



## A REVIEW

# 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 single-celled protozoa to more complex bacteria and rickettsiae. 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 tough 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. This manuscript briefly defined various clinical cases of parasitic infections of canines with the aid of haemogram for the accurate diagnosis and differential diagnosis of canine blood parasitic diseases.

**Key Words :** Blood 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; Macpherson, 2013) 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 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.

Conventional, immuno and molecular diagnostic methods were in practice to diagnose various blood parasites of canines, in which, the hemogram analysis was given lesser importance. In this study, the importance 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 one test or one 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 (Nicole 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. Examine samples within three hours of collection or refrigerate them at 39° F (4° C) to

avoid artificially increased PCV, mean cell volume and decreased mean cell hemoglobin concentration. Preparing blood smears within one 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 mandatory to evaluate RBCs 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 (total leukocyte count), erythrogram, thrombogram (platelet count) 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 (Berend Houwen, 2000).

### Case study and hemogram interpretation:

Routine screening of Leishman stained peripheral blood smears was carried out to diagnose the presence of blood parasites and for detailed differential leukocyte

Case study: No.1.	
Case history	Description
Breed: Beagle; Sex: Male; Age: One year and three months. Reported with the symptoms of listless, anorectic and pale mucous membrane (m/m). Packed cell volume (PCV): 22% Hemoglobin (Hb): 6.0 g/dl Total erythrocyte count (TEC): $2.8 \times 10^6/\mu\text{l}$ Total Protein (TP): 3.8 g/dl Platelet: Thrombocytosis with presence of mega platelets Total Leukocyte Count (TLC): $10000 \times 10^3/\mu\text{l}$	<b>DLC:</b> Anisocytosis and poikilocytosis with moderate number of RBC fragments. Numerous RBC had large pale centers. <b>TLC:</b> Marginal lymphopenia and mild monocytosis. Marked microcytic hypochromic anaemia with thrombocytopenia.
<b>Differential Leukocyte Count (DLC):</b> Neutrophil (N): 7000/ $\mu\text{l}$ , Lymphocyte (L): 1000/ $\mu\text{l}$ Monocyte (M): 1500/ $\mu\text{l}$ , Eosinophil (E): 500/ $\mu\text{l}$	
<b>Interpretation:</b> Inflammatory leukogram with superimposed stress and tissue necrosis. Monocytosis indicated inflammation and tissue necrosis. Marginal lymphopenia indicated superimposed stress. Hypochromic red blood cells are more fragile than normal and hence increased number of RBC fragments was noticed. The anaemia could be non-regenerative, Iron deficiency anaemia. Thrombocytopenia with megaplatelets likely indicated reactive thrombocytopenia in response to the erythropoietin.	
<b>Diagnosis:</b> Severe hook worm infection with iron deficiency anemia resulting from chronic blood loss.	

**Case study: No.2**

**History:** Breed: German Shepherd; Sex: Male; Age: One year and three months. Listless, anorectic, high temperature (105.5F), the animal was collapsed within a day of examination. The case was suspected for haemoprotozoan parasites.

PCV: 49.6; Hb: 16.9 g/dl; TEC:  $8.2 \times 10^6/\mu\text{l}$ ; TP: 6.8 g/dl; A/G ratio: 0.2;

Globulin: 6.8g/dl

**TLC:**  $40.4 \times 10^3/\mu\text{l}$

**DLC:** N: 92, L: 7, M:1

MCV, MCH and MCHC were found in normal range

**Interpretation:** Inflammatory leukogram with superimposed stress and severe Leukocytosis with neutrophilia indicated the bacterial induced septicemia. Due to the septic shock the animal might have collapsed. The 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 are the major cause in induction of blood clots which might have induced the production of emboli and multi organ failure. The condition of disseminated intravascular coagulation due to production of megaplatelets (Immature) from bone marrow was occurred and it is an unfortunate occurrence in this case.

**Diagnosis:** Bacteria induced septic shock due the Severe disseminated intravascular coagulation throughout the blood stream. Found negative for haemoprotozoan parasites.

**Differential diagnosis:**

1. In canines, *Ehrlichia canis*, *Anaplasma platys*, *Anaplasma phagocytophilum*, *Babesia canis*, *Trypanosoma evansi* are the commonly reported blood parasites. Elevation of monocyte count, Leukopenia and thrombocytopenia are common finding in canine Ehrlichiosis and anaplasmosis. In this case, though there was a reduction in platelet count, higher range of total leukocytes, neutrophilia along with normal range of monocytosis had given clear indication about the severity of bacteria induced septicemia.
2. Reduction in haemoglobuline and anaemia is the common symptoms of canine babesiosis. Since, there was a normal range of haemoglobuline (15 to 17 gm%), the possibility of occurrence of babesiosis found nil.
3. Radiography of chest and abdomen could have been performed in this case to rule out the Cardiomyopathy and foreign body occurrence in gastric system.
4. Due to the presence of higher range of total protein and hyper gammaglobulinemia, chronic inflammation is 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 is narrow due to the absence of clinical symptoms viz. hepato renal encephalopathy and jaundice.

