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Case Report

First report of *Trypanosoma evansi* in a canine in Argentina

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ABSTRACT

An Argentinian Dogo which suffered from anorexia, lymphadenopathy, cachexia and paresis of the hind limbs was diagnosed with trypanosomiasis in Argentina in 2013. In this study, we describe the clinical profile and its evolution as well as the molecular method employed to identify and quantify *Trypanosoma evansi*.

1. Introduction

Trypanosoma evansi is a salivarian hemoparasite species within the class *Mastigophora* with a widespread distribution around the world. It affects a great number of domestic and wild mammals as well as lab animals. Equines, camelids, dogs, deer and Asian elephants are the most frequently affected animals, although it can also be found in swine, small ruminants, buffaloes and bovines (Brandão et al., 2002; Dávila & Silva, 2000; Desquesnes, 2004; Franke et al., 1994; Herrera et al., 2005). In Argentina, *T. evansi* was described in equines (Monzón et al., 1995) and in capybaras (Eberhardt et al., 2014). It is transmitted mechanically by hematophagous insects from the genera *Stomoxys* and *Tabanus*, and also from *Glossina* spp. in Africa. In Central and South America it can also be transmitted by *Desmodus rotundus*, which acts as both vector and reservoir. In addition to mechanical transmission, *T. evansi* can be transmitted through milk, during sexual intercourse, or orally by licking or by ingestion of meat and blood infected with the parasite (Brun et al., 1998; Hoare, 1965; OIE Terrestrial Manual, 2010; Raina et al., 1985).

There have been reports of infection with *T. evansi* in canines in Iran (Hosseininejad et al., 2007), India (Ravindran et al., 2008), Colombia (Correa-Salgado et al., 2010) and Afghanistan (Aref et al., 2013). The natural infection by *T. evansi* in canines shows severe, and potentially fatal signs. In the acute phase, the clinical signs in dogs infected naturally include intermittent fever, progressive anemia, anorexia, cachexia, edema of head and throat, lymphadenopathy, paresis of the hind limbs, ataxia, uncoordinated movement, lethargy, ocular signs with corneal opacity, vascular signs and nervous system alterations with opisthotonos and tonic-clonic seizures (Ian et al., 2004). In the chronic phase, there is more compromising of the general condition as the associated symptoms intensify.

The present study describes a case of natural infection by *T. evansi* in a canine from the rural area of Concordia, Entre Ríos. The canine was taken to the laboratory of parasitology at the School of Veterinary Sciences in Esperanza, Santa Fe. It showed anorexia, linfadenopathy, cachexia and paresis of the hind limbs, among other signs. Blood smears were performed and showed flagellate forms, which were confirmed as *T. evansi* by the PCRs.

2. Materials and methods

2.1. Animal and clinical investigation

A fourteen years old male Argentinian Dogo, who lived in the rural area of Los Charruas (31°10′00″S 58°11′00″W), province of Entre Rios, presented to the consultation with a history of dullness, depression (Fig. 1A), progressive emaciation, poor appetite, enlargement of the popliteal and prescapular lymph nodes, pale mucous membranes (Fig. 1B and C) and pyrexia (41 °C).

