ALTERNATION OF HEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILES IN DOGS NATURALLY INFECTED WITH *HEPATOZOON CANIS* IN SOUTH WESTERN GUJARAT, INDIA

***B.R. Maharana[1]. Binod Kumar[2]. B. J. Thakre[3], N. D. Hirani[4], and T. K. Patbandha[5]**

[1, 2, 4] Department of Veterinary Parasitology, [3]Teaching Veterinary Clinical Complex, [5]Polytechnic in Animal Husbandry, College of Veterinary Science & A.H, JAU, Junagadh (Gujarat)- 362001, India
*Corresponding Author Email: drbiswaranjanmaharana@gmail.com

ABSTRACT

This study represents Hepatozoon canis infection in three among seventy eight dogs referred to Teaching Veterinary Clinical Complex (TVCC), Veterinary College, Junagadh from January to April 2013 with prior history of anorexia, anemia, depression and difficulty in locomotion. Significant clinical findings included higher body temperature (106°F), enlarged lymph nodes and congested mucus membrane. Blood smears were stained with Giemsa stain revealed capsule shaped gametocytes of H. canis in the cytoplasm of approximately 15 to 20% circulating neutrophils.This is the first time documentation of hepatozoonosis in dogs from south western Gujarat, India and hence placed on record.

Key Words: *Biochemical, Dog, Hematological, Hepatozoon canis*.

INTRODUCTION

Hepatozoonosis, is a debilitating, tick-borne disease of carnivores caused by *Hepatozoon canis*. It spreads by ingesting infected brown dog tick, *Rhipicephalus sanguineus* which has been considered as the main vector (Baneth *et al*., 2003). Dog, the definitive host, exhibits high parasitaemia, even approaching upto 100 % of the peripheral blood neutrophils (Baneth, 2001). *H. canis* was firstly reported in India in 1905 (James, 1905) which is considered as the recognized

1. **Biswa Ranjan Maharana,** Asst. Professor, Dept. of Veterinary Parasitology, College of Veterinary Science & A.H., JAU., Junagadh, Gujarat, 362001, Email: drbiswaranjanmaharana@gmail.com, Mobile: +91-8650924354/+91-8128154595
2. **Binod Kumar,** Asst. Professor, Dept. of Veterinary Parasitology, College of Veterinary Science & A.H., JAU., Junagadh, Gujarat, 362001, Email: drkumarbinod@gmail.com, Mobile: +91-9725560977
3. **Bhupendrakumar Jamsubhai Thakre**, Asst. Professor, Teaching Veterinary Clinical complex, College of Veterinary Science & A.H., JAU., Junagadh, Gujarat, 362001, Email: drbhupendrakumarthakre@gmail.com, Mobile: +91-7069412676
4. **Nitin Damodarbhai Hirani,** Asst. Professor, Dept. of Veterinary Parasitology, College of Veterinary Science & A.H., JAU., Junagadh, Gujarat, 362001, Email: nitinbhaihirani@gmail.com, Mobile: +91-9408789575
5. **Tapas Kumar Patbandha,** Polytechnic in Animal Husbandry, Junagadh (Gujarat)- 362001, India, JAU., Junagadh, Gujarat, 362001, E Mail: patbandhavet@gmail.com, Mobile:+91-720664754

species causing canine hepatozoonosis presently prevalent in the various regions of India (Banerjee *et al*., 2008; Eljader *et al*., 2012; Palanivel *et al*., 2010). The clinical manifestation of the disease may range from being asymptomatic (due to low parasitaemia), to a severe disease with clinical symptoms of fever, anorexia, weight loss, anaemia, ocular discharge, weakness of the hind limbs and signs of lethargy and cachexia corresponding to high parasitaemia (Baneth, 2001).The purpose of this study is to retrospectively describe the clinical and haemato biochemical findings in dogs naturally infected only by *H. canis* in dogs in Gujarat state.

MATERIAL AND METHODS:

The study zone:

The study was conducted in and around Junagadh district of Gujarat state, in the south western region of India. Junagadh has a tropical wet and dry climate, with three distinct seasons observed, a mild winter from November to February, a hot summer from March to June, and a monsoon from July to October. These environmental conditions provide favourable and conducive conditions for the survival and propagation of vectors which play an important role in transmission of haemoprotozoan diseases.

