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Therapeutic management and Haemato-biochemical alterations in diarrhoeic dogs affected with ancylostomosis

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Abstract
The canine hookworm, *Ancylostoma caninum* is the most prevalent gastrointestinal nematode parasite of dogs throughout the globe and a common cause of diarrhoea in dogs. A study was carried out to assess haemato-biochemical response and anthelmintic therapy in diarrhoeic dogs diagnosed with Ancylostomosis at Veterinary Clinical Complex, Nagpur Veterinary College, Nagpur. A total of 213 dogs with symptom of diarrhoea were screened of which 51 were found positive for *Ancylostoma* spp. infection. Therapeutic management was carried out in three treatment groups comprising 12 dogs in each group. Group I was treated with Pyrantel pamoate (10mg/kg, PO, single dose), Group II was treated with Fenbendazole (50mg/kg, PO, for 3 consecutive days) and Group III was treated with Doramectin (0.4 mg/kg, SC, single dose). Therapeutic response was assessed in terms of reduction in EPG on 7th and 15th day post-treatment. All three treatment groups showed a complete reduction of egg burden in faeces on day 7th and day 15th post-treatment. The study revealed a decrease in Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) (microcytic hypochromic type of anaemia), eosinophilia, hypoproteinaemia, hypoalbuminemia, hypoglobulinemia and decreased blood urea nitrogen in Ancylostoma infected dogs. Improvement in haemato-biochemical parameters were observed following treatment.

Keywords: Diarrhoea, dog, ancylostomosis, pyrantel, fenbendazole and doramectin

Introduction
Diarrhoea in canines is the most commonly encountered disease of dogs by veterinary practitioners and many often it is related to viral gastroenteritis, neglecting other causes. The canine hookworm, *Ancylostoma caninum* is the most prevalent gastrointestinal nematode parasite of dogs throughout the globe and a common cause of diarrhoea in dogs (Mirjam *et al.*, 2016) [14]. Stray and owned dogs play an important role in the transmission of these diseases. The transmission of these zoonotic roundworms could be through direct contact via penetration through intact skin or through indirect contact via contaminated food and water (Eguia-Aguilar *et al.*, 2005) [8]. Transplacental spread can be possible in pregnant bitches and lactogenic spread can be seen in new born puppies. In puppies less than 6 months of age, the infection can be extremely severe and fatal. Whereas many often it goes unnoticed in adults without clinical signs. Clinical symptoms of ancylostomosis in dogs observed are pale gums, weakness, weight loss, sometimes itchy paws, poor growth rate and rough or poor hair coat. Iron-deficiency anaemia, hypoalbuminemia, and acute enteritis characterized by diarrhoea that may contain fresh blood or digested blood are pathological signs of *Ancylostoma* spp. infection (Kalkofen, 1987) [12].
The problem becomes more important from zoonotic point of view causing cutaneous larva migrans or creeping eruptions in humans (Ashraf *et al.*, 2008) [2]. The drugs febantel, moxidectin, milbemycin oxime, fenbendazole and pyrantel are approved to treat the *Ancylostoma* spp. infection. Whereas, Jimenez Castro *et al.* (2019) [11] documented the development of multiple anthelmintic resistance in multiple isolates of Ancylostoma in the USA. The rising concern of anthelmintic resistance is being fuelled by the misuse and overuse of these medications in India, where there is no compliance with drug regulations. The present study was carried out to observe haematological and biochemical alterations during the course of the disease and after treatment with pyrantel pamoate, fenbendazole and doramectin as periodical assessment of the therapeutic response of the drug is essential.

Materials and Methods

Faecal sample were collected from 213 dogs at veterinary clinical complex, Nagpur Veterinary College, Nagpur. The dogs presented with symptoms of diarrhoea. The samples were qualitatively examined (by direct smear and sedimentation techniques) for ova and were identified on the basis of morphological characteristics (Soulsby, 2005) [19] and was further confirmed by PCR. The *Ancylostoma spp*. positive samples were analysed by Stoll's technique for the quantification of eggs before and after treatment.

Positive dogs were randomly divided into 3 treatment groups with twelve animals in each group: Group I, Group II, and Group III. Infected dogs in Group I were treated with pyrantel pamoate @ 10 mg/kg body weight, PO, single dose. Dogs in Group II were treated with Fenbendazole @ 50mg/kg body weight, PO, for 3 consecutive days and dogs in Group III were treated off-label with Doramectin @ 0.4 mg/kg body weight, SC, single dose. Faecal samples were collected on the 0^{th}, 7^{th} and 15^{th} day post-treatment. Reduction in faecal egg count was studied to determine the therapeutic response of these drugs. Supportive treatment with fluid therapy (Inj. Ringer's Lactate, Inj. Dextrose Normal Saline as per requirement), antibiotic (Inj. Ceftriaxone @ 25 mg/kg body weight, BID, IV), antiemetic (Inj. Ondansetron @ 0.2 mg/kg body weight, BID, IM) and styptic (Inj. Etamsylate @ 250 mg/kg body weight, BID, IM) were administered.

