



DIAGNOSTIC EXERCISE

From The Davis-Thompson Foundation*

Case #: **233**; Month: **March**; Year: **2024**
Answer Sheet

Title: American trypanosomiasis (Chagas disease) in a dog

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Clinical History: A 7-month-old female Boxer dog was referred to a veterinary hospital 4 days after showing increased abdominal volume and diarrhea, despite normal appetite. The dog lived in a semi-rural area on the outskirts of the town. On physical examination, there was dehydration of 8%, pale mucous membranes, a rectal temperature of 32° C, heart rate of 90 beats per minute, weak femoral pulse, capillary reperfusion time greater than 2 seconds, dyspnea, subcutaneous edema in the limbs, increased abdominal volume, and feces adhered to the hair in the perianal region. Results for blood counts and serum biochemistry are summarized in Table 1. There was hypochromic normocytic anemia, subnormal plasma proteins (5.6g/dL), and an unremarkable leukogram except for some hypersegmented neutrophils. Serum biochemistry showed azotemia, hypoalbuminemia, and a slight alanine aminotransferase (ALT) increase. Radiological examinations of the chest and abdomen confirmed pleural and abdominal effusion and cardiac dilation.

Table 1 Serum biochemistry values of the dog from this case

Parameter	Value	Reference range
Albumin	1.21 g/dL	2.6-3.3 g/dL
Alanine aminotransferase	138 IU/L	21-86 IU/L
Alkaline phosphatase	116 IU/L	20-156 IU/L
Creatinine	2.1 mg/dL	0.5-1.5 mg/dL
Blood urea nitrogen	230 mg/dL	21.4-59.92 mg/dL

An abdominocentesis was performed, and approximately 500ml of serosanguinous fluid with a density of 1022 and characteristics of modified transudate was removed

(Fig.1). Treatment with furosemide (4 mg/kg IV) was instituted, but the dog died 36 hours after admission.

Gross Findings: At gross examination, the heart was enlarged and globose (Figs 2A and 2B), the ventricular walls were thinned (Fig.2C), and multifocal irregular white or yellow pale areas were seen in the myocardium. There was an accumulation of approximately 200 ml of slightly cloudy fluid free in the pericardial cavity and 500 ml with the same characteristic in the thoracic cavity. The abdominal cavity was markedly distended by 2.3 L of serosanguinous, turbid fluid. There were free fibrin filaments adhered to the capsular surface of the liver (Fig. 3A), which was markedly congested oozing a large amount of blood at the cut surface. The liver parenchyma had clear areas interspersed with red areas (nutmeg liver), which could be better appreciated at the cut surface (Fig. 3B).

Gross Images:



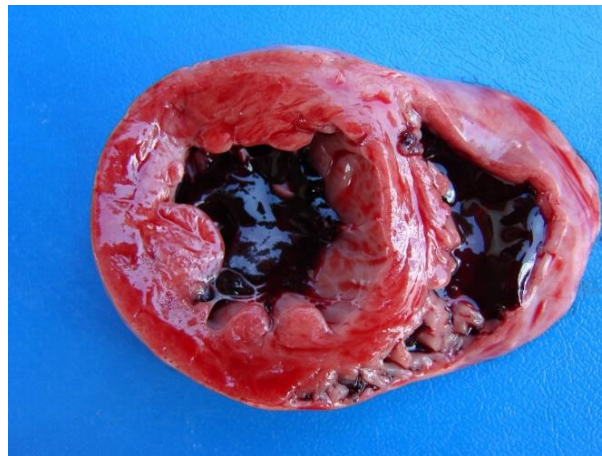
Figure 1. Dog, Chagas disease. Serosanguinous fluid (modified transudate) drained from the abdominal cavity.



A

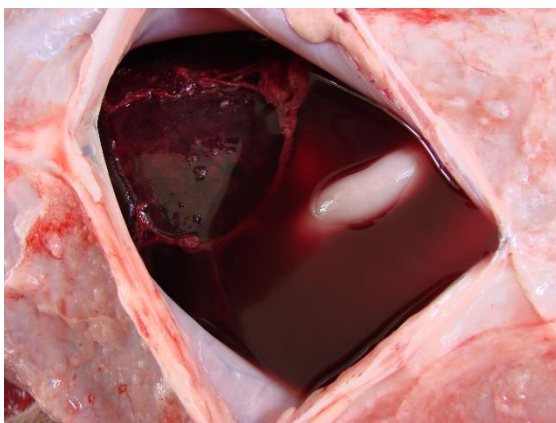


B



C.