Sample collection and processing:

The samples were collected from dogs presented at Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh. Total seventy eight dogs were referred to TVCC, from January to May 2013 with prior history of anorexia, anemia, depression and presence of brown dog tick on body surface. Clinical findings included higher body temperature (106^{o}F), enlarged lymph node and congested mucus membrane. Blood sample were collected and sent to Veterinary Parasitology Laboratory for hematological, biochemical analysis and to ascertain the presence of haemoprotozoan parasite, if any. Few numbers of ticks were collected from the infected dogs and processed for parasitological identification. The hematology of the whole blood was done with fully automated analyzed haematology system (Mindrey, China) as per instructions of the manufacturer. Samples were processed by Giemsa stain following standard protocol (Soulsby, 2005) for detection of haemoprotozoan infection, if any. Briefly, the blood smears were prepared, air dried, fixed in methanol, stained with Giemsa, and examined under oil immersion lens of compound binocular microscope (100X) for the detection of haemoprotozoan parasite. Total protein, albumin, globulin, serum alkaline phosphatase (ALP), L-alanineaminotransferase (ALT), aspartate amino transferase (AST), blood urea nitrogen (BUN) and creatinine were estimated spectrophotometrically with semi-automated blood biochemistry analyzer (Merc Pvt. Ltd.). The values of the erythrogram and leukogram parameters were compared with the reference values as described in Merck Veterinary Manual (Kahn and Line, 2010).

The infected dogs were treated with inj. Meloxicam 2 ml s/c (Zydus, Animal Health @ 0.5 mg/kg b.w) and fluid therapy (Intalyte ®, Intas Pharmaceuticals) @ 250 ml intravenously along with 3.5% colloidal intravenous infusion solution (Haemaccel®, DexaMedica, India) @ 50 ml intravenously for three days. Tab. Doxycycline @ 10mg/kg b.w. (Ranbaxy, India) was given orally b.i.d for twenty one days along with injection of hepatobiliary drug (Belamyl, Sarabhai Zydus, India) @ 0.25ml intramuscularly, multivitamins (Neurobion, Merc, India) @ 1 tab and haematinics (Haemup®, CadilaPharma, India) @ 20 ml orally as supportive therapy for another twenty days. The blood samples from treated animals were collected 21 days post treatment with the intention of evaluating the efficacy of therapeutic regimen.

RESULTS & DISCUSSION

This study represents *Hepatozoon canis* infection in three among seventy eight dogs presented at TVCC, Junagadh. The observed clinical findings include elevated body temperature (106^{o}F), enlarged lymph node and congested mucus membrane. Similar clinical findings are reported earlier by various workers (Chhabra *et al*., 2013;

Sarma *et al*., 2012; Sakuma *et al*., 2009). Blood samples stained by Giemsa stain revealed capsule shaped gametocytes of *H. canis* in the cytoplasm of approximately 15 to 20% circulating neutrophils. Levels of *H. canis* parasitaemia observed by previous researchers ranged from as low as 0.5 % (Gondim *et al*., 1998) and as high as 90 % (Baneth *et al*., 1995). The disease severity was greater in the present cases with high parasitaemia which is in concurrence with Baneth and Weigler, 1997. The collected ticks were morphologically identified as *Rhipicephalus sanguineus* which could have played a role in transmission of canine hepatozoonosis since infection occurs due to ingestion of such infected ticks (Nordgren and Craig, 1984) containing sporulated oocysts (Pawar, 2003). In the present study, *Ehrlichia canis* was ruled out by examining the blood smear for *E. canis* morulae. Conversely, simultaneous infection of hepatozoonosis with canine monocytic ehrlichiosis has been reported by various workers (Sarma *et al*., 2012; Sasaki *et al*., 2008).

The hemogram revealed anemia, moderate thrombocytosis and leukocytosis. A complete blood count (CBC) was also performed and the result indicated moderate neutrophilia. The disturbances observed in haemogram may be attributed to myelosupressive effect of *H. canis* infection. The direct effect of *H. canis* on thrombocytosis is doubtful, but the detailed mechanism of this process is unknown, as described earlier (Baneth, 2006). Similar findings have also been reported by Pawar and Gatne (2004). The neutrophilia and anaemia are probably subordinate to necrosis and inflammation in the liver, lungs, spleen and lymph nodes (Gaunt, 2000) and these findings are consistent with those previously reported (Sarma *et al*., 2012; Elias and Homans, 2008).

Serum biochemical analysis indicated moderate increase in ALT, ALP, AST, BUN, creatinine, globulin and decrease in total protein and albumin (Table 1). Increased ALP, AST and ALT activity may be associated with increased osteoblastic activity or hepatic lesions (Kraft and Durr, 1995).This results are in agreement with the findings reported by Barton *et al*. (1985). Blood urea nitrogen (BUN) increase was comparable to the findings of Pawar and Gatne (2005) which might be as a result of dehydration or owing to renal amyloidosis or secondary glomerulonephritis in the chronic stage of the disease (Sarma *et al*., 2012). Hypoalbuminemia might be attributed to an acute phase protein response, decreased protein intake, chronic inflammation, increased globulin production or protein-losing nephropathy (Sarma *et al*., 2012; Medici and Heseltine, 2008).

