

Buffy Coat Smear from a Puppy

Karen E. Russell, Kirsten F. Barnhart, Jennifer S. Fryer, Thomas M. Craig

**What Is Your
Diagnosis?**

Case Presentation

An 8-week-old intact female Australian Heeler was presented to the Texas Veterinary Medical Center on emergency. The puppy was from south Texas and was referred for a 2-day history of progressive lethargy and possible heart and liver disease. The puppy was anorexic and had loose stools; there was no history of vomiting. Upon presentation, the puppy was quiet but alert, with a temperature of 37.7°C, a pulse rate of 144 beats/min, and a respiratory rate of 72 breaths/min. Mucous membranes were pale. On auscultation, heart sounds were quiet, and no murmur was detected. The abdomen was distended, and a fluid wave was detected.

Results of a CBC revealed normocytic, hypochromic, nonregenerative anemia (PCV 27%, reference interval 37-55%; MCV 75.8 fL, reference interval 60.0-77.0 fL; MCHC 31.1 g/dL, reference interval 32.0-36.0 g/dL; reticulocytes 1.6% [56,960/ μ L]), and leukocytosis (23,600 cells/ μ L; reference interval, 6000-17,000 cells/ μ L) characterized by neutrophilia (16,992 cells/ μ L; reference interval, 3000-11,500 cells/ μ L), and monocytosis (1888 cells/ μ L; reference interval, 150-1350 cells/ μ L). The lymphocyte count was 4720 cells/ μ L (reference interval, 1000-4800 cells/ μ L), and the plasma protein concentration was decreased at 4.9 g/dL (reference interval, 6.0-8.0 g/dL). Moderate numbers of reactive lymphocytes were observed in the peripheral blood film. The platelet count was 225,000 cells/ μ L (reference interval, 200,000-500,000 cells/ μ L).

Radiographs revealed decreased detail within the abdomen and an enlarged cardiac silhouette and left atrium. Findings were consistent with ascites and cardiomegaly. Echocardiography revealed severe right heart dilatation. Abdominal fluid was collected and analyzed. The fluid was orange and opaque, and the super-

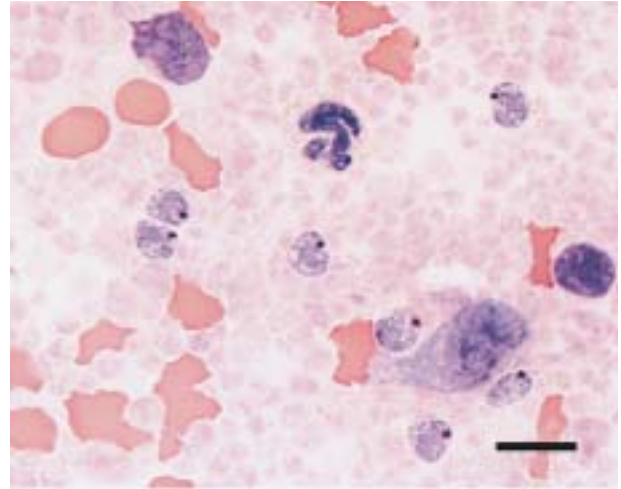


Figure 1. Buffy coat smear from a puppy. Wright-Giemsa, bar = 10 μ m.

natant was light yellow and hazy. The fluid had a total nucleated cell count of 1330 cells/ μ L and an erythrocyte count of 18,000 cells/ μ L. Total solids were 3.2 g/dL. The nucleated cell population consisted of approximately 75% nondegenerate neutrophils, 15% large mononuclear cells, 6% lymphocytes, and 4% plasma cells. Many of the lymphocytes were large and reactive. These findings were consistent with a modified transudate. The reactive lymphocytes and the plasma cells in the fluid were consistent with chronic antigenic stimulation.

Given the age of the puppy, clinical findings, and the geographic area where the puppy was from, buffy coat smears were prepared for microscopic examination (Figure 1). The buffy coat smears were made from the initial blood sample; however, the sample had been stored at 4°C for approximately 10-12 hours.

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From the Departments of Veterinary Pathobiology (Russell, Barnhart, Craig) and Small Animal Medicine and Surgery (Fryer), College of Veterinary Medicine, Texas A&M University, College Station, Tex. Corresponding author: Karen E. Russell, DVM, PhD, Department of Veterinary Pathology, 4467-TAMU, Texas A&M University, College Station, TX 77843-4467 (krussell@cvm.tamu.edu).