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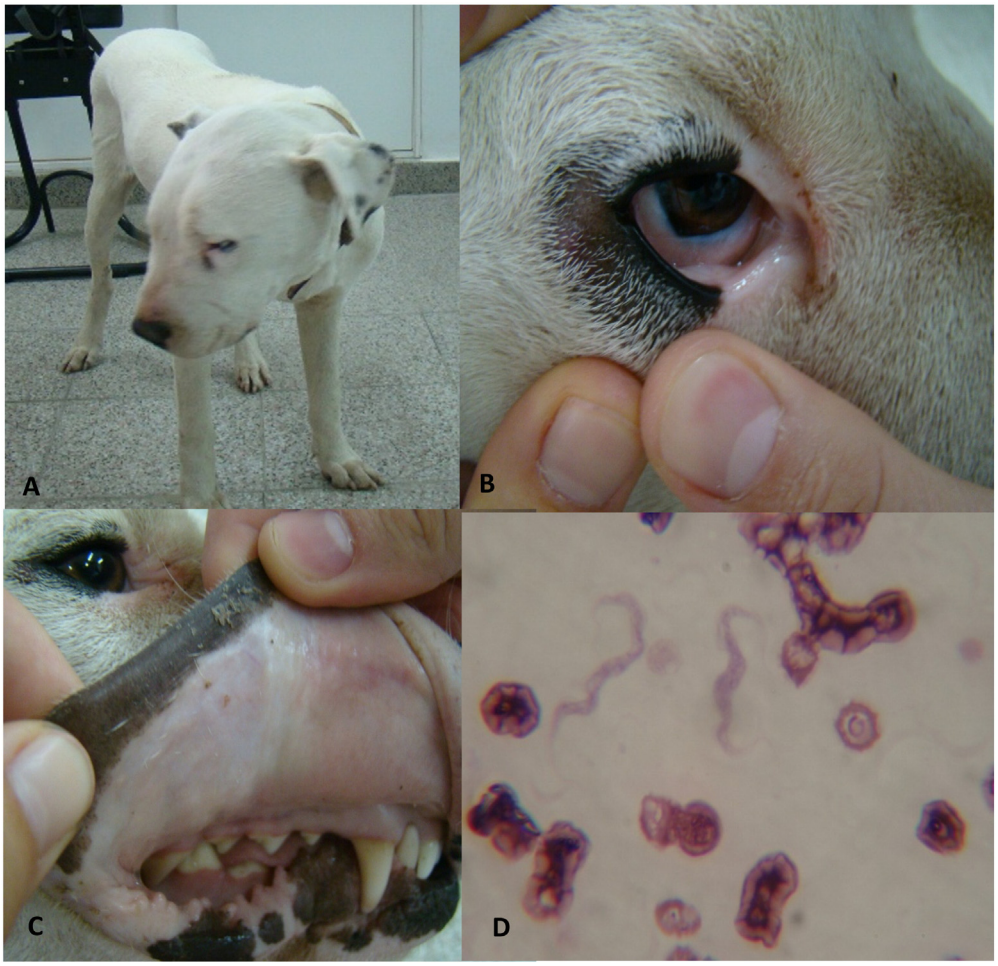

Fig. 1. Dull and depressed dog (A). Note the ocular conjunctiva and oral mucous pale (B and C). Giemsa stained blood smear showing *Trypanosoma evansi* (1000×) (D).

2.2. Sampling

Blood samples were taken and then used to make smears, which were fixed, stained with Giemsa and observed under an optical microscope at 1000×. Complete blood count and biochemical analysis were performed.

2.3. DNA extraction

Total genomic DNA was obtained using 200 μl of blood clot as previously described (Eberhardt et al., 2014). Briefly, the blood clot was mixed with 400 μl of lysis buffer (10 mM Tris-Cl, 100 mM EDTA, 0.5% SDS, pH 8.0) and proteinase K was added (200 μg/ml). Samples were incubated in a water bath at 50 °C overnight, proteinase K was inactivated by boiling for 5 min and DNA was extracted according to standard phenol/chloroform methods (Sambrook & Russell, 2001). A negative extraction control containing molecular grade water instead of blood was included. The DNA pellet was washed, air dried and resuspended in 50 μl of sterile TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0). Genomic DNA concentration and purity were assessed using the SPECTROstar Nano and the MARS Data Analysis Software (BMG Labtech, Germany).

2.4. Real-time PCR and sequence analysis

Two primer pairs targeting different molecular markers were used for the detection of *T. evansi* DNA in the blood sample, ITS1-TeRT (Eberhardt et al., 2014) and TeRoTat (Konnai et al., 2009). Primers ITS1-TeRT are derived from the *T. evansi* ITS1 sequence and TeRoTat primers are derived from the *T. evansi* Rode Trypanozoon antigen type (RoTat) 1.2 Variable Surface Glycoprotein (VSG) gene. This is a specific DNA region lacking homology to other known VSG genes in trypanosomes, but it is highly conserved among *T. evansi* strains (Claes et al., 2004). All real-time PCRs were performed in an Applied Biosystems StepOne™ thermocycler as previously described (Eberhardt et al., 2014).