Figure 2. Dog, Chagas disease. The heart is enlarged (**A**), globose (**B**), and the walls of the ventricles are thinned with large coagula in both ventricular cavities (**C**).



A.



B.

Figure 3. Dog, Chagas disease. The liver is voluminous, and its capsule covered by a film of fibrin (**A**). There is a nutmeg aspect to the cut surface of the liver (**B**).

Histologic and Ultrastructural Description: Microscopically, the heart showed multifocal to coalescent inflammatory infiltrate of histiocytes, lymphocytes, plasma cells, and neutrophils in the myocardium with degeneration and necrosis of cardiac myocytes. Within the cytoplasm of the cardiac myocytes in the areas of the cardiac lesions, there were pseudocysts containing amastigotes with approximately 3 μm in diameter and with morphology compatible with *Trypanosoma cruzi* (Fig. 4). The liver had centrilobular sinusoidal congestion and dilatation and centrilobular hepatocellular degeneration, necrosis, and hemorrhage. Myocardial preparations examined by transmission electron microscopy (Fig. 5) revealed the presence of flagellated amastigote protozoan organisms; this morphology was compatible with *T. cruzi*.

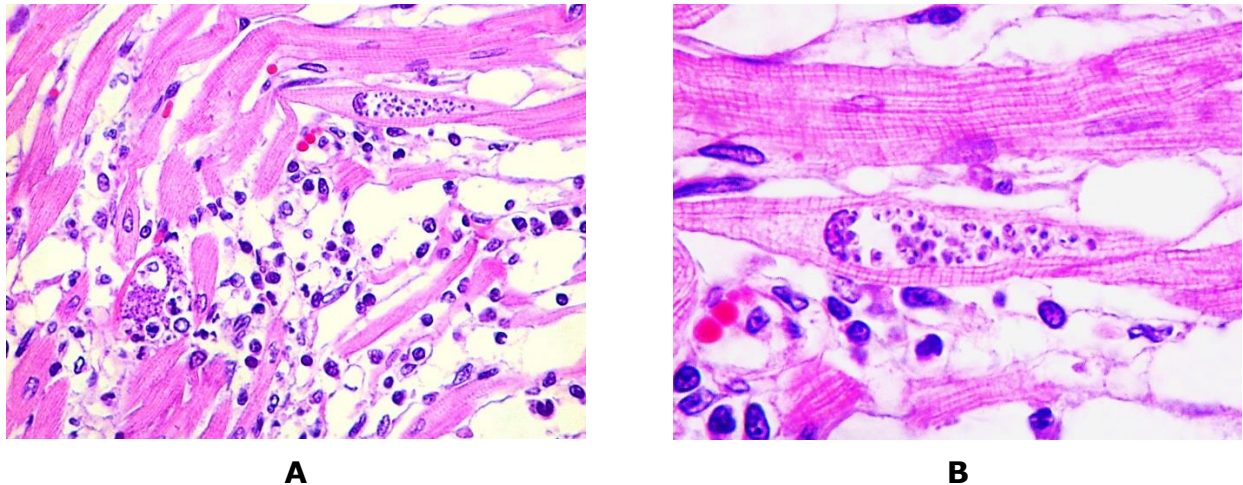


Figure 4. Dog, Chagas disease. Histiocytic and lymphoplasmacytic myocarditis caused by *Trypanosoma cruzi*. Note the pseudocyst containing multiple amastigotes in two cardiac myocytes (A). Higher magnification of the pseudocyst (B).