The infected dogs were treated with Meloxicam (2ml S/C, Zydus Animal Health, @ 0.5mg/kg b.w) to reduce pain and inflammation. Tab. Doxycycline (@10mg/kg b.w.,Ranbaxy,India) was prescribed for three weeks for complete removal of parasites. Fluid therapy along with haemacel transfusion, was given to deliver an instantaneous replacement for blood loss and to prevent ischemic necrosis and organ failure. Multivitamins along with hematinics were given to encounter anaemia. Crude liver extracts was given in order to protect liver from damage. The dogs promptly responded to therapy within 72 hours. Blood smears showed no gametocytes and haematological and serum biochemical parameters showed remarkable improvement (Table 1) on 21th day after post treatment. Similar line of treatment has been reported by various workers (Sarma *et al*., 2012; Elias *et al*., 2008).

The results of the present investigation substantiate that *H. canis* causes hematobiochemical alternations in varied intensity. Presently there appears to no published report on occurrence of hepatozoonosis from southwestern Gujarat,India. This manuscript is a plausible indication of the parasitological evidence of *Hepatozoon canis* infection in this geographical part of the country.

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REFERENCES

Banerjee, P.S., Mylonakis, M.E., Garg, R., Vatsya, S. and Yadav, C.L. (2008) Concurrent hepatozoonosis, monocytic and granulocytic ehrlichiosis in a dog. *Journal of Veterinary Parasitology*, **22**: 9-11.

Baneth, G. (2001). Canine hepatozoonosis. In: Encyclopedia of Arthropod-transmitted infections of Man and Domesticated Animals. Ed M.W. service. CABI Publishing, Wallingford. pp 215-220.

Baneth, G. (2004.) Canine Hepatozoonosis - Two Different Diseases. Rhodes, Greece: 29th World Congress of the World Small Animal Veterinary Association.

Baneth, G. (2006). Hepatozoonosis. In: Infectious Diseases of the Dog and Cat, 3rd ed. (Greene, C. E. ed.), Saunders, Philadelphia. pp. 698-705.

Baneth, G., Aroch, I. and Presentey, B. (1997). *Hepatozoon canis* infection in a litter of Dalmatian dogs. *Veterinary Parasitology,* **70**:201-206.

Baneth, G., Harmelin, A. and Presentey, B.Z. (1995). *Hepatozoon canis* infection in two dogs. *Journal of American Veterinary Medical Association*, **206:**1891-1894.

Baneth, G., Mathew, J.S., Shkap, V., Macintire, D.K., Barta, J.R. and Ewing, S.A. (2003). Canine hepatozoonosis: two disease syndromes caused by separate Hepatozoon species. *Trends in Parasitology*, **19**: 27-31.

Barton, C.L., Russo, E. A., Craig, T.M. and Green, R.W. (1985). Canine hepatozoonosis: a retrospective study of 15 naturally occurring cases. *Journal of American Animal Hospital Association*, **21**:125-134.

Chhabra, S., Uppal, S.K. and Singla, L.D. (2013). Retrospective study of clinical and hematological aspects associated with dogs naturally infected by *Hepatozoon canis* in Ludhiana, Punjab, India. *Asian Pacific Journal of Tropical Biomedicine*, **3**(6): 483-486.

Elias, E. and Homans, P.A. (2008). *Hepatozoon canis* infection in dogs: clinical and haematological findings; treatment. *Journal of Small Animal Practice*, **29**: 55-62.

Eljadar, M.S.M., Singla, L.D., Mustafa, R.A.A. and Uppal, S.K. (2012). Morphometric variations in gametocytes of *Hepatozoon canis* from naturally infected dogs. *Journal of Parasitic Disease*, **37**(1): 143-147.

Gaunt, S.D. (2000). Extreme neutrophillic leucocytosis. In: Schalm's Veterinary Haematology. 5th Ed. Eds B.F. Feldman, J.G. Zinkel and N.C. Jain. Lippincott, Williams & Wilkins, Baltimore., Pg. 347-349.

Gondim, L.F.P., Kohayagawa, A., Alencar, N.X., Biondo, A.W., Takahira, R.K. and Franco, S.R. (1998). Canine hepatozoonosis in Brazil: description of eight naturally occurring cases. *Veterinary Parasitology,* **74**: 319-323.

James, S.P. (1905). On parasite found in the white corpuscules of the blood of dogs. Scientific Memoirs by the Officers of the Medical and Sanitary Departments of the Government of India, pg.1-12.

Kahn Cynthia, M. and Line, S. (2010). The Merck Veterinary Manual. 10th ed. Whitehouse Station, N.J., USA.

