Australian Journal of Basic and Applied Sciences, 9(27) August 2015, Pages: 425-428

ISSN:1991-8178

Australian Journal of Basic and Applied Sciences

Journal home page: www.ajbasweb.com

A Case of *Trypanosoma Cruzi* Infection in an Old Dog from Federal District, Brazil

[1]Mariana A. Lima, [1]Wanessa A.C. da Silva, [1]Filipe T. Carneiro, [1]Maia A. Abrahim, [1]Simone P. Mesquita, [1]Marcela C. Scalon, [2]Marina S. Nascente, [3]Mariana M. Hecht, [2]Gláucia B. Pereira-Neto, [3]Nadjar Nitz, [1]Giane R. Paludo

[1]*University of Brasilia, College of Agronomy and Veterinary Medicine, Veterinary Clinical Pathology Laboratory, Brasilia, Brazil*
[2]*University of Brasilia, College of Agronomy and Veterinary Medicine, Veterinary Clinics, Brasilia, Brazil*
[3]*University of Brasilia, College of Medicine, Interdisciplinary Laboratory of Biosciences, Brasilia, Brazil.*

ARTICLE INFO

Article history:
Received 23 June 2015
Accepted 25 August 2015
Available online 2 September 2015

Keywords:
Chagas disease, canine, Trypanosomiasis, diagnosis

ABSTRACT

Background: Dogs are considered important reservoirs for Chagas disease in urban areas and improving the diagnosis of Trypanosoma cruzi infection in dogs is very important to evaluate the risk of transmission of infection to the human population. **Objective:** The main objective of this paper is to describe a naturally occurrence of Chagas disease in dogs at Federal District. **Results:** An old dog was presented to Small Animal Veterinary Hospital from University of Brasilia with anorexia, weakness, depression, dehydration and soft dark faeces. On auscultation, a mitral systolic grade 3 heart murmur was observed. ECG rhythm showed sinus arrhythmia with first degree atrioventricular block. Alterations in laboratorial tests included: hematocrit 25%, leukocytes 24.6 x 103/µl, platelets 83 x 103/µl, icteric plasma and increase on ALP activity. Trypomastigotes of Trypanosoma cruzi were observed at blood smear and confirmed by PCR. On day three the patient died at home. **Conclusion:** This is the first report of a naturally Chagas disease in a dog at Federal District.

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To Cite This Article: Mariana A. Lima, Wanessa A.C. da Silva, Filipe T. Carneiro, Maia A. Abrahim, Simone P. Mesquita, Marcela C. Scalon, Marina S. Nascente, Mariana M. Hecht, Gláucia B. Pereira-Neto, Nadjar Nitz, Giane R. Paludo, A Case of Trypanosoma Cruzi Infection in an Old Dog from Federal District, Brazil. ***Aust. J. Basic & Appl. Sci.,*** *9(27): 425-428, 2015*

INTRODUCTION

The protozoan parasite *Trypanosoma cruzi* is the etiologic agent of American Trypanosomiasis, also known as Chagas disease. More than 150 species of mammals, mostly wild species, maintain *T. cruzi* infection in nature. The distribution of vectors and wild reservoirs of *T. cruzi* is restricted to South America, Central America, Mexico and southern United States, being that the Caribbean islands are free of the disease (Rassi & Resende, 2012).

The most common way of infection is through contact with the faeces of an infected triatomine bug from the genera *Triatoma*, *Rhodnius* or *Panstrongylus*, an insect that feeds on humans and animals blood. Other ways of infection include vertical transmission, transfusions, organ transplant from an infected donor, laboratory accident and oral ingestion, with ingestion of blood or meat of infected animals or ingestion of food or drinks contaminated with feces or urine from infected triatomine (Eloy & Lucheis, 2009; Rassi & Resende, 2012).

Acute Chagas disease occurs immediately after infection and may persist up to a few weeks or months and is more observed in young dogs than in adult ones. During this phase parasites may be found in the circulating blood and infection may vary from asymptomatic to mild, when there may be anorexia, generalized lymphadenopathy, diarrhea, myocarditis and sudden death, caused by serious cardiac arrhythmia (Eloy & Lucheis, 2009).

After the acute phase, infected patients may display an indeterminate asymptomatic form of disease during which few or no parasites are found in the blood and no clinical signs or residual changes from the acute phase of the disease are shown (Andrade *et al*., 1997).

