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Hemogram Interpretation and Diagnosis of Blood Parasites of Canines

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ABSTRACT: The organisms that live in the blood of their animal hosts are called as blood parasites and these parasites can range from bacteria and rickettsiae to single-celled more complexed protozoa. The transmission varies, depending on the parasite, but often they are transmitted through the bites of ticks or flies. Various blood parasites such as *Ehrlichia canis, Babesia canis, Hepatozoon canis* and *Trypanosoma evansi* can cause serious threat in canines. The routine diagnosis in detection of various developmental stages of intracellular parasites with Romnowsky's staining method would be a greatest task of an expert parasitologist. Further, each blood parasite is associated with specific hematological variations and identification of these hematological alterations during routine laboratory screening of blood samples from dogs displaying clinical signs would be essential for diagnosing blood parasitic infections. In this manuscript twenty two canine blood samples suspected for various hemoprotozoan parasites obtained from a private veterinary clinic at Vijayawada over a period of 7 months (March to September) was screened by Leishman's staining technique. Complete blood profile was carried out manually and detailed haemogram analysis was carried out for the accurate diagnosis and differential diagnosis of various canine blood parasites of veterinary importance. Out of 22 canines examined, a total of 6(27.3%), 2(9.1%), 2(9.1%), 1(4.6%) samples were found positive for *Ehrlichia canis, Trypanosoma evansi, Babesia* sp. and *Hepatozoon canis* respectively. Since the sample size was very lesser amount (22), we could not able to discuss in detail about the prevalence data; only the haemogram of specific blood parasite was given prime importance in this study for the diagnosis of blood parasites.

Keywords: Blood parasite, Haemoprotozoan parasite, Canine, haemogram analysis.

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INTRODUCTION

Canines are important reservoirs of many zoonotic pathogens including several gastrointestinal (GI) and tick borne parasites (Robertson and Thompson, 2002). The roundworm *Toxocara canis* is one of the most common zoonotic GI parasites acquired from dog (Lee *et al.,* 2010) followed by hookworms *i.e*. *Ancylostoma* and *Uncinaria* spp. (Bowman *et al*., 2010). In addition, the tick-borne diseases got evolved as a growing threat to both canine and human health. The most important tick-borne diseases that flare-up on dogs around the globe are Lyme disease, Ehrlichiosis, Anaplasmosis, Rocky Mountain Spotted Fever, Babesiosis, Bartonellosis, and Hepatozoonosis (Shaw *et al*., 2001). All can have serious health consequences for dogs and many can have serious health consequences for people as well. Here, we reported the occurrence and diversity of *Trypanosoma* sp., *Babesia* sp., *Hepatozoon canis*. and *Ehrlichia* canis of canines and analyzed the haemogram with the clinical symptoms for the accurate diagnosis in the absence of shaw specific blood parasites in the Leishman's stained blood smear.

Conventional, immuno and molecular diagnostic methods were in practice to diagnose various blood parasites of canines, in which, the hemogram analysis was given lesser important. In this study, the important of hemogram in diagnosis and differential diagnosis of various parasitic diseases of canines was portrayed. The complete blood count (CBC), serum chemistry profile, and urinalysis are the cornerstones of clinical laboratory assessment and it is important to perform all the tests concurrently on presentation, particularly in sick patients.

The peripheral blood serves as the transport medium between the bone marrow and the tissues; consequently, a CBC acts as a snapshot of the hematopoietic system at a specific point in time. Interpreting a test or a group of tests without the others is prone to diagnostic errors. The goal of this manuscript is to help small-animal practitioners to develop a systematic approach that enables them to logically interpret hemogram data in any clinical situation for the diagnosis of blood parasites.

