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Investigation of haematological alterations in ancylostomosis in dogs of Thrissur, Kerala

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Abstract
The study investigated haematological alterations in dogs infected with *Ancylostoma* spp. in Thrissur district, Kerala, over a one-year period. Blood samples from 50 infected dogs and 10 healthy controls were analysed for complete blood count (CBC) using a five-part automatic haematological analyser. Significant changes were observed in several parameters: infected dogs exhibited elevated neutrophil and basophil counts, along with reduced red blood cell count (RBC), haemoglobin (Hb), volume of packed red cells (VPRC) and mean corpuscular haemoglobin (MCH). Platelet distribution width (PDW) was also significantly increased in infected animals. The findings indicated that dogs with ancylostomosis suffer from normocytic hypochromic regenerative anaemia, likely due to chronic blood loss and nutrient deficiencies. These results provide valuable insights into the haematological impact of canine ancylostomosis and its associated anaemia, aiding in the diagnosis and management of infected animals.

Keywords: *Ancylostoma*, Ancylostomosis, Haematology and Anaemia

Introduction
Hookworms are the major canine gastrointestinal parasites worldwide causing ancylostomosis. *Ancylostoma caninum*, *Ancylostoma braziliense* and *Uncinaria stenocephala* are common hookworm species of dogs. Transmission of ancylostomosis mainly occurs through contaminated soil and transcolostral route. Puppies under three months of age are more susceptible to infection (Dos Santos *et al*., 2020) [4]. Each *A. caninum* worm can ingest up to 0.5 mL of blood per day (Traversa, 2012) [9]. Among hookworms *A. caninum* is the most pathogenic (Dantas-Torres *et al.* 2020) [3] and have large buccal capsule with three pairs of ventral teeth and a pair of dorsal teeth (Uppal *et al*., 2017) [10]. Ancylostomosis in dogs causes a range of symptoms from mild enteritis to fatal haemorrhagic diarrhoea accompanied by anaemia and weight loss. The severity depends on various factors, such as the age of the host, the parasitic load and the specific species of hookworm involved (Taylor *et al*., 2016; Campos *et al.* 2017) [8, 2]. Severe anaemia may develop in adult animals. However, its clinical presentation is often linked to malnutrition and the presence of concurrent chronic diseases. *Ancylostoma* spp. present significant public health risks due to their zoonotic potential, causing cutaneous larva migrans in humans following exposure to environmental third-stage larvae (L_3), as well as eosinophilic enteritis.
The study on haematotological changes in canine ancylostomosis was limited in Kerala even though the prevalence was high. So the current study was conducted to examine and correlate haematological alterations between infected and control dogs of Thrissur, providing insights into infection-induced blood parameter variations.

Materials and Methods
The study was conducted on dogs in different areas of Thrissur district, Kerala from August 2023 to August 2024. Fifty dogs with ancylostomosis as well as 10 healthy animals were selected and two millilitre of blood collected aseptically using sterile syringe and needle and transferred to vacutainer tubes containing ethylene diamine tetraacetic acid (EDTA) and CBC

was taken using a five part automatic haematological analyser Orphee Mythic 5 vet PRO on the same day of collection at University Veterinary hospital, Kokkalai, Thrissur for haematological analysis. Statistical analyses for comparison of each parameter was done by independent t-test.

Results

Haematological analysis revealed significant differences between infected dogs and control dogs. Among leukocytic parameters, the mean neutrophil and basophil counts were significantly elevated in infected animals. The mean neutrophil count was notably higher in the infected group ($12.55 \pm 0.95 \times 10^9$/L) compared to the control group ($6.59 \pm 0.93 \times 10^9$/L), with a t-value of 4.477 and a P-value of < 0.001. Similarly, the basophil count was significantly elevated in the infected group ($0.457 \pm 0.082 \times 10^9$/L) compared to the control group ($0.102 \pm 0.054 \times 10^9$/L), with a t-value of 3.642 and a P-value of 0.001.

