

Clinico-Pathological Changes in Hemoprotozoan Infections in Dogs

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ABSTRACT

A total of 1200 blood samples were examined from the dogs for screening hemoprotozoan infection in and around Navsari district of south Gujarat, India. Out of these cases 18 were found positive for *Trypanosoma cruzi*, 27 for *Dirofilaria immitis* and 8 for *Hepatozoon canis*. The clinico-pathological changes included gastrointestinal and haematological disturbances including anaemia, ascites, fever, cardiomyopathy and altered serum urea and creatinine values. The information derived on hemoprotozoan diseases could be helpful for further subsequent diagnostic, preventive and therapeutic care in South Gujarat.

Key words: Clinico-pathology, Dogs, Hemoprotozoa.

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INTRODUCTION

Reports on hemoprotozoan diseases in dogs are at low frequency as compared to large animals due to their strict habitat and diet (Bitton *et al.*, 2012). Moreover such diseases may go unnoticed due to the absence of laboratory diagnosis. Diagnosis of these diseases is very important for the effective treatment of the affected dogs. Hence, clinico-pathology of hemoprotozoan infections was studied in dogs of South Gujarat.

MATERIALS AND METHODS

In all, 1200 blood samples were examined from the dogs presented at Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Navsari, Gujarat (India) during the period from November 2016 to May 2017 for screening hemoprotozoan infections. Out of these, 53 dogs or samples were found positive for different hemoprotozoan spp. From each positive case, approx. 2 mL blood was collected in K₃EDTA vial for haemogram on an automated haematological analyzer (NiHonKohden, Model: MEK-6450,4 Part, Vet). The thin blood smears were made using fresh blood (without anticoagulant) and stained with Eosin-Methylene blue stain. Blood samples (4 mL) were also collected in clot activator vials without anticoagulant. These were allowed to clot and serum separated following centrifugation was stored at -20 °C until analyzed. The serum biochemical parameters, *viz.*, urea and creatinine were measured by a semi-automatic biochemistry analysis (Nova-2002 model). The liver function tests, *i.e.* total protein and alanine aminotransferase were also performed to assess the effects of hemoprotozoan infections. Selected cases were also screened by ultrasonography for organopathy.

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RESULTS AND DISCUSSION

Hepatozoon Cases

There were total 8 positive cases out of 1200 screened for hepatozoon infection with symptoms of vomiting and diarrhoea. Among them 3 were male and 5 were females with age ranging from 3-12 months. Clinical examination revealed fever, pale anaemic mucous membrane and tensed abdomen along with gastrointestinal disorders. The blood smears of the dogs affected with hepatic disorders revealed the presence

Table 1: Hematological and biochemical changes in dogs with hemoprotzoan diseases

Hemoprotzoan diseases	Hb (g/dL)	TLC ($10^3/\mu\text{L}$)	N	L	E	M	ALT (U/L)	Creatinine (mg/dL)	Total protein (g/dL)	Urea (mg/dL)
Normal Reference value)*	11.9-18.9	5.0-14.1	58-85	8-21	0-9	2-10	10-109	0.5-1.7	5.4-7.5	8-28
<i>Hepatozoon canis</i> (n=7)	4.5 ±3.20	25800 ±48.23	89 ±2.4	08 ±1.45	01 ±4.31	03 ±3.53	430 ±32.14	1.70 ±1.10	4.73 ±1.15	42.36 ±10.28
<i>Dirofilaria immitis</i> (n=27)	5.2 ±0.11	16100 ±30.23	80 ±2.5	14 ±1.32	05 ±1.10	01 ±1.32	324 ±33.21	1.64 ±2.10	4.49 ±3.21	36.03 ±5.23
<i>Trypanosoma cruzi</i> (n=18)	3.0 ±1.31	22500 ±16.10	85 ±4.5	10 ±4.00	01 ±1.20	04 ±0.50	256 ±39.00	1.83 ±1.13	4.30 ±1.10	50.63 ±6.23
Mean	4.1 ±1.11	19300 ±32.00	82.5 ±2.51	12 ±2.31	3 ±2.10	2.5 ±1.51	290.0 ±34.01	1.735 ±0.10	4.395 ±0.10	43.33 ±7.3

*Merck Veterinary Manual (Loos Scarth L. 2006). Hb=Hemoglobin, TLC=Total leucocytes count, N=Neutrophils, L=Lymphocytes, E=Eosinophils, M=Monocytes, ALT= Alanine amino-transferase.

of gametocytes of *Hepatozoon canis* in leucocytes. The gametocytes displaced the nucleus of the leucocytes giving a typical appearance. The prominent central clear area was indicative of a moderate anaemic condition. The haemato-biochemical findings of these cases are presented in Table 1. There was increase in ALT and urea and decrease in total protein as compared to the normal reference value in the affected cases of *Hepatozoon canis* in our study.

In similar study, Mundin *et al.* (2008) and O'Dwyer *et al.* (2006) found anaemia as a common laboratory abnormality in dogs suffering from hepatozoon infections. The ultrasonographic examinations also revealed hepatitis characterized by swelling of the liver in our study. Our findings on haemato-biochemical findings were similar to those of Khoshnegah *et al.* (2009), who also reported *Hepatozoon canis* gametocytes within neutrophils in Giemsa-stained peripheral blood smears and bone marrow smear. Increased count of neutrophils indicating neutrophilia reported in the current investigation have been also indicated in the past in *Hepatozoon canis* infection (Gondim *et al.*, 1998; Gavazza *et al.*, 2003; Mundim *et al.*, 2008).