Kraft, W. and Durr, U.M. 1995. Klinische Labor diagnostic in der Tiermedizin. 3rd Ed. W. Kraft and U.M. Durr. Schattauer, Stuttgart.

Medici, E.M. and Heseltine, J. (2008). American canine hepatozoonosis. *Compendium*, **30**: E1-E9.

Palanivel, K.M., Vijayalingam, T.A. and Selvasubramanian, S. (2010). Perspective studies on canine hepatozoonosis in Chennai.

Tamilnadu Journal of Veterinary and Animal Science, **6**: 291-293.

Pawar, S.D. and Gatne, M.L.(2005). Some haematological and biochemical profiles in canine hepatozoonosis. *Journal of Veterinary Parasitology*, **19**: 171-172.

Pawar, S.D. and Gatne, M.L.(2004). Haematological and biochemical profiles in dogs infected with *Hepatozoono canis*. *Journal of Veterinary Parasitology*, **18**: 185-186.

Sakuma, M., Nakahara, Y., Suzuki, H., Uchimura, M., Sekiya, Z., Setoguchi, A. and Endo, Y. (2009). A case report: a dog with acute onset of *Hepatozoon canis* infection. *The Journal of veterinary medical science*, **71**: 835-838.

Smith, T.G. (1996). The genus Hepatozoon (Apicomplexa: Adeleina). *Journal of Parasitology,* **82**: 565-585.

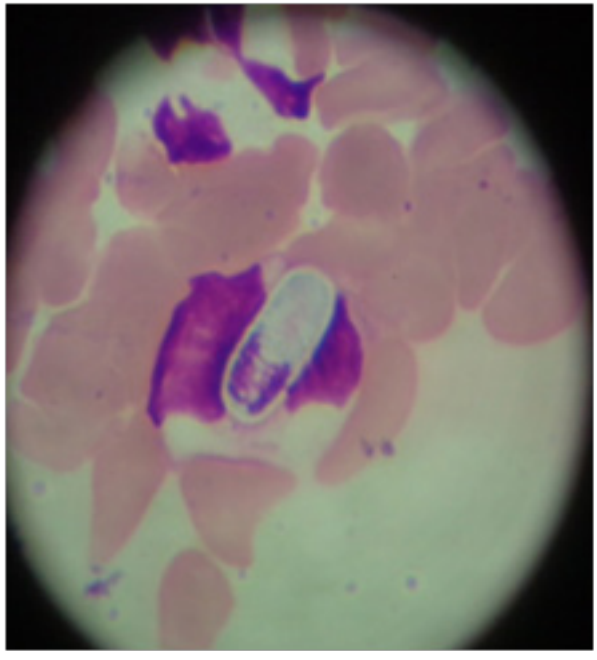

Fig. 1. Gamonts of *Hepatozoon canis* in the neutrophil (HP 400x)

Tab 1: Hemato-biochemical profile before and after therapy (n=3)

Type of Parameter	Variables (n=3) Parameters	Pre-treatment	Post treatment (Day 21)	Reference Values
Hematological	Hb (gm%)	8 ± 0.17	10.9 ± 0.26	12-19
	PCV (%)	29 ± 0.58	32 ± 1.73	35-57
	TEC (x10^6/cu mm)	4.9 ± 0.06	5.1 ± 0.23	5-7.9
	TLC (x10^3/cu mm)	8.1 ± 0.12	7.2 ± 0.17	5-14.1
	Platelets (x10^3/µl)	5.2 ± 0.12	4.1 ± 0.17	1.4-4.4
	DLC (%)			
	Neutrophil	83 ± 2.15	73 ± 1.15	58-85
	Lymphocyte	11 ± 1.15	20 ± 1.73	8-21
	Monocyte	3 ± 0.0	4 ± 0.58	2-10
	Eosinophil	2 ± 0.0	2 ± 0.0	0-9
	Basophil	1 ± 0.0	1 ± 0.0	0-1
Serum-biochemical	Total Protein (g/dl)	4.8 ± 0.12	5.7 ± 0.12	5.4-7.5
	Albumin (g/dl)	1.8 ± 0.12	2.4 ± 0.06	2.3-3.1
	Globulin (g/dl)	3.0 ± 0.06	3.3 ± 0.06	2.4-4.4
	ALP (U/l)	200 ± 2.31	119 ± 2.31	1-114
	ALT (U/l)	120 ± 5.77	80 ± 1.15	10-109
	AST (U/l)	18 ± 1.15	14 ± 0.58	13-15
	BUN (mg/dl)	31.5 ± 0.87	9.5 ± 0.12	8-28
	Creatine (mg/dl)	1.4 ± 0.12	1.3 ± 0.06	0.5-1.7
	Parasitaemia of neutrophils (%)	20 ± 2.89	00	----------