes were also observed. The lesions were microscopically characterized by severe multizonal lymphohistiocytic cellular infiltrates in the myocardium interstice and amastigotes nests surrounded by mononuclear cells (Fig. 2). Additionally, multifocal nonsuppurative meningoencephalitis, characterized by lymphocytic cellular infiltrates and moderate mononuclear perivascularitis with pseudocysts in the cerebral cortex, cerebellum, and brain stem, were identified. Other findings included sinusoids hepatic congestion of the central vein, mild mononuclear hepatitis, vacuolization of hepatocytes, severe spleen congestion, and diffuse lymph nodes hyperplasia. No macroscopic or microscopic lesions were found in the digestive tract.



**Fig. 1** - Canine. Heart. Remarkably globoid aspect and white foci of epicardium surface in the right ventricle.



**Fig. 2** - (A) Histological analysis revealing presence of amastigotes surrounded by mononuclear cells in myocardium interstice (arrow), stained with hematoxylin-eosin, 100x magnifications; and (B) severe multizonal lymphohistiocytic cellular infiltrates in the myocardial interstitium, 40x magnification.

Amastigotes forms of *T. cruzi* were also observed in sections of the liver, lymph nodes, and kidneys.

The *T. cruzi* infection was confirmed by nested PCR on the 18S rDNA region<sup>19</sup>, and sequencing of the amplified products revealed 100% homology with *Trypanosoma cruzi* strain MT3869 (GenBank accession number AF303660.1). The sequence obtained in this study was deposited in the GenBank (accession number JQ912643).

## DISCUSSION

Dogs might serve as valuable sentinels for the presence of Chagas disease because once infected, they are susceptible and can develop pathological alterations that are similar to those detected in humans that coexist in the same environment<sup>5</sup>. Moreover, they are considered the experimental model of choice for human Chagas disease<sup>4,20</sup>. Clinical signs such as limb edema, flaccid paralysis, and acute death were associated with cardiac and central nervous failure. Myocarditis and meningoencephalitis, in humans and animals, often culminate in death due to myocarditis<sup>16,18</sup>.

Serum biochemistry and blood changes are not specific in Chagas disease; however, in the acute phase, an increased activity of liver enzymes and azotemia occur<sup>8</sup>. The occurrence of leukocytosis has not been reported in *T. cruzi* infections, however, the decrease in total blood cell count due to bone marrow hypoplasia is observed<sup>13</sup>. Regardless of the presence of amastigotes in the bone marrow sample, in this case, the abortion might have caused neutrophilia, possibly due to parasite infection. Congenital infection has been described in infected dogs<sup>17</sup>.

No increase of total serum protein was detected as similar to the results described by BARR *et al.*<sup>4</sup>. The identification of hypoalbuminemia in this case can be due to liver disorders, because of the presence of bilirubinuria and hepatitis on the histopathology results.

The diagnosis of Chagas disease is often missed because of the characteristics of a multisystem infection; however, the number of trypomastigote forms in the acute phase supports the diagnosis<sup>4</sup>. The treatment with diminazene diaceturate shows better results in *T. evansi* infections<sup>12</sup>; nevertheless a significant reduction in the number of trypomastigotes in the bloodstream was observed in this case. This treatment was not sufficient to cure this animal and resulted in its death.

According to BAHIA *et al.*<sup>2</sup> (2002), the clinical presentation might

be related to the type of strain involved in the infection. *T. cruzi* circulates in nature as a heterogeneous population complex presenting intraspecific diversity. The PCR analysis of the microorganism of this study showed 100% homology with the MT3869 strain that belongs to the *T. cruzi* ZIII strain, described primarily as wild strain. The habit of hunting reinforces the hypothesis that the infection has been acquired during this activity.

However, reports of human infection with different clinical presentations of the disease associated with this strain have been reported<sup>9</sup>. Despite the fact that there are no records of acute Chagas disease in humans in the city of Cuiabá, this is the first report of the disease in dogs in this region representing an alert to health professionals and authorities on the possibility of transmission of this zoonosis.

## RESUMO

### Infecção natural por *Trypanosoma cruzi* em um cão na região central do Brasil: relato de caso

*Trypanosoma cruzi*, infecta cerca de 10 milhões de pessoas, principalmente na América Latina e mais de 25 milhões apresentam-se em risco de adquirir a doença nas áreas endêmicas. Os cães são considerados importantes reservatórios representando fator de risco para a população humana. Este relato descreve caso de doença de Chagas em cão na cidade de Cuiabá. O diagnóstico foi feito a partir do exame direto, apresentando inúmeras formas tripomastigotas em esfregaço sanguíneo. Amastigotas foram visualizadas na microscopia de medula óssea, linfonodo, rins, fígado e cérebro. A infecção por *T. cruzi* (ZIII) foi confirmado através do sequenciamento de produtos amplificados pela PCR. O animal apresentou sinais multissistêmicos, evoluindo para óbito. Apesar da doença de Chagas aguda em humanos não ser descrita em Cuiabá, este é o primeiro relato de um caso canino nessa região, fato que, constitui alerta aos profissionais da saúde e autoridades sanitárias para a possibilidade da transmissão desta zoonose em Cuiabá.

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