Each PCR run included a positive control (100 ng of DNA from *T. evansi*-infected capybara blood) and a negative control with blood-free DNA extraction. In order to confirm the identity of the infecting pathogen, complete ITS1 was amplified and sequenced as previously described (Eberhardt et al., 2014).

3. Results

When the canine entered the laboratory, it was in a poor general condition, lethargic and with no appetite. At the clinical exam, it had a temperature of 41 °C, pale mucosa, enlarged prescapular and popliteal lymph nodes and paresis of the hind limbs. The biochemical analysis and blood count showed a hematocrit value of 16%, hypochromic

Table 1
Blood count showed a hematocrit value, hypochromic anemia, thrombocytopenia and an inflammatory leukogram.

HB g%	GR mm^3	GB mm^3	Nb %	Ns %	E %	B %	L %	M %	Platelets mm^3
5.5	2,855,000	9100	6	68	0	0	12	14	165,000

Table 2
Blood biochemistry showed slight increase in uremia, a rise in hepatic enzymes, hypoalbuminemia, hypergammaglobulinemia and an increase in CPK.

Urea mg/dl	Creatinine mg/dl	ALT[a] Ul/l	AST[b] Ul/l	FAS[c] Ul/l	GGT[d] Ul/l	PT[e] g%	Alb[f] g/dl	CPK[g] Ul/l	Brr.D[h] mg/dl	Brr.T[i] mg/dl
89.64	1.63	427	211	287	18	9.2	2.65	1579	0.17	0.57

[a] ALT: alanine aminotransferase.
[b] AST: aspartate aminotransferase.
[c] FAS: alkaline phosphatase.
[d] GGT: gamma-glutamyl transpeptidase.
[e] PT: total proteins.
[f] Alb: albumin.
[g] CPK: phosphokinase creatinine.
[h] Brr.D: direct bilirubin.
[i] Brr.T: total bilirubin.

anemia, thrombocytopenia and an inflammatory leukogram (Table 1). A slight increase in uremia, a rise in hepatic enzymes, hypoalbuminemia, hypergammaglobulinemia and an increase in CPK were also present in the biochemistry (Table 2). The blood smears showed 25 and 30 μm long exoglobular clusters with an undulating membrane and a free flagellum, congruent with *Trypanosoma* spp. (Fig. 1D). After 3 days, jaundice, hepatomegaly, splenomegaly and dilated cardiomyopathy were observed. The histopathology showed hepatitis with severe bile stasis, splenitis with hemosiderosis, myeloid metaplasia and also atrophy of cardiac myofibrils surrounded by inflammatory cells. Membranous glomerulonephritis, diffuse tubular degenerations with hyaline casts and multifocal tubular necrosis were observed in the liver.

Both *T. evansi* specific real-time PCRs were positive and quantitative analysis using standard curves previously generated (Eberhardt et al., 2014) showed a parasitaemia level of about 20 parasites per μl of blood. Amplification of the complete ITS1 region yielded a single PCR product of approximately 470 bp and ITS1 sequence itself was found to be 340 nucleotides in length and 100% similar to sequences of *T. evansi* previously reported infecting capybaras in the Iberá wetlands (KC988260-KC988263, (Eberhardt et al., 2014)), 300 km north to the location were the present case was originated. No DNA contamination was detected in the blood-free extractions. Sequence generated herein was deposited on GenBank (Accession number: KX926429).

4. Discussion

T. evansi is the causative agent of "surra" in wild and domestic animals and unlike *T. brucei*, it does not need a cyclic transmission and can be spread mechanically by fly bites.

The signs described in the present study suggest that the animal was in the acute phase of the disease, which coincides with descriptions in previous research. Even though the transmission is produced by hematophagous insect bites, oral transmission by ingestion of raw meat from infected carnivores or rodents is not discarded.

The canine under study was living in a rural area with its mother and two other canines which had died a year before. Before death, these canines suffered from severe anemia but they lacked a certain diagnosis.

To date, *T. evansi* has only been described in equines and capybaras in Argentina. Therefore, the utter relevance of this report is undeniable. We believe that further studies as regards the prevalence of this hemoparasite in Argentina should be performed in the near and medium term given that although it has not been considered to be a human pathogen, there are confirmed cases in Africa and in India.

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