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Haematological studies of stray dogs infected with *Hepatozoon canis* in Andhra Pradesh

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Abstract

To evaluate the hematological parameters of stray dogs naturally infected with *Hepatozoon canis* presented to various veterinary dispensaries of Andhra Pradesh. The results of present haematological study of all infected dogs showed the remarkable decrease in the level of Hb, RBC and PCV values. These observations indicate development of severe erythrocytopenia and anaemia. The decrease in the level of platelet count indicates severe thrombocytopenia. The decrease in MCH and MCHC values indicates anaemia also observed the neutrophilia and lymphocytopenia.

Keywords: Haematological parameters, stray dogs, anaemia, erythron-cytopenia, thrombocytopenia

Introduction

In vector-borne diseases, tick-borne diseases (TBDs) are considered as emerging and re-emerging diseases and are of zoonotic important. Hence, it is valuable to identify the tick-borne pathogens in dogs and elucidate the factors that determine their prevalence. The most common tick-borne diseases of dogs are babesiosis, ehrlichiosis and hepatozoonosis (Homer *et al.*, 2000; Baneth *et al.*, 2003) [8, 2] and are endemic in India (Shaw *et al.*, 2001) [19]. Canine hepatozoonosis, caused by *Hepatozoon canis* is another tick-borne protozoan disease, and is characterised by fever, anaemia, and lethargy (Baneth *et al.*, 2003) [2] and thrombocytopenia, lymphadenopathy, ocular signs, bleeding diatheses, and irreversible bone marrow destruction (Skotarczak, 2003) [21]. Asymptomatic cases of *H. canis* with low level of parasitaemia often go unnoticed. Infection is common in dogs in Asia and co-infection of *H. canis* with *Ehrlichia*, *Babesia*, *Leishmania* and *Anaplasma* is more frequent (Shaw *et al.*, 2001; Mylonakis *et al.*, 2004) [19, 14]. Survey of canine tick-borne pathogens in India revealed that *H. canis* was the most common tick-borne pathogen (Abd Rani *et al.*, 2011) [1]. The present study was conducted in the Department of Veterinary Parasitology to describe the haematological findings of stray dogs infected naturally with *Hepatozoon canis*, in view of the lack of such information about this disease in Andhra Pradesh.

Materials and Methods

A total of 25 stray dogs showing symptoms of high fever, anaemia, lymphadenopathy, thrombocytopenia, ocular signs were presented to various veterinary dispensaries during May to June 2020 from different districts of Andhra Pradesh were selected and blood samples and serum samples were collected. Seven normal dogs were selected and considered as control group. Peripheral blood smears were prepared and stained with Giemsa stain after methanol fixation (Soulsby, 1983) [20]. The stained blood smears were screened for haemoprotozoa under light microscope. The blood samples collected in EDTA coated vacutainers were kept undisturbed for 2 hours and then, centrifuged at 3000 rpm for 5 minutes and the serum separated was immediately decanted in 2ml Eppendorf tubes. Haematological analysis was carried using auto haematology analyser (Mindray Shenzhen Monday Biomedical electronic Co Ltd., China). All the haematological values were compared with normal reference values and interpreted accordingly.

Results

The climate of AP is generally hot and humid. Mean annual temperature of the state is 33.2 °C. In summer, the temperature generally ranges between 20 °C and 40 °C. At certain places the temperature is as high as 45 °C on a summer day. The summer season in this state generally extends from March to June. During these months the moisture level is quite high.

Coastal Andhra region experiences relatively low temperatures during winter and summer seasons but higher temperatures during SWM and NEM seasons compared to the Rayalaseema region of the state. On a monthly basis, state as a whole experiences high morning humidity during October (84%) and least humidity is recorded during May (66%). The summer is followed by the monsoon season, which starts during June and continues till September. The Haematological

alterations viz., Red blood cells (RBC), White blood cells (WBC), Haemoglobin (Hb), Lymphocytes, Neutrophils, Eosinophils, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelets of white blood samples of all *Hepatozoon canis* positive dogs (25) and apparently healthy dogs (7) were evaluated and presented in the below table