Following this stage comes the chronic phase of the disease. Infected patients may present signs of cardiac insufficiency such as ventricular arrhythmias and myocardial dilation. These signs are initially seen on the right side, but progression to biventricular insufficiency is common, mostly due to chronic, severe myocarditis (Andrade *et al*., 1997; Eloy & Lucheis, 2009).

The diagnosis of American Trypanosomiasis can be made by observation of the parasite in a blood smear by microscopic examination. However, a blood smear works well only in the acute phase of infection when parasites are seen circulating in blood. Diagnosis is also possible through isolation of the parasite, serology and molecular techniques (Eloy

Corresponding Author: Giane Regina Paludo, University of Brasilia, College of Agronomy and Veterinary Medicine, ICC Sul, Campus Universitário Darcy Ribeiro, Post office box 4508, Brasília, DF 70910-970, Brazil. Tel: +55 61 31072850, E-mail: giane@unb.br

& Lucheis, 2009).

Dogs are commonly used as a model of Chagas disease in humans, for similarly developing the disease. However, there are few studies in naturally infected animals, even though they are considered an important reservoir of the parasite (Eloy & Lucheis, 2009). The first study regarding *Trypanosoma cruzi* using PCR technique from Southern Bahia, the state with the highest level of human infection in Brazil, found two positive dogs with no clinical signs from 272 studied (0,7%). These results show that dogs may have an important role in maintaining the parasites in the environment (Santana *et al*., 2012).

Despite Federal District is not considered an endemic area for Chagas disease, research shows the presence of the synanthropic triatomine, both in intradomicile and peridomicile environments, including kennels. It illustrates the potential risk for transmission of *Trypanosoma cruzi* infection in this region (Maeda *et al*., 2012).

Case report:

November 2013, an 11-year-old intact female Cocker Spaniel with traveling history to the north of Brazil was presented to the Small Animal Veterinary Hospital from University of Brasilia with anorexia, weakness, depression, and soft dark faeces. Her vaccination and worming were up to date. Upon examination, the patient was quiet but responsive, as well as mildly dehydrated. On auscultation, a mitral systolic grade 3 heart murmur was observed. ECG showed sinus arrhythmia with first degree atrioventricular block, left atrium overload, suppression of R wave amplitude, left axis deviation and T wave was 25% > R suggesting myocardial hypoxia. Stool examination detected *Ancylostoma* eggs. Abnormal findings in hemogram included: hematocrit 25% (37-55%), leukocytes 24.6 x 10^3/µl (6-17 x 10^3/µl), platelets 83 x 10^3/µl (200-500 10^3/µl) and an icteric plasma (Marcondes *et al*., 2000). An increase in alkaline phosphatase activity [597 UI/L (20-156 UI/L)] (Meyer *et al*., 1992) was the only abnormal finding in biochemistry. Trypomastigotes of *Trypanosoma cruzi* were visualized at blood smear (Fig. 1).

PCR was performed with whole blood DNA extraction product and the result was positive for *Trypanosoma cruzi* infection, using the following nucleotides: TCZ1 (5'-GAG CTC TTG CCC CAC ACG GGT GCT -3') and TCZ2 (5'CCT CCA AGC AGC GGA TAG TTC ACG-3') previously described (Moser *et al*., 1989). PCR products were hybridized with specific radiolabelled probe as proposed in another study (Hech *et al*., 2010).

On day three the patient did not return to the hospital and the owner informed its death. Since the patient did not come back, no post mortem examination or tests were performed.

Discussion:

In this case, the dog was presented to the Hospital with inespecific clinical signs and there was visualization of trypomastigotes of *Trypanosoma cruzi* in the blood smear, confirmed by PCR. This could either mean that the animal presented a recent infection by the agent or a previous infection with reacutization, since the animal has travelled to endemic areas of the disease. Recent infection could be confirmed by IgM titer, but unfortunately, it was not possible to accomplish due to the sudden death of the animal (Rassi & Resende, 2012).