Sample collection and management: To ensure accurate hematologic interpretation, minimizing artifacts as much as possible would be mandatory. Poor blood collection techniques, inadequate sample volumes, prolonged sample storage, and delayed sample analysis will provide opportunities for artifact formation. Common vein puncture sites in dogs include the jugular and cephalic veins and the lateral saphenous vein as it crosses the tarsus. Ethylenediaminetetraacetic acid (EDTA) is the anticoagulant of choice for blood film preparation because it preserves cellular detail better than other anticoagulants like heparin, and citrate (Lam *et al*., 2004). Analyzing hematologic samples as soon as possible prevents artifacts created by exposure to anticoagulants and from cell deterioration due to storage and shipment. It is essential to examine the blood samples within three hours of collection or refrigerate them at 39º F (4º C) to avoid artificially increased PCV, mean cell volume and decreased mean cell hemoglobin concentration. Preparing blood smears within an hour of collection would prevent various morphological artifacts *viz*. erythrocyte crenation, neutrophil hypersegmentation, and lymphocytic nuclear distortion, which commonly encountered in aged blood samples (Adewoyin and Nwogoh, 2014). Further, it would be essential to evaluate RBC for evidence of anisocytosis, poikilocytosis, polychromasia and other intra cellular parasites. Important erythrocyte morphologic abnormalities include spherocytes, schistocytes, acanthocytes, and burr cells, among others. Further, it is also essential for the analysis of various abnormalities viz. neutrophils for toxic change and the presence or absence of a left shift (elevated band neutrophils), lymphocytes for reactivity, and monocytes for phagocytosed organisms. Comparative analysis of leukogram, erythrogram, thrombogram along with the overall morphological abnormalities of various white blood cells by Leishman's staining method would yield an optimum interpretation for the effective diagnosis (Houwen, 2000).

Case study and hemogram interpretation: Routine screening of Leishman's stained peripheral blood smear was carried out to diagnose the presence of blood parasites and for detailed differential leukocyte count. Majority of the blood parasites induces various hematologic changes but detection of specific developmental stages of suspected blood parasites would be an expert's task at the field level. Hence, in the current case study, the correlation of clinical history, clinical symptoms along with typical morphological abnormalities observed in red and white blood cells were performed for the effective diagnosis of blood parasites and also to correlate the nature of the disease as acute or chronic.

Blood Parasites	No. of Canines found positive / total no. tested (%)	
Ehrlichia canis	06/22	(27.3%)
Trypanosoma evansi	02/22	(9.1%)
Babesia sp.	02/22	(9.1%)
Hepatozoon canis	01/22	(4.6%)

Case study: No.1.

History: A male German Shepherd of One year and three months old was reported with listless, anorectic and high temperature (105.5°F). The animal was collapsed within a day of examination. It was a chronic case, suspected for haemoprotozoan parasites and was treated for ehrlichiosis since last 15 days with oral doxycycline and parenteral amikacin.	
PCV: 49.6; Hb: 16.9 g/dl; TEC: 8.2 x 10^6/µl; TP: 7.8 g/dl; A/G ratio: 0.2; Globulin:5.8g/dl **Total Leukocyte Count (TLC):** 40.4 × 10^3/ µl **Differential Leukocyte Count (DLC):** N: 92, L: 7, M:1	**Description:** Hemoglobin, PCV and TEC were found in normal range. **TLC**: Severe leukocytosis with neutrophilia was noticed. Relative thrombocytopenia was observed by Leishman's stained blood smear.

Interpretation: Inflammatory leukogram with superimposed stress indicated severe leukocytosis and neutrophilia with left swift, indicated the bacterial induced septicemia. Relative thrombocytopenia along with the raise in total leukocyte count could have induced an acute septic shock. Further, the reduction in platelet count and presence of immature platelets in the circulation might have induced the formation of blood clots, emboli and multi organ failure. The condition of disseminated intravascular coagulation due to production of megaplatelets (Immature) from bone marrow was an unfortunate occurrence in this case.

Diagnosis: Due to the severe disseminated intravascular coagulation throughout the blood stream, the animal might have collapsed due to the bacterial induced septic shock. Found negative for haemoprotozoan and Rickettsial organisms.

Differential diagnosis:

Ehrlichia canis, *Anaplasma platys*, *Anaplasma phagocytophilium*, *Babesia canis*, *Trypanosoma evansi* and *Hepatozoon canis* are the commonly reported blood parasites of canines. Elevation of monocyte count, leukopenia and thrombocytopenia were the frequent finding of canine Ehrliciosis and anaplasmosis. In this case, although there was a reduction in platelet count, higher range of total leukocytes, neutrophilia along with normal range of monocytosis have given clear indication about the severity of bacteria induced septicemia.

The hemoglobin concentration was found to be in normal range (16.9gm%) and therefore the possibility of occurrence of babesiosis found nil.

Radiography of chest and abdomen was not performed in this case to rule out the cardiomyopathy and foreign body occurrence in the gastric system.

Due to the presence of higher range of total protein and hyper gammagloulinemia, chronic inflammation was clearly established.

Opinion and interpretation:

Citing the raise in TLC, neutrophilia, hyperprotenemia and history of poor weight gain, warranted to follow histo-pathological investigation to detect the chronic intrinsic bone marrow disease.