**Case study: No.3**

**History:** Breed: Labrador; Sex: Female; Age: Two and half year; Anorectic, high temperature (106°F), 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:  $4.2 \times 10^6/\mu\text{l}$ , TP: 6.2 g/dl

A/G ratio: 0.5, Platelet: 70000/  $\mu\text{l}$ , TLC: 4250/  $\mu\text{l}$

**DLC:**

N: 82%, L: 7%, M:11%

**Description:** Reduction in Haemoglobuline, PCV and TEC was noticed.

**TLC:** Marginal Leukopenia, relative Neutrophilia, monocytosis, severe reduction in platelet count and more number of immature platelets was observed.

**Interpretation:**

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

**Diagnosis:** Canine monocytic ehrlichiosis.

Case study: No.4	
<b>History:</b> Breed: German Shepherd; Sex: Male; Age: Two year: Inappetance, transient fever (104°F to 105 °F), anaemia, ventral edema and lethargy. Habit of feeding raw beef was noticed.	
PCV: 19.0, Hb: 7.6 g/dl, TEC: $3.8 \times 10^6/\mu\text{l}$ , TP: 3.2 g/dl	<b>Description:</b> Reduction in Haemoglobuline, PCV and TEC was observed.
Platelet: 250000/ $\mu\text{l}$ , TLC: 4850/ $\mu\text{l}$	<b>TLC:</b> Marginal Leukopenia.
<b>DLC:</b> N: 78%, L: 12%, M:05%, E:05%	
<b>Interpretation:</b> Lower erythrogram values were noticed with anaemia (Regenerative).	
<b>Diagnosis:</b> Number of <i>Trypanosoma evansi</i> organisms were been detected in the Leishman's stained blood smear.	

Case study: No.5	
<b>History:</b> Breed: Beagle; Sex: Male; Age: Five year; Inappetance, marginal raise in temperature (104°F), anaemia with 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: $3.4 \times 10^6/\mu\text{l}$ , Platelet: 300000/ $\mu\text{l}$ , TLC: 19550/ $\mu\text{l}$	<b>Description:</b> Reduction in Haemoglobuline, PCV and TEC was noticed.
<b>DLC:</b> N: 57%, L: 36%, M:06%, E:01%	<b>TLC:</b> Marginal Leukocytosis.
<b>Interpretation:</b> Lower erythrogram values were noticed with anaemia (Regenerative). 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.	
<b>Diagnosis:</b> Gamonts of <i>Hepatozoon canis</i> was detected from few neutrophils by Leishman's staining technique.	

Case study: No.6	
<b>History:</b> Breed: Beagle; Sex: Male; Age: Six year; high temperature (105.5°F) since four days with pale mucus membrane.	
PCV: 20.0; Hb: 5.8 g/dl;	<b>Description:</b>
TEC: $3.2 \times 10^6/\mu\text{l}$ , Platelet: 120000/ $\mu\text{l}$	Erythrogram: Severe Reduction in Haemoglobuline, PCV and TEC was noticed. There was a marginal macrocytic (MCV: 75 fl) hypochromic (MCHC:30%) anaemia.
MCV: 75 fl: MCHC:30%	<b>TLC:</b> Marginal Leukopenia, Lymphopenia and Thrombocytopenia.
TLC: 5950/ $\mu\text{l}$	
<b>DLC:</b> N:78%, L: 09%, M:07%, E:06%,	
<b>Interpretation:</b> As per data drawn from two cases of 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 thrombocytopenia were the common finding in <i>B. canis</i> infections.	
<b>Diagnosis:</b> Peripheral blood smear was found positive for <i>Bebesia canis</i> organism.	

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.

Canine babesiosis, hepatozoonosis and ehrlichiosis

are important tick-borne diseases that infect canine worldwide (Shaw *et al.*, 2001). Microscopic examination of blood films associated with hematological profiling is performed routinely by most of Veterinary Hospitals to diagnose blood parasitic infections. By this approach, it was reported that *E. canis* was the most common blood parasite infecting canines in India, followed by *H. canis* and *B. canis* (Fleischman, 2012). Further, the reports on *Trypanosoma evansi* might be scanty and can't be ignored. This study also reported hematological profile

and haemogram analysis for each detected organism, which can be used as an effective way for the diagnosis of blood parasites without expecting the show specific organism in the stained blood smear.

In canine ehrlichiosis, normocytic normochromic anemia was commonly noticed, which is non-regenerative due to bone marrow dysfunction (Fleischman, 2012) 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 (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 is 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 is indicative of leukocytosis and which is 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 (Sorawat *et al.*, 2020).

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 is most likely the direct consequence of parasitizing *Babesia* organisms and damaging RBCs (Fleischman, 2012). Thrombocytopenia was found to be a predominant characteristic of *B. canis* positive cases (Eichenberger *et al.*, 2016). Mild leucopenia and neutropenia also were detected with canine babesiosis, as previously found by other studies (Wongsawang *et al.*, 2018).

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.

### Conclusion:

The various case reports given an important hemogram indications for the diagnosis of blood parasites even in the absence of specific organism in the blood cells. Majority of the Canine monocytic ehrlichiosis cases were presented with thrombocytopenia, monocytosis, and anemia. Hepatozoonosis were reported with anemia, leukocytosis, neutrophilia, thrombocytopenia and monocytosis. In contrast, anemia, thrombocytopenia, eosinopenia, and lymphopenia were 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 conclude that higher risk of blood parasitic infections were noticed in canines with lower RBC, Hb, PCV, and platelet values than the normal hematological profiles.

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