Table 1: Haematological alterations in dogs infected with *H. canis*

Parameters & Units	Mean \pm Se		Minimum		Maximum		Standard Deviation		Reference Value
	INF	CNTL	INF	CNTL	INF	CNTL	INF	CNTL	
RBC ($10^6/\mu\text{L}$)	3.09 \pm 0.47*	5.96 \pm 0.10	1.71	5.59	9.17	6.25	2.12*	0.24	5.0-8.5
WBC ($10^6/\mu\text{L}$)	12.07 \pm 0.13	13.13 \pm 0.26	4.05	8.90	20.5	17	5.19	3.07	5.5-14.6
Hb(g/dL)	6.25 \pm 0.99*	14.17 \pm 0.36	1.2	12.8	18.9	15	4.42*	0.87	12-18
Platelets ($10^5/\mu\text{L}$)	0.45 \pm 0.8*	3.52 \pm 0.61	0.1	2.38	1.16	6.4	0.37*	1.5	2-5($10^5/\mu\text{L}$)
Lymphocytes (%)	20.81 \pm 0.50*	30.53 \pm 0.27	9	25.0	30.6	34	6.73*	3.1	10-35%
Neutrophils (%)	74.541 \pm 0.58*	63.82 \pm 0.11	63.5	60.0	86.9	68	7.07*	2.73	60-70%
Eosinophils (%)	4.31 \pm 0.61	5.3 \pm 0.46	0.9	4.00	9.3	7	2.74	1.11	4-9
PCV (%)	18.37 \pm 0.95*	39.54 \pm 0.92	3.6	36.5	56.7	42.5	13.18*	2.25	37-55
MCV (fL)	69.22 \pm 0.41	67.98 \pm 0.01	61.9	65.0	86.9	70.8	6.30	2.48	60-77
MCH (pg)	18.82 \pm 0.47	23.81 \pm 0.66	15	22.1	23.85	26.5	1.76*	2.1	19.5-24.5
MCHC (%)	27.48 \pm 0.88*	33.98 \pm 0.66	21.5	32.0	35.54	36.1	2.93*	3.92	32-36

Note: SE-Standard Error, *-indicate the significant values at $p<0.05$.

Red blood cells: The estimated value for RBC in the dogs of *H. canis* infected group was 3.09 \pm 0.47 and 5.96 \pm 0.01, respectively. The minimum and maximum RBC values in infected dogs was observed as 1.7 & 9.17 and 5.59 & 6.25 with standard deviation of 2.12 and 0.24, respectively. There was a significant difference between infected & control group ($p<0.05$). The RBC values in infected group were less than the reference range in the control group as shown in the table.

White blood cells: The estimated mean value for WBC in the dogs of *H. canis* infected group was found to be 12.07 \pm 0.13 whereas in control group it is 13.13. The minimum and maximum values of WBC in infected was observed as 4.05 and 20.5 with standard deviation of 5.19. There was no significance difference between the infected group and control group ($p<0.05$). The WBC values in infected and control group was within normal range (Table).

Haemoglobin values: The estimated mean value of Hb in the dogs of *H. canis* infected group was found to be 6.25 \pm 0.99, where as in control group, Hb was 14.17 \pm 0.36. The minimum and maximum Hb values in infected and control groups were observed as 1.2 & 18.90 and 12.80 & 15 with the standard deviation of 4.42 and 0.87, respectively. There was a significant difference between the infected and control group ($p<0.05$). The Hb concentration in infected group was less than the reference range and within normal range in the control group as shown in the Table.

Lymphocytes (%): The estimated mean value for lymphocytes in the dogs of *H. canis* group was found to be 20.81 \pm 0.50, whereas in control group lymphocytes were 30.53 \pm 0.27. The minimum and maximum lymphocytes percent in infected and control group were observed as 9 & 30.6 and 25.00 & 34 with the standard deviation of 6.73 and 3.1, respectively. There was a significant difference between the infected and control group ($p<0.05$). The lymphocytes percent in infected and control group was less than the reference range and within normal range in the control group.

Neutrophils (%): The estimated mean value for neutrophils in

the dogs of *H. canis* infected group was found to be 74.541 \pm 0.58, whereas in control group neutrophils were 63.82 \pm 0.11. The minimum and maximum neutrophil values in infected and control group were observed as 63.5 & 86.9 and 60 & 68 with the standard deviation of 7.07 and 2.73, respectively. There was a significance difference between the infected group and control group ($p<0.05$). The neutrophils percent in infected group was more than the reference range and within normal range in the control group as shown in the table.