The mean red blood cell count (RBC) was significantly lower in the infected group ($4.9 \pm 0.2 \times 10^6$/µL) than in the control ($6.82 \pm 0.24 \times 10^6$/µL), with a t-value of 4.212 and a P-value of < 0.001. The mean Hb levels were also reduced in the infected group (10.49 ± 0.48 g/dL) compared to the control group (15.26 ± 0.5 g/dL), with a t-value of 4.158 and a P-value of < 0.001. The mean VPRC was markedly lower in the infected group (30.6 ± 1.33%) compared to the control group (45.75 ± 1.34%), with a t-value of 8.026 and a P-value of < 0.001. The mean corpuscular haemoglobin (MCH) in infected animals (22.08 ± 0.7 pg) was significantly lower than in control animals (25.65 ± 1.5 pg), with a t-value of 2.086 and a P-value of 0.041. Furthermore, the mean red cell distribution width-standard deviation (RDW-SD) was significantly reduced in the infected group (32.904 ± 0.358 fL vs. 38.244 ± 0.601 fL), with a P-value of < 0.001. The percentage of nucleated red blood cells (NRBC) was significantly higher in the infected group (388.09 ± 99.44%) compared to the control (14.63 ± 3.77%), with a t-value of 3.753 and a P-value of < 0.001. The mean value of large immature cell (LIC) was significantly decreased in infected animals (0.461 ± 0.039) with a t- value of 2.330 and a P- value of 0.023.

The mean PDW was significantly increased in the infected group (16.17 ± 0.72) compared to the control group (11.01 ± 1.94), with a t-value of 2.829 and a P-value of 0.006. No significant differences were observed for the other parameters. The Comparison of haematological parameters between infected and control animals is given in Table 1.

Table 1: Haematological parameters of *A. caninum* infected and control animals

Parameters	Infected (n=50)	Control (n=10)	t-value	P-value
WBC (10^9uL)	14.73 ± 1.09	11.93 ± 0.86	2.014^{ns}	0.051
LYM %	13.92 ± 0.99	13.32 ± 1.32	0.260^{ns}	0.796
MON %	6.98 ± 0.42	7.22 ± 0.6	0.247^{ns}	0.806
NEU %	72.51 ± 1.32	70.2 ± 1.91	0.748^{ns}	0.457
EOS %	2.96 ± 0.42	3.95 ± 0.52	1.020^{ns}	0.312
BASO %	0.294 ± 0.024	0.413 ± 0.093	1.752^{ns}	0.085
LYM# (10^9/L)	2.12 ± 0.16	2.09 ± 0.33	0.074^{ns}	0.942
MON# (10^9/L)	1.22 ± 0.12	1.2 ± 0.12	0.082^{ns}	0.935
NEU# (10^9/L)	12.55 ± 0.95	6.59 ± 0.93	4.477**	< 0.001
EOS# (10^9/L)	0.716 ± 0.096	1.647 ± 0.962	0.963^{ns}	0.360
BASO# (10^9/L)	0.457 ± 0.082	0.102 ± 0.054	3.642**	0.001
RBC (10^6uL)	4.9 ± 0.2	6.82 ± 0.24	4.212**	< 0.001
HGB (g/dL)	10.49 ± 0.48	15.26 ± 0.5	4.158**	< 0.001
VPRC (%)	30.6 ± 1.33	45.75 ± 1.34	8.026**	< 0.001
MCV (fL)	65.37 ± 1.09	66.98 ± 1.12	1.033^{ns}	0.310
MCH (pg)	22.08 ± 0.7	25.65 ± 1.5	2.086*	0.041
MCHC (g/dL)	34.3 ± 0.71	32.05 ± 2.09	1.223^{ns}	0.226
RDW-CV	15.62 ± 0.35	14.28 ± 0.63	1.612^{ns}	0.112
RDW-SD	32.904 ± 0.358	38.244 ± 0.601	6.314**	< 0.001
PLT (10^3uL)	311.762 ± 20.154	314.7 ± 30.527	0.062^{ns}	0.951
MPV	9.43 ± 0.3	9.46 ± 0.4	0.068^{ns}	0.946
PDW	16.17 ± 0.72	11.01 ± 1.94	2.829**	0.006
PCT	0.266 ± 0.019	2.31 ± 2.085	0.980^{ns}	0.356
P-LCR	28.32 ± 1.8	31.81 ± 5.74	0.738^{ns}	0.464
P-LCC	77.66 ± 5.69	104.94 ± 24.34	1.091^{ns}	0.301
NRBC %	388.09 ± 99.44	14.63 ± 3.77	3.753**	< 0.001
NRBC#	163.73 ± 23.11	205.76 ± 82.23	0.667^{ns}	0.507
ALY %	0.084 ± 0.013	0.114 ± 0.056	0.521^{ns}	0.613
ALY#	0.040 ± 0.010	0.023 ± 0.01	0.784^{ns}	0.436
LIC %	0.461 ± 0.039	33.371 ± 32.737	2.330*	0.023
LIC#	0.070 ± 0.012	0.061 ± 0.019	0.323^{ns}	0.748