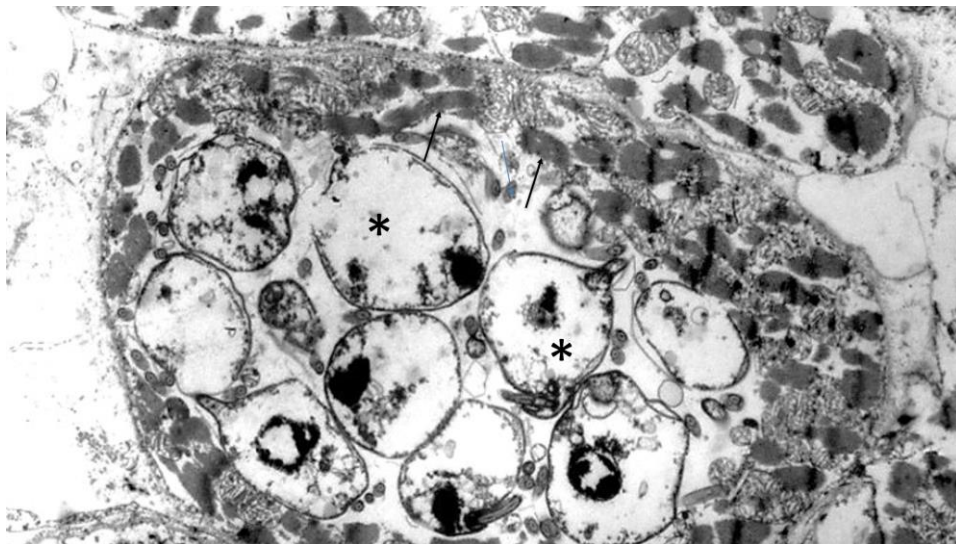


Figure 5. Dog, Chagas disease. Ultrastructural characteristics of cardiac myocytes parasitized by *T. cruzi*. Fragments of muscular bands (arrows) of a cardiac myofiber are distended by intracytoplasmic amastigotes (*). No membrane separates the cytoplasmic constituents of the cardiac myocyte and the amastigotes.