Eosinophils (%): The estimated mean value for eosinophils in the dogs of *H. canis* infected group was found to be 4.31 \pm 0.61, whereas in control group eosinophils were 5.3 \pm 0.46. The minimum and maximum eosinophils values in infected and control group were observed as 0.9, 9.3 and 4.00 and 7 with standard deviation of 2.74 and 1.11 respectively. There was no significance difference between the infected group and control group ($p<0.05$). The eosinophils percent in infected group and within range in the control group as shown in the table.

Mean corpuscular Haemoglobin concentration (%): The estimated mean value of MCHC in the dogs of *H. canis* infected group was found to be 29.13 \pm 0.66, whereas in control group MCHC was 33.98 \pm 0.66. The minimum and maximum MCHC values in infected and control group were observed as 22.4 & 35.54 and 32.00 & 36.10 with standard deviation of 2.96 and 1.62, respectively. There was a significance difference between the infected and control group ($p<0.05$). The MCHC in infected group was less than the reference range and within normal range in the control group as shown in the table.

Platelets ($10^5/\mu\text{L}$): The estimated mean value for platelets in the dogs of *H. canis* infected group was found to be 0.45 \pm 0.8, whereas in control group platelets were 3.52 \pm 0.61. The minimum and maximum MCHC values in infected and control group were observed as 0.1 & 1.16 and 2.38 & 6.4 with standard deviation of 0.37 and 1.15,

respectively. There was a significance difference between the infected group and control group ($p < 0.05$). The platelet count in infected group was less than the reference range and within normal range in the control group as shown in the table. The results of present haematological study of all infected dogs showed the remarkable decrease in the level of Hb, RBC and PCV values. These observations indicate development of severe erythrocytopenia and anaemia. The decrease in the level of platelet count indicates severe thrombocytopenia. The decrease in MCH and MCHC values indicates anaemia. Also observed the neutrophilia and lymphocytopenia.

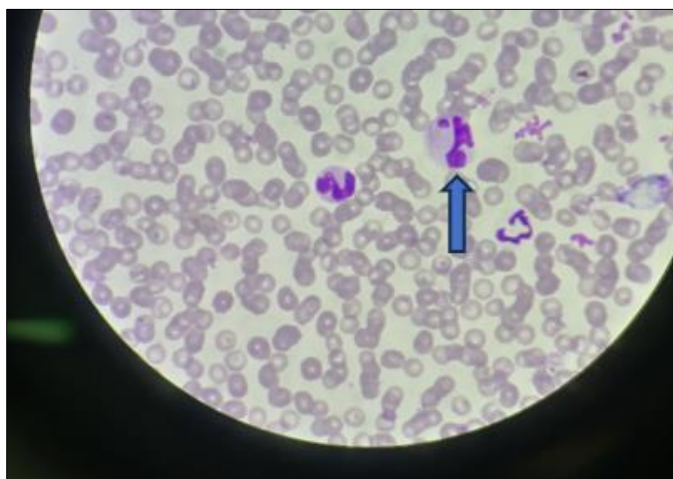


Fig 1: Microscopic picture of *Hepatozoon canis* under 1000x

Discussion

The estimated mean values of haematological parameters *viz.*, Red blood cells (RBC) ($10^6/\mu\text{L}$), White blood Cells (WBC) ($10^3/\mu\text{L}$) Haemoglobin (g/dL), Lymphocytes (%), Neutrophils (%), Eosinophils (%), Packed Cell Volume (%), Mean Corpuscular Volume (fL), Mean Corpuscular Haemoglobin (pg), Mean Corpuscular Haemoglobin concentration (%), and Platelets ($10^5/\mu\text{L}$) of *Hepatozoon canis* infected dogs were 3.09 ± 0.47 , 12.07 ± 0.16 , 6.25 ± 0.99 , 20.81 ± 0.15 , 74.54 ± 0.58 , 4.31 ± 0.61 , 18.37 ± 0.95 , 69.22 ± 0.41 , 18.82 ± 0.47 , 27.48 ± 0.88 and 0.45 ± 0.8 and of control group were 5.96 ± 0.10 , 13.13 ± 0.126 , 14.17 ± 0.36 , 30.53 ± 0.127 , 63.82 ± 0.11 , 5.3 ± 0.46 , 39.54 ± 0.92 , 67.98 ± 0.101 , 23.81 ± 0.66 , 33.98 ± 0.66 and 3.52 ± 0.61 , respectively.