** Significant at 0.01 levels; * Significant at 0.05 levels; ^{ns} non-significant

Discussion

In the present study, out of the 31 parameters checked 10 parameters showed significant difference between infected and control group. The mean total leucocyte (WBC) count was higher in infected animals though the difference was not statistically significant. This finding is consistent with studies by Brahmbhatt *et al.* (2015) [1] and Campos *et al.* (2017) [2], were they reported elevated WBC counts in dogs infected with *Ancylostoma* spp. Brahmbhatt *et al.* (2015) [1] attributed this increase to the host's immune response to infection. In the present study, neutrophil counts were significantly elevated in infected dogs indicating an inflammatory response at feeding site of hookworms in the intestine. This observation aligns with Brahmbhatt *et al.* (2015) [1], who similarly reported a high neutrophil percentage (74.42%) in dogs with ancylostomosis. Conversely, Nwoha and Anene (2016) [6] observed a reduction in WBC and neutrophil counts in cases of mixed infection with *A. caninum* and *Trypanosoma brucei* in Nigeria.

In the current study, no significant changes were noted in monocyte or eosinophil counts between infected and control dogs, despite eosinophilia being commonly associated with parasitic infections. Brahmbhatt *et al.* (2015) [1] and Campos *et al.* (2017) [2] reported significantly elevated eosinophil counts in infected animals. The absence of significant eosinophilia in this study could be attributed to differences in the stage of infection. Basophil counts, however, were significantly elevated, corroborating to the findings of Held and Mochizuki (2023) [5], who found a significant association between basophilia and parasitic infections. They linked basophilia to elevated leukocyte counts in hookworm infections. Current study observed a decrease in the mean percentage of LIC in infected animals, a parameter that has not been analysed in previous researches. The reduction in LIC may be associated with recovery from infection-induced stress, as chronic blood loss and nutritional deficiencies in ancylostomosis could impair bone marrow function, leading to reduced production of immature cells.

The mean RBC count was significantly lower in infected dogs indicative of anaemia. The mean Hb levels were also significantly lower in the infected group. This finding aligns with previous studies by Qadir *et al.* (2011) [7], Nwoha and Anene (2016) [6] and Campos *et al.* (2017) [2], who reported decreased Hb levels, further supporting the presence of anaemia. Furthermore, the mean VPRC was significantly reduced in infected dogs reflecting a marked decrease in erythrocyte count. This finding aligns with the observations of Qadir *et al.* (2011) [7], Brahmbhatt *et al.* (2015) [1] and Nwoha and Anene (2016) [6], who reported significant reductions in red blood cell counts in infected dogs, which are indicative of anaemia. The mean corpuscular haemoglobin (MCH) was significantly lower in infected dogs consistent with findings by Brahmbhatt *et al.* (2015) [1] and Campos *et al.* (2017) [2], indicating hypochromic anaemia. The study also reported a significant increase in mean NRBC% in infected animals suggesting a regenerative response as the body attempted to compensate for erythrocyte loss, indicating regenerative anaemia. The marked reduction in RDW-SD in infected dogs suggests a uniform red blood cell size, while the decreased RBC count indicates the presence of normocytic anaemia. Overall, the data suggested that infected dogs exhibited normocytic hypochromic regenerative anaemia.

With regard to platelet parameters, the study found a significantly higher PDW in infected dogs indicating a marked variation in platelet size, with both small and large platelets present in peripheral circulation. This finding has not been extensively explored in other studies. No significant differences were observed in platelet count (PLT) or mean platelet volume (MPV) between the groups.

In conclusion, the findings of the present study on leucocyte and erythrocyte parameters largely align with previous research, particularly regarding increased neutrophils and decreased RBC count, Hb, MCH, RDW-SD and VPRC in infected dogs. Although this study did not find significant differences in eosinophil counts, which are typically elevated in parasitic infections, the overall pattern of anaemia and altered immune cell profiles is consistent with previous findings. The significant increase in PDW suggests potential alterations in platelet function in response to ancylostomosis.

Conclusion

Significant alterations in haematological parameters were observed between *A. caninum* infected and control groups. Infected animals exhibited reduced RBC, Hb, VPRC, MCH and RDW-SD, alongside elevated neutrophil and basophil counts, NRBC % and PDW. The anaemia in infected animals was characterised as normocytic, hypochromic and regenerative.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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