Possibility of occurrence of leptospirosis in this case was nil due to the absence of clinical symptoms viz. jaundice and hepato renal encephalopathy.

Case study: No. 2.

History: A female Labrador of two and half years old was reported with the symptoms of high temperature (106ºF), anorectic, anaemia, ecchymosis in the ventral abdominal region with severe tick infestation. The case was suspected for haemoprotozoan parasites.

PCV: 23.0, Hb: 8.2 g/dl, TEC: 4.23 × 10^6/µl, TP: 6.2 g/dl A/G ratio: 0.5, Platelet: 70000/ µl, TLC: 4250/ µl **DLC:** N: 82%, L: 7%, M:11%	**Description**: Reduction in Hemoglobin, PCV and TEC was noticed. **TLC**: Marginal Leukopenia, relative neutrophilia, monocytosis with severe reduction of platelet count and presence of more number of immature platelets were observed.

Interpretation:

Severe monocytosis with thrombocytopenia and presence of megaplatelets suggested ehrlichiosis induced acute inflammation. Appearance of immature platelets in the peripheral circulation was the cause for ecchymosis. Bone marrow hypoplasia and impairment of bone marrow production for all blood elements could be the definite grounds for pancytopenia, which resulted in induction of non-regenerative normocytic normochromic anaemia.

Diagnosis: Canine monocytic ehrlichiosis.

Case study: No. 3.

History: A German Shepherd male dog with 2 years age was reported to the veterinary clinic with inappetance, transient fever (104ºF to 105ºF), pale mucous membrane, anaemia, ventral edema and lethargy. Habit of feeding raw beef was noticed.

PCV: 19.0, Hb: 7.6 g/dl, TEC: 3.8 × 10^6/µl, TP: 3.2 g/dl Platelet: 250000/ µl, TLC: 4850/ µl **DLC:** N: 78%, L: 12%, M:05%, E:05%,	**Description:** Reduction in Hemoglobin, PCV and TEC was observed. **TLC**: Marginal leukopenia.

Interpretation:

Lower erythrogram values noticed with presence of few nucleated red blood cells indicated regenerative hypochromic anaemia.

Diagnosis: Leishman's stained blood smear was found positive for *Trypanosoma evansi*.

Case study: No. 4.

History: A Beagle male dog, five years aged was reported with inappetance, pyrexia (104ºF), anaemia and edemated hind limb. Skeletal and cardiac myositis was suspected and the radiography of chest revealed cardiomyopathy with Vertebral Heart Size (VHS) of 11.	
PCV: 21.0, Hb: 6.8 g/dl, TEC: 3.42 x 10^6/µl, Platelet: 300000/ µl, TLC: 19550/ µl **DLC:** N: 57%, L: 36%, M:06%, E:01%.	**Description**: Reduction in Hemoglobin, PCV and TEC was noticed. **TLC**: Marginal leukocytosis.
Interpretation: Lower erythrogram values with presence of few nucleated RBC were the indicator of regenerative anaemia. Skeletal and cardiac myositis was the reason behind the hind limb muscular degeneration, edema and cardiomyopathy. Further the pet was manifestation with disseminated periosteal bone proliferation. **Diagnosis:** Gamonts of *Hepatozoon canis* was detected in the neutrophils by Leishman's stained blood smear.	

Case study: No.5.

History: A Beagle, male dog of six years old was reported with high temperature (105.5ºF) since four days with pale mucus membrane.	
PCV: 20.0; Hb: 5.8 g/dl; TEC: 3.2 x 10^6/µl, Platelet: 120000/ µl Mean Corpuscular Volume (MCV): 75 fl: Mean Corpuscular Hemoglobin concentration (MCHC):30% TLC: 5950/ µl **DLC:** N:78%, L: 09%, M:07%, E:06%,	**Description:** **Erythrogram:** Severe reduction in hemoglobin, PCV and TEC was observed. There was a marginal macrocytic (MCV: 75 fl) hypochromic (MCHC: 30%) anaemia. **TLC**: Relative Leukopenia, lymphopenia and thrombocytopenia.
Interpretation: As per data drawn from two cases suspected for canine babesiosis in this study, both the dogs were presented with regenerative anemia, as demonstrated by lower RBC, Hb, and RBC volumes when compared to reference ranges. Macrocytic (high MCV) hypochromic (low MCHC) anemia with relative thrombocytopenia were the common findings in *B. canis* infections. **Diagnosis:** Peripheral blood smear was found positive for *Bebesia canis* organism.	