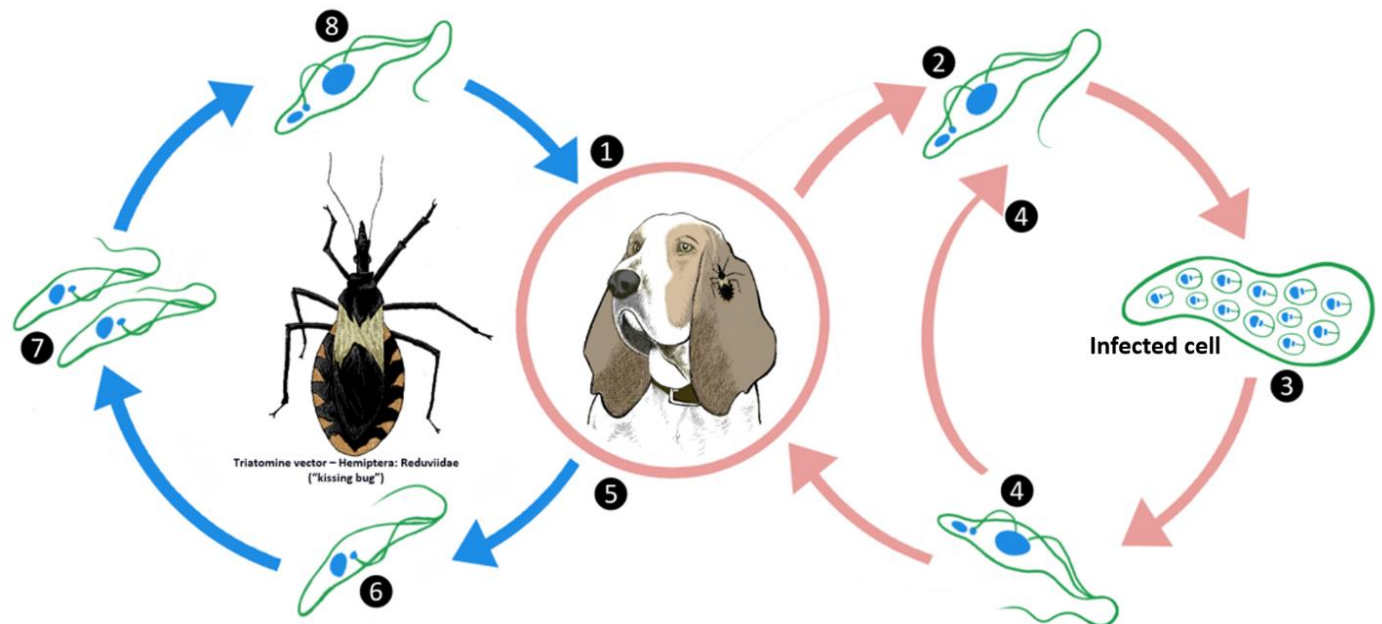


Figure 6. Infection cycles of American trypanosomiasis in dogs. (1) Infected triatomine insect bites the host, feeds on blood, and deposits its feces containing metacyclic trypomastigotes in the vicinity of the bite. (2) After the entry of several cells at the site of the bite, the metacyclic trypanosomes transform into amastigotes. (3) Amastigotes multiply by binary division within the host. Trypomastigotes can infect other cells and transform into intracellular amastigotes, spreading the infection. (4) Intracellular amastigotes transform into trypomastigotes, break the cell membrane and enter the bloodstream. (5) Metacyclic trypomastigotes can be ingested in blood meals made by uninfected triatomine insects. Trypomastigotes transform into epimastigotes, (6) which are initially located in the midgut of these insects, (7) multiply there by binary fission, and (8) are moved to the hindgut where they transform into trypomastigotes, closing the cycle. (Drawing by Mario Assis Neto)

Morphologic Diagnoses:

1. Myocarditis, histiocytic, lymphoplasmacytic, and neutrophilic, multifocal to coalescent marked, with intracytoplasmic amastigotes
2. Dilated cardiomyopathy
3. Hydropericardium, hydrothorax, and ascites
3. Hepatic centrilobular congestion, necrosis, and hemorrhage

Cause: *T. cruzi*

Name of the Condition: American trypanosomiasis (Chagas disease)

Comments: American trypanosomiasis (Chagas disease) is caused by the protozoan parasite *T. cruzi*. Both the parasite and the infection in humans were first

described in 1909 by the Brazilian physician and researcher Carlos Chagas (3). *T. cruzi* is found mainly in blood-sucking triatomine insects (6), which are primarily of the *Triatoma* spp. (family *Reduviidae*, subfamily *Triatominae*) in South America. These giant insects (adults can reach 4.5 cm in length) are colloquially called "kissing bugs" (2). *T. cruzi* is distributed where insect vectors also occur (5). The parasite is also found in small mammals in a sylvatic cycle that is enzootic from the southern and southwestern United States to central Argentina and Chile (9).

T. cruzi exists in three forms (1,5): (i) the **trypomastigote**, found in the blood circulation of the parasitized host, is 15-20 μ m long, with a centrally located nucleus and a single flagellum originating near a large subterminal kinetoplast (posterior to the nucleus); (ii) the **amastigote**, found intracellularly in the host, is roughly a spheroid, approximately 1.5-4.0 μ m in diameter, and contains a nucleus and kinetoplast. The tiny flagellum is rarely prominent under light microscopy; (iii) the **epimastigotes** are found in the insect vector. They are flagellated and spindle-shaped, with the kinetoplast anterior to the nucleus.

Stercorarian transmission (stercorarian = growing or living in dung) occurs when the infected triatomine insect defecates on or near the host during or shortly after the blood meal. Subsequently, the fecal material containing trypomastigotes is rubbed into the wound produced by the bite, in abrasions on the skin, or mucous membranes.

When the vector is involved in the cycle, infection occurs upon the insect vector's feces containing trypomastigotes being deposited at the vector bite site or near it, as occurs in human infections in South America, but this may not be the main route of infection in veterinary Chagas disease. Oral *T. cruzi* transmission can also occur and may be the most important way of acquiring the disease (5) through ingesting infected bugs or consuming infected reservoir animals through predation or scavenging. In humans, consuming contaminated food or drink has led to devastating outbreaks of acute Chagas disease (5). Ingestion of infected insect vectors causes the parasite to be released into the mouth of the dog (2). Blood transfusion and transplacental transmission can also occur, and transmission by ingestion of milk from infected lactating dogs is suspected (1). Also, congenital transmission is estimated at 5% to 10% of infected pregnant women and probably also occurs in dogs with an undetermined prevalence (5).