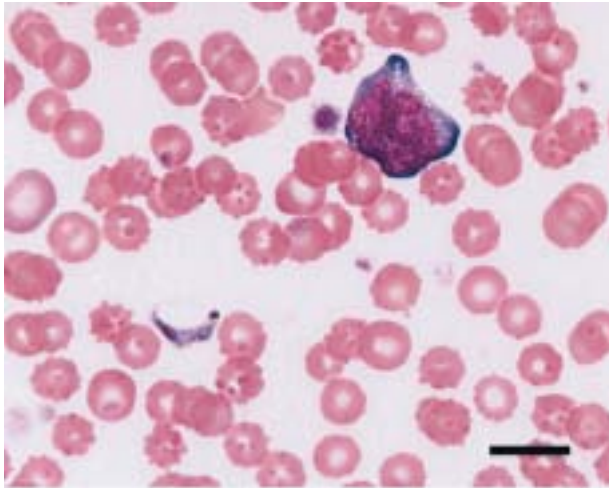


Figure 2. Blood smear from a puppy with trypanosomiasis. This smear was made at the time of initial presentation, 10-12 hours before buffy coat smears were prepared. A single trypomastigote is visible, as well as a large, reactive lymphocyte. Wright-Giemsa, bar = 10 μ m.

Cytologic Interpretation

The buffy coat preparation was very cellular, with a densely packed background of platelets over some areas of the smear (Figure 1). Many round structures, approximately 3-5 μ m in diameter, were seen. The vast majority of structures were extracellular, but rare ones were found intracellularly, within monocytes. The structures contained granular light blue cytoplasm with a single small, dark, eccentrically located magenta dot and a centrally located round, light pink spot. Blood smears prepared at the same time as the buffy coat preparation also contained these structures, which were presumably organisms. Reevaluation of the blood smear made at the time of the initial CBC revealed many small, flagellated, extracellular organisms, which had an undulating membrane, a kinetoplast, and a nucleus (Figure 2). The cytologic interpretation was trypanosomiasis, consistent with *Trypanosoma cruzi* infection.

Gross Pathology and Histopathology

Because of the poor prognosis associated with *T cruzi* infection, the puppy was euthanized. On gross necropsy, clear reddish fluid was present in the thoracic and abdominal cavities and in the pericardial sac. The liver was enlarged and congested. The heart was globose, and the right ventricle was enlarged and flaccid. There were small light tan areas in the right ventricular wall and papillary muscle of the left ventricle that were suggestive of myocarditis. No congenital malformation of the vessels or heart structure was found. The gross pathologic findings were most consistent with acquired right-

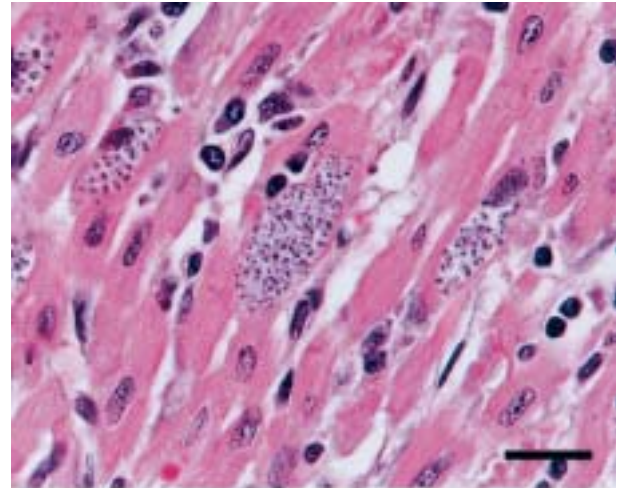


Figure 3. Histologic section of cardiac muscle with numerous amastigote-containing pseudocysts of *Trypanosoma cruzi*. Hematoxylin and eosin, bar = 20 μ m.

sided congestive heart failure.

Histopathologic evaluation of the heart revealed severe nonsuppurative myocarditis with many amastigote-containing pseudocysts of *T cruzi* (Figure 3). Mild nonsuppurative myositis also was observed in the diaphragm and the temporal muscle. Amastigote forms of the parasite were found in the diaphragm, but none were found in the temporal muscle. Marked congestion, mild pulmonary edema, and discrete intravascular coagulation were seen in the lung.

Discussion

Trypanosoma cruzi is the etiologic agent of Chagas' disease or American trypanosomiasis. Parasite distribution extends from South and Central America to the southern United States, with most canine cases occurring in Texas and Louisiana.¹⁻⁶ Insect vectors belong to the family Reduviidae (Hemiptera) and are commonly called kissing, cone-nosed, or assassin bugs. Reservoir mammals include dogs, raccoons, opossums, and armadillos.