DISCUSSION

Canine ehrlichiosis, babesiosis, trypanosomiasis and hepatozoonosis are the important vector borne diseases that infects canine worldwide (Shaw *et al*., 2001). Microscopic examination of blood films associated with hematological profiling was performed routinely by most of the veterinary hospitals for the diagnosis of blood parasitic infections. In this study Out of 22 canines examined, *Ehrlichia canis*, *Trypanosoma evansi*, *Babesia* sp. and *Hepatozoon canis* was found positive for 27, 9.1, 9.1 and 4.6 percent respectively. *E. canis* was the most common blood parasite followed by *T. evansi*, *Babesia* sp. and *Hepatozoon canis*. Similar to our findings, Fleischman, (2012) also reported *E. canis* as a most common blood parasite infecting canines in India, followed by *H. canis* and *B. canis*. Normocytic normochromic anemia was noticed in the later phase of chronic canine ehrlichiosis, which was non-regenerative due to the bone marrow dysfunction and the anemia could be as a result of antibody production against erythrocytes, in combination with immune mediated hemolytic anemia (IMHA) (Fleischman, 2012). Furthermore, monocytosis and thrombocytopenia was the main WBC abnormality in dogs with ehrlichiosis, followed by leukopenia which was similar to findings from other reports (Nair, 2016). Increased platelet consumption during the acute phase of infection, as a result of inflammatory mechanism could be the probable reason behind thrombocytopenia in *E. canis* infection (Solano, 2016).

Hepatozoonosis was associated with anemia in this study. The RBC indices (RBC count, HGB, HCT, MCH, and MCHC) were below the normal reference range in infected dogs, when compared to healthy animals, which was characteristic of normocytic anemia (Baneth *et al*., 2001). In contrast, WBC counts were increased in the majority of *H. canis* infected dogs, when compared to healthy animals, which was indicative of leukocytosis and which was consistent with the previous findings (Salakij *et al*., 1999). These elevated cell numbers were higher than those observed in other canine blood parasitic infections, which may be due to the inflammatory response induced by tissue invasion and multiplication of *Hepatozoon* organism.

In most canine babesiosis cases, infected dogs presented with regenerative anemia, as demonstrated by lower RBC, hemoglobin and PCV volumes when compared to reference ranges. Macrocytic (high MCV) hypochromasia (low MCHC) anemia and heterogeneous cell volume were also associated with *B. canis* infections, which was most likely the direct consequence of parasitizing *Babesia* organisms and damaging RBCs (Fleischman, 2012). Relative thrombocytopenia was found to be a predominant characteristic of *B. canis* positive cases (Eichenberger *et al*., 2016). Mild leucopenia and neutropenia were also been detected with canine babesiosis, as previously found by other studies.

Further, the reports on canine trypanosomiasis might be scanty and can't be ignored. The patho-physiology of anaemia in *Trypanosomosis* is complex and it is due to mechanical injury to erythrocyte, occurs by the lashing action of the powerful locomotory flagella and microtubule reinforced bodies of the millions of the organisms during parasitaemia. Severity of anaemia usually reflects the intensity and duration of parasitaemia and reported that anemia was the main haematological findings of *T. evansi* in dogs (Gunaseelan *et al*., 2009). These data supported the fact that hematological abnormalities are a hallmark for the identification of blood parasites of canines. The fundamental study of hematological profile and haemogram analysis depicted in the present case report for each blood parasites can be effectively utilized at the field level diagnosis.

CONCLUSION

The various case reports indicated the importance of hemogram for the diagnosis of blood parasites even in the absence of specific organism in the blood cells. Majority of the Canine monocytic ehrlichiosis was presented with thrombocytopenia, monocytosis, and anemia. Hepatozoonosis was reported with anemia, leukocytosis, neutrophilia, thrombocytopenia and monocytosis. In contrast, anemia, thrombocytopenia and lymphopenia were the blood abnormalities of canine babesiosis. Severe to moderate reduction in TEC, PCV, Hb and TLC values with marginal neutrophilia, eosinophilia and lymphocytopenia was reported with *T. evansi* infection. Further, the study concluded that the canines with lower RBC, Hb, PCV, and platelet values than the normal hematological profiles were under higher risk for blood parasitic infections.

FUTURE SCOPE

It's not always possible to obtain specific blood parasites in the stained blood smear for the diagnosis of blood protozoa/Ricketsial organisms in the field condition. Hemogram analysis along with the clinical symptoms would be an ideal tool for the diagnosis of accurate stage of pathogenesis and course of the disease.

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CONFLICT OF INTEREST

Nil.

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