There was a significant difference ($p < 0.05$) between the mean values of RBC ($10^6/\mu\text{L}$), Haemoglobin (g/dl), Lymphocytes (%), Neutrophils (%), Packed cell Volume (%), Mean Corpuscular Haemoglobin (pg), Mean Corpuscular Haemoglobin Concentration (%) and Platelets ($10^5/\mu\text{L}$) in the *Hepatozoon canis* infected and control group of dogs, respectively. These observations confirm the development of severe erythrocytopenia, thrombocytopenia, haemolytic anaemia, neutrophilia and lymphocytopenia in the infected dogs. The haematological alterations recorded in the dogs infected with *H. canis* are in accordance with the earlier reports of Sarma *et al.*, (2012) [17], Inokuma *et al.*, (2002) [9], Revathi *et al.*, (2022) [16], Mandal *et al.*, (2021) [12], Chhabra *et al.*, (2021) [6], Kaur *et al.*, (2012) [11], Qarmar *et al.*, (2016). However, there was no significance of difference ($p < 0.05$) of the mean values of WBC ($10^3/\mu\text{L}$), Eosinophils (%) and MCV (fL) between the infected and control group of dogs. Lilliehook (2019) [18], Sarma *et al.*, (2012) [17] also observed no changes in the WBC and MCV values in canine

hepatozoonosis. In contrast to the present observations, Mandal *et al.*, (2021) [12] demonstrated the development of lymphocytosis, lowered PCV indicating normocytic anemia in dogs infected with hepatozoonosis. In the present study normocytic haemolytic anaemia was mainly found. Baneth G *et al.*, (2006) [4] and Gondim *et al.*, (1998) [7] reported that anaemia as the commonest primary haematological sign observed in most of the hepatozoonosis infected dogs, Mundim *et al.*, (2008) [13] and Voyoda *et al.*, (2004) [22] stated that Neutrophilia and leucocytosis alterations were more frequent in the *hepatozoon* infected dogs and this study findings are consistent with the findings of recorded lowered WBC, RBC and haemoglobin values. Karagene *et al.*, (2006) [10] stated that concurrent infections with pathogens such as *Babesia canis* and *Ehrlichia* spp. will aggravate the clinical condition of dogs infected with *Hepatozoon canis*. These minor differences in the haematological values in the infected dogs might be due to the virulence of Hepatozoon species, age, health and nutritional status of the dogs act. Investigation of the haematological alterations in *Hepatozoon* infected dogs could be helpful to estimate the disease prognosis.