During the life cycle of the parasite, 3 morphologic forms or stages occur: epimastigote, trypomastigote, and amastigote. The epimastigote occurs in the insect vector and is elongated and flagellated. This form multiplies by binary fission in the midgut of the vector and then develops into the trypomastigote form, which moves into the hindgut of the insect. Infection of a host occurs when trypomastigotes from feces of the insect vector are deposited at a bite site, in an open wound, or on mucous membranes of a susceptible host.⁶⁻⁸ The trypomastigote is the infectious stage and is the form found in blood. It is flagellated and has an undulating membrane that extends the entire length of the organ-

ism. It is typically elongated and approximately 15-20 μm in length. After hematogenous spread, the trypomastigote is phagocytized by macrophages or enters myocytes, where it transforms into the amastigote stage. This intracellular form is round and approximately 2-4 μm in diameter and has a large round nucleus and a dark rod-shaped kinetoplast. The flagellum is small and not visible by light microscopy. This stage of the parasite also divides by binary fission and forms pseudocysts within tissues. Within the pseudocyst, the parasite transforms from the amastigote stage back into the trypomastigote stage. When the pseudocyst ruptures, trypomastigotes enter peripheral blood.

The time between infection and development of acute disease varies. In experimental infections, this period is approximately 2-4 weeks. Young dogs tend to develop disease earlier than older animals. In the acute phase of the disease, rupture of pseudocysts with release of organisms from the host's cells, especially cardiac myocytes, causes the clinical signs associated with severe myocarditis and cardiac dysfunction. Typical clinical signs, many of which were seen in the puppy in this case, include pale mucous membranes, lethargy, ascites characterized as a modified transudate, hepatomegaly, splenomegaly, and tachyarrhythmia. Clinical signs of right-sided heart failure, including cardiac dilatation, pleural effusion, and pulmonary edema, further support a diagnosis of acute trypanosomiasis. In this case, cardiomegaly, severe acute right-sided heart failure and ventricular tachycardia were observed.

Organisms may be found in ascitic fluid or lymph node aspirates. Hematology often reveals lymphocytosis, and trypomastigotes may be identified in the blood during acute disease. This puppy had lymphocyte numbers at the upper end of the reference interval for our laboratory and many large and reactive lymphocytes. The moderate nonregenerative anemia, mature neutrophilia and monocytosis were consistent with ongoing inflammation and antigenic stimulation. The hypoproteinemia and modified abdominal transudate were most likely secondary to right-sided heart failure, which increases hydrostatic pressure extending to the liver, resulting in loss of fluid and protein into the abdomen. Although many organisms were seen in blood films from this puppy, this is not always the case, and parasitemia may be low. Concentration techniques, such as buffy coat preparations, increase one's chance of finding and identifying the organism.

In this case, buffy coat smears prepared 10-12 hours after blood collection contained many round organisms; no other forms, including elongated trypomastigotes, were found. The round form of the organism in the buffy coat smears may have represented either a time-related morphologic change in the trypomastigotes, or an in

vitro transformation of the trypomastigote stage to the amastigote stage. A time-related change is possible because of the substantial time lag between blood sample submission and buffy coat preparation in this case. *T. cruzi* can aerobically acquire energy from glucose, amino acids, and peptides, most likely from the tricarboxylic acid cycle.^{7,9} During the elapsed time, glucose and other nutrients would have been depleted, leaving the organism in an energy-deficient state. The round forms of the parasite probably were not degenerative forms because all the organisms had intact plasma membranes, and internal structures were demonstrable by light microscopy.

The hypothesis that the round forms may have been amastigotes, formed by the in vitro transformation of trypomastigotes, is supported by the following morphologic characteristics: the round shape of the parasite, the presence of a small dark kinetoplast and lighter nucleus, and the lack of a visible flagellum. A change in temperature or pH of the blood or a combination of both may have contributed to this transformation. Experimentally, many amastigote forms of the parasite are found in the midgut contents harvested from insects 72-96 hours after an infective meal.¹⁰ This phenomenon seems related to temperature, at least in part, because higher numbers of amastigote forms are observed when insects are kept at 23°C or 26°C compared with 28°C.¹⁰ Generally, when organisms are propagated in vitro at room temperature, the epimastigote form predominates. Amastigote forms can predominate in culture; however, the specific factors that contribute to this are not understood.¹¹ Specific media, elevated temperature, and the number of passages appear to be important in determining which form of the organism predominates in vitro.¹¹

This case is significant because we discovered a unique, alternative morphologic form of *T. cruzi* in the dog. Identification of this new form is especially important for diagnosticians who routinely receive blood that may be several hours or days old. Although it is important to always make blood films from freshly drawn blood when submitting samples for a CBC, concentrated preparations such as buffy coat smears usually are not made. We are uncertain whether this morphologic form of *T. cruzi* represents a time-related change or a stage of the parasite; however, when it is seen in concentrated smears or on blood films made from older blood, trypanosomiasis should be suspected. \diamond

Key Words: Blood smear, buffy coat, canine, Chagas' disease, *Trypanosoma cruzi*, trypanosomiasis

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