Gametocytes of *H. canis* were encountered during routine blood smear examination and the pathogenicity of the infection in relation to alterations in haematological and biochemical parameters in the host as reported by Gavazza *et al.* (2003), Pawar and Gatne (2005), Chhabra *et al.* (2013) and Mundim *et al.* (2008).

Filaria Cases

Among the total reported 27 positive cases out of 1200 dogs screened for filarial parasites (*Dirofilaria immitis*) 4 were female and 23 were males with an age range varying from 3 to 10 years. These dogs had a history of swelling of both hind limbs and ventral abdomen, and were confirmed on presentation at the clinics. Abdominocentesis in 12 cases revealed turbid fluid. Muffled heartbeat and tachycardia were also recorded. In 3 cases echocardiography revealed

an enlarged heart (all 4 chambers) and ascitic floating viscous fluid in ultrasonography. 3 cases were tentatively diagnosed to have dilated cardiomyopathy with pericardial effusion suggestive of hemoprotzoan disease. From the cases suffering from ascites, the thin blood smears revealed the presence of microfilaria (Nematode also known as Heartworm). The fresh blood drop when examined at 1000X magnification revealed the vigorous movement of worms (Fig. 1). The mosquitoes act as vectors to transfer 3rd stage larvae of microfilaria to the skin of the dog, subsequently, after two moults, the adult worms move into the pulmonary vein and pulmonary artery (Soulsby,1986).

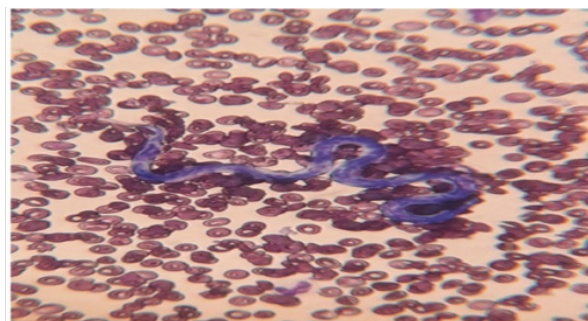


Fig. 1: Microfilaria (embryo of the *Dirofilaria*) in blood smear of dog (1000X)

The worm lodged in the heart and blood vascular system may obstruct blood flow resulting in ascites. Heartworm disease is directly related to the number of worms the animal is infested with, the duration of the infection, and the host's immune response. Animals can often be asymptomatic. Clinical signs are exercise intolerance and coughing. Cachexia, syncope, and ascites can be seen in severely affected dogs. This is due to pulmonary artery embolism because of the worms (Shapiro and Mandel, 2010). There was reduction in haemoglobin and total protein, and increase in total leucocytes, ALT and blood urea nitrogen in the filaria affected cases compared to the normal reference value in our study (Table 1). In similar cases, Radhika *et al.* (2001) reported a significant increase in erythrocyte sedimentation rate and total

leucocyte count, and a significant decrease in haemoglobin, total erythrocytes count and packed cell volume in microfilaria infected dogs. The circulating larvae of *Dirofilaria repens* might cause tissue damage and destruction of RBCs leading to low TEC and Hb content as opined by Yousif *et al.* (1990). Kumar (1980) and Kumar *et al.* (1984) associated the decrease in erythrocyte production in microfilariasis due to damage caused by microfilaria to the liver tissues resulting in loss of blood and depression of erythropoiesis.

Trypanosome Cases

A total of 18 dogs out of 1200 screened, consisting of 10 males and 8 females were found to have trypanosome infections. The majority of them had a history of chronic vomiting and black-coloured faeces. Clinical signs consisting of a pale conjunctival mucous membrane, labored breathing, abducted elbow and cachectic body condition were recorded. These cases also showed clinical signs of renal disturbances.

The blood drop examination under the microscope of these dogs revealed the presence of *Trypanosoma cruzi* in extracellular fluid (Fig. 2). The trypanosoma incidences in dogs are at low frequencies as compared to cattle and buffaloes. But the vector bites of the dogs and contaminated meat eating could be playing the important role in trypanosome infection.

There was reduction in haemoglobin, increase in total leucocytes and increase in blood urea nitrogen in the affected cases of trypanosoma. In similar study, Aquino *et al.* (2002) reported significant decrease ($p < 0.01$) in red blood cell count, haemoglobin concentration and packed cell volume in the trypanosoma infected animals. Barr *et al.* (1991) reported increased leucocytes count in trypanosomiasis in dogs. The increased ALT and urea values and decreased total protein as compared to the normal reference values in the affected cases of *Trypanosoma cruzi* was noticed in our study. The changes in serum biochemistry including decreased albumin, globulin and hypoglycemia, were also observed in the trypanosoma infected dogs by Aquino *et al.* (2002).

In conclusion, the incidences of hemoprotzoan diseases found in dogs of South Gujarat indicate the need for rigorous laboratory diagnosis comprising blood smear examination, haemogram and kidney-liver function tests. The prompt and correct laboratory diagnosis could be useful for effective treatments and can also be useful for providing epidemiological information to veterinarians.

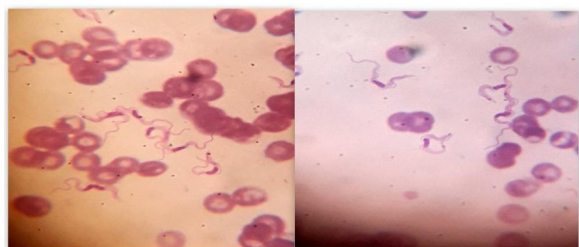


Fig. 2: Trypanosoma in dog blood smears (1000x)

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