After infection, trypomastigotes enter cells and transform into amastigotes, which multiply by binary fission or remain free in circulation to spread from the local site of infection. After hematogenous spread, cardiac myocytes become infected with trypomastigotes, which, after transforming into amastigotes, multiply and change back into trypomastigotes before rupturing and escaping from the cell back into circulation (2).

The vector becomes infected by ingesting circulating trypomastigotes, which transform into epimastigotes and multiply by binary fission. Transformation of the epimastigotes back into trypomastigotes occurs in the vector's hindgut before the trypomastigotes are passed in the feces.

Several risk factors predispose to *T. cruzi* infection (5). These factors are associated with encounters with the triatomine insect vector in the environment and include: (i) Residing in, or traveling to, an area with infected insect vectors; (ii) lifestyles that include prolonged exposure to the outdoors, including outdoor work or housing; (iii) multidog kennel environments; (iv) having an infected littermate or dog at the same premises; (v) being born to an infected mother; (vi) behavior of consuming insects; (vii) young dogs are more likely to develop severe clinical outcomes; and (viii) seroprevalence increases with dog age because older dogs have had a longer time for more exposures. We could detect three (i, ii, and vii) of these factors that might have influenced the disease in the dog of this report.

Parasitemia in dogs might appear at three days post-infection (PI), peak at 17 days PI, and usually be subpatent by one month PI. If signs of acute myocarditis occur, they typically develop about two weeks PI, with recovery occurring around one month PI. Rapid intracellular multiplication cycles ensure a quick rise in parasitemia before effective immunity develops (2).

There are three phases in the clinical course of American trypanosomiasis in dogs: acute, indeterminate (or latent), and chronic. (1). We classified the case reported here as acute. The disease occurred in a young dog puppy with myocarditis characterized by non-suppurative myocarditis with multiple cardiac myocytes parasitized by pseudocysts containing *T. cruzi* amastigotes and absence of fibrosis. In dogs, these changes are strong evidence of acute cases of Chagas disease in dogs (2,5-7,9). Myocarditis results from the rupture of cardiac myocytes, causing an inflammatory reaction with more cellular destruction and inflammation (5).

Cardiac enlargement and myocardial failure lead to signs of biventricular heart failure, i.e., generalized congestion (ascites, pleural effusion, jugular distension, pulmonary edema, nutmeg liver), typical signs of dilated cardiomyopathy (5). We observed these lesions in the dog in the current report. The aspect of the enlarged liver (nutmeg liver) covered by a thick fibrin film is typical of right heart failure in the dog, as it is the serosanguineous ascitic fluid.

The anemia and hypoproteinemia seen in the dog of our case possibly resulted from the loss of red blood cells (RBCs) and proteins to the abdominal and thoracic cavity; this loss occurs due to the imbalance in Starling's forces, more precisely due to the increase in hydrostatic pressure in the capillaries due to cardiac failure, causing a more significant outflow of fluid containing RBCs into body cavities (8); the exact mechanism would also produce the subcutaneous edema.

The increased activity of the ALT occurs due to hepatic necrosis induced by systemic congestion due to failure of the right ventricle to pump blood, thus leading to stasis in the large vessels and passive congestion in organs such as the liver. Other authors also report an increase in ALT in the acute phase of canine Chagas disease (1). Urea and creatinine levels were elevated, possibly due to a pre-renal disorder, as the kidneys filter an average of 20% of the cardiac output, and heart failure results in low cardiac output, with probable renal hypoperfusion, which

reduces the filtration rate and the excretion of creatinine and urea. Low cardiac output explains the weak femoral pulse and increased capillary reperfusion time.

Some authors consider that, at least in some regions, contact with an infected dog is not an immediate zoonotic risk of American trypanosomiasis (5). In contrast, in some areas of South America, dogs participate significantly in the domestic transmission of *T. cruzi*, as the presence of infected dogs or cats in the household is closely associated with the human prevalence and incidence of *T. cruzi* (4). Also, dogs can serve as sentinels of vector exposure risk for humans (5).

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