Hematological alterations consisted in microcytic normochromic anemia, leukocytosis with neutrophilia and lymphopenia, along with thrombocytopenia (Meyer *et al*., 1992). The most prominent feature known of canine trypanosomosis is anemia. Acute *Trypanosoma cruzi* infection is associated with bone marrow hypoplasia, which explains the observed anemia and thrombocytopenia (Marcondes *et al*., 2000). Leukocytosis with neutrophilia can be associated with either the acute or reacutized inflammatory process due to infection of *T. cruzi* (Santana *et al*., 2012). Lymphopenia is usually related to corticosteroid response due to stress (Meyer *et al*., 1992). The only biochemical profile abnormality found was an increase in alkaline phosphatase (ALP) serum activity (Moser *et al*., 1989), which also could be related to hypercortisolism secondary to stress. It has been noted that there are some discrepancies in biochemistry alterations in canine trypanosomosis. Increased serum activity of alanine transaminase (ALT) and aspartate transaminase (AST) have been reported in infected dogs (Barr *et al*., 1991). The activity of AST was not determined, but ALT activity was normal.

In the case of a naturally infected pregnant dog from Cuiabá, in central western Brazil in 2013, a study (Almeida *et al*., 2013) found hypocromic normocytic anemia, leukocytosis, neutrophilia and lymphopenia. They associated the neutrophilia to an abortion due to parasite infection and the anemia to bone marrow hypoplasia. Another study (Barr *et al*., 1991) has realized one experimental study in 1991 in dogs infected with *T. cruzi*. Dogs developed clinical signs of severe acute myocarditis, lymphadenopathy and lymphocytosis. Biochemical alterations found were increased serum ALT and AST activities and urea nitrogen concentration, and low concentration of glucose before the death of animals from one of the groups studied, which was different from what was observed in this case.

In the acute form of the disease no echocardiographic changes are observed. In this case an echocardiography was scheduled, but the animal died before performing it. On the other hand, electrocardiographic alterations are often noted and can include first, second and third degree atrioventricular block, right bundle branch block,

sinus tachycardia, and depressed R wave amplitude. Moreover, the presence of conduction disturbances predicts sudden death, which may also occur (Ware, 2014). These conduction disturbances were observed in this patient and could lead to sudden death, although we consider this is just a hypothesis, since we are not sure about myocardium performance.

Even though there are no records of vectorial transmission of Chagas disease in the Federal District, infected triatomines, humans and animals still can be found. Therefore, dogs are considered important reservoirs for Chagas disease in urban areas and the improvement of the diagnosis of *Trypanosoma cruzi* infection in this species is very important to evaluate the risk of transmission of the disease to both animal and human populations. This is the first description of naturally Chagas disease in dogs in Brasilia, DF.

ACKNOWLEDGMENTS

The authors wish to thank MEC (Ministério da Educação) and Capes (Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for their support with regard to scholarship funding.

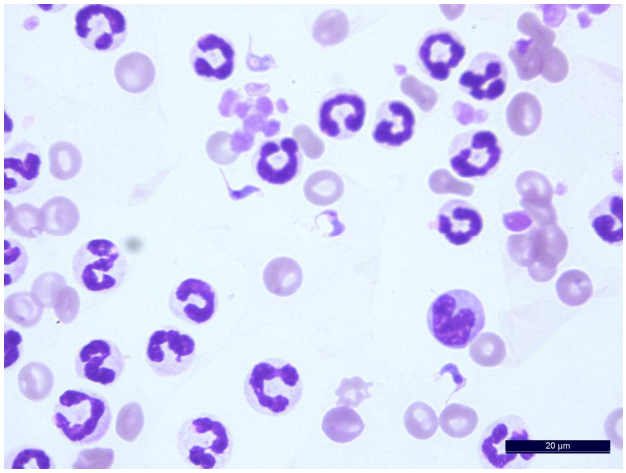

Fig. 1: Trypomastigote forms of *Trypanosoma cruzi* at the blood smear of a dog. Diff-Quik. 1000x.

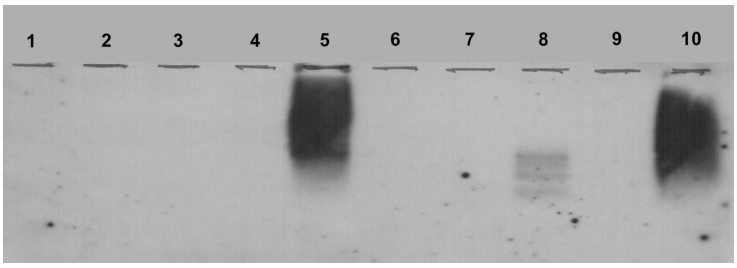

Fig. 2: *Southern blot* results for *Trypanosoma cruzi* using TCZ1 and TCZ2. Lanes: 1: negative control (water); 3: negative animal; 5 positive animal; 6: negative animal; 8: positive control; 10: purified *T. cruzi* DNA

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