References

1. Abd Rani PAM, Irwin PJ, Coleman GT, Gatne M, Traub RJ. A survey of canine tick- borne diseases in India. *Parasites and Vectors*. 2011;4(141):1-7.
2. Baneth G, Mathew JS, Shkap V, Macintire DK, Barta JR, Ewing SA. Canine hepatozoonosis: two Disease syndromes caused by separate *Hepatozoon* spp. *Trends Parasitology*. 2003;19(1):27-31.
3. Baneth G, Weigler B. Retrospective case control study of hepatozoonosis in dogs in Israel. *J Vet. Intan. Med*. 1997;11:365-370.
4. Hepatozoonosis BG. In: Green CE, editor. *Infectious diseases of the dog and cat*. 3rd ed. Philadelphia: WB Saunders; c2006. p. 608-705.
5. Bilwal AK, Mandali GC, Tandel B. Clinico-pathological alterations in naturally occurring *Babesia gibsoni* infection in dogs of middle-South Gujarat, India. *Veterinary world*. 2017;10(11):1227-1232.
6. Chhabra S, Uppal SK, Singla LD. Retrospective study of clinical and hematological aspects associated with dogs naturally infected by *Hepatozoon canis* in Ludhiana, Punjab, India. 2013;3(6):483-486.
7. Gondim LFP, Kohayagawa A, Alencar NX, Biondo AW, Takahica RF, Franco SRV, *et al.* Canine hepatozoonosis in Brazil: description of eight naturally occurring cases. *Vet Parasitol*. 1998;74:319-323.
8. Homer MJ, Aguilar-Delfin I, Telford SR III, Krause PJ, Persing DH. Babesiosis. *Clinical Microbiology Review*. 2000;13:451-469.
9. Inokuma H, Okuda M, Ohno K, Shimoda K, Onishi T. Analysis of the 18SrRNA gene sequence of a *Hepatozoon* detected in two Japanese dogs. *Veterinary Parasitology*. 2002;106:265-271.
10. Karagene IT, Pasa S, Kirli G, Hosgor M, Bilgic HB, Ozon YH, *et al.* Parasitological, molecular and serological survey of *Hepatozoon canis* infection in dogs around the Aegean coast of Turkey. *Vet Parasitol*. 2006;135:113-119.
11. Kaur P, Deshmukh S, Singh R, Bansal BK, Randhawa CS, Singla LD, *et al.* Para-clinico-pathological observations of insidious incidence of canine hepatozoonosis from a mongrel dog a case report. *J*

- Parasit Dis. 2012;36(1):135-8. doi:10.1007/s12639-011-0092-x.Epub 2012 Jan 21.
12. Mandal M, Maity DA, Mandal PS, Jana M, Roy MC, Pakhira F, *et al.* Diagnosis and Therapeutic Management of Hepatozoonosis in dog. Int. J Curr. Microbiol. App. Sci. 2021;10(8):550-555. Doi: <https://doi.org/10.20546/ijcmas.2021.1008.065>
 13. Mundim AV, Morais IA, Tavares M, Cury MC, Mundim MJS. Clinical and hematological signs associated with dogs naturally infected by *Hepatozoon* sp. And with other hematozoa: A retrospective study in Uberlandia, Minas Gerais. Brazil Vet Parasitol. 2008;153:3-8.
 14. Mylonakis ME, Koutinas AF, Baneth G, Polizopoulou Z, Fytiannou A. Mixed *Ehrlichia canis*, *Hepatozoon canis*, and presumptive *Anaplasma phagocytophilum* infection in a dog. Veterinary Clinical Pathology. 2004;33(4):249-251.
 15. Qamar M, Malik MI, Latif M, Ain QU, Aktas M, Shaikh RS, *et al.*, Molecular detection and prevalence of *Hepatozoon canis* in Dogs from Punjab (Pakistan) and Hematological profile of infected dogs. Vector Borne Zoonotic Dis. 2017;17(3):179-184.
 16. Revathi P, Bharathi MV, Madhanmohan M, Latha BR, Rani KV. Molecular epidemiology, characterisation of *Hepatozoon canis* in dogs as well as in ticks and haemato- biochemical profile of the infected dogs in Chennai. Indian Journal of Animal Research; c2022. p. 1-7.
 17. Sarma K, Mondal DB, Saravanan M, Kumar A, Mahendran K. Haemato-biochemical changes in *Hepatozoon canis* infected dogs before and after therapeutic management. Journal of Veterinary Parasitology. 2012;26(1):35-38.
 18. Lilliehock I, Vedten T, Petters HW, Baneth HK, G. *Hepatozoon canis* infection causing a strong monocytosis with intra monocytic gamonts and leading to erroneous leukocyte determinations. Veterinary Clinical Pathology. 2019;48(3):435-440.
 19. Shaw ES, Michael JD, Birtles RJ, Edward BB. Tick-borne diseases of dogs. Trends Parasitology. 2001;17(2):74-80.
 20. Soulsby EJJ. Helminths, Arthropod and Protozoa of Domesticated Animals, 7th ed. Bailliere Tindal and Cassell Ltd., London UK; c1982. p. 35-740.
 21. Skotarczak B. Canine ehrlichiosis. Annals of Agricultural and Environmental Medicine. 2003;10(2):137.
 22. Voyoda H, Pasa S, Under A. Clinical *Hepatozoon canis* infection in dog in Turkey. J Small Anim. Pract. 2004;45:613-617.