Haemato-biochemical analysis

The Haemato-biochemical profiles of all infected dogs were studied on 0^{th} and 14^{th} day post-treatment. About 2 ml of blood was collected from the saphenous or cephalic veins in the EDTA and clot activator vial for hematological biochemical observations respectively.

Estimation of blood parameters

The haematological changes with respect to Haemoglobin, Packed Cell Volume, Total Erythrocyte Count, Total Leukocyte Count, Differential Leucocyte Count, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration and Platelets were estimated using Haematology Analyser (Horiba Make ABX Micros ESV-60) based on principle of photometry numeric integration and electronic impedance variation methods. Estimation of serum biochemical parameters Total protein, Albumin, Globulin and Blood Urea Nitrogen assay were done by using Microlab 300 Biochemical Semi-Auto Analyser.

Results and Discussion

Fifty-one out of 213 dogs were positive for ancylostomosis by concentration methods i.e. Sedimentation and Floatation technique (by using 33% $ZnSO_4$ solution). The positive infected dogs were randomly divided into three treatment groups comprising 12 animals in each group.

Overall mean Haemoglobin (Hb gm/dl), Packed Cell Volume (PCV %) and Total Erythrocyte Count (TEC $10^6/mm^3$) were lower than normal on day 0^{th} in all three treatment groups. In all treatment groups Hb, PCV and TEC values showed a significant increase on day 14^{th} post treatment (Table 1). The findings are in agreement with the studies of Baba *et al*. (2017) [4], Campos *et al.* (2017) [5], and Aziz *et al.* (2020) [3], who also reported low mean Hb in parasitised dogs than healthy dogs. The adult worms of *Ancylostoma* spp. can suck up to 0.5 ml of blood per day (Epe, 2009) [9]. The adult changes the feeding sites causing small ulcers in the intestinal mucosa leading to continuous bleeding that might aggravate the anaemia (Christodoulou *et al*., 2010) [6]. The low levels of Hb, PCV and TEC could be explained by the voracious blood-sucking nature of the adult worm and bleeding in the intestinal lumen, leading to depletion of iron reserves.

The RBC indices MCV (fl), MCH (pg) and MCHC (g/dl) showed lower mean values in all three treatment groups on day 0^{th} which showed significant variation with an increasing tendency on day 14^{th} post-treatment (Table 1). The present study's finding is in agreement with the study of De *et al*. (2016) [7] and Qadir *et al*. (2010) [16]. Normocytic normochromic anaemia can be detected in early cases of bleeding which can progress into microcytic hypochromic anaemia in later stages on the basis of stage and severity of infection. In the present studies, the anaemia could be the result of severe loss of iron due to excessive blood losses both by feeding of worms and bleeding through bite injuries.

In all three treatment groups, Mean Total Leucocyte Count and mean values of differential leucocyte count did not show any significant variation, while neutrophilia and higher eosinophil count (within the normal range) observed on 0^{th} day before treatment. Improvement in the values were noticed on day 14^{th} post treatment (Table 1). In earlier studies, Campos *et al*. (2017) [5] and Qadir *et al*. (2010) [16] reported no significant differences in the number of neutrophils in parasitised and non-parasitised dogs contrary to the present study. The neutrophilia in the present study could be attributed to concurrent bacterial infection.

Baba *et al*. (2017) [4] and Qadir *et al*. (2010) [16] observed eosinophilia in Ancylostoma infected dogs. Whereas the reports of present findings are contrary to the finding of De *et al.* (2016) [7] who reported non-significant variation in eosinophils count in *Ancylostoma* spp. positive dogs. The eosinophilia in the present study might be attributed to the biting adult worms in the intestine and migrating larval stages in somatic tissue. The eosinophils are potent effector cells associated with the parasite in the histological section as evidenced by Vardhani, 2003 [20]. In the present study, platelets did not show any correlation with the infection of *Ancylostoma* spp.

Table 1: Haematobio chemical Parameters of different groups on day 0^{th} and day 14^{th}

	Group I		Group II		Group III	
Parameters	**Day 0**	**Day 14**	**Day 0**	**Day 14**	**Day 0**	**Day 14**
Hb (g/dl)	9.38 ± 0.36^{a}	11.37 ± 0.30^{b}	9.13 ± 0.51^{a}	11.61 ± 0.53^{b}	8.78 ± 0.52^{a}	10.88 ± 0.47^{b}
PCV (%)	29.26 ± 0.99^{a}	36.43 ± 1.79^{b}	28.28 ± 1.33^{a}	34.78 ± 1.61^{b}	26.98 ± 1.51^{a}	33.02 ± 1.33^{b}
TEC (10^6/ml)	4.77 ± 0.18^{a}	5.21 ± 0.17^{b}	4.61 ± 0.20^{a}	5.24 ± 0.26^{b}	4.50 ± 0.25^{a}	4.97 ± 0.19^{b}
TLC (10^3/ml)	9.60±1.05	11.18±0.86	11.31±1.33	8.67±0.96	7.89±1.58	10.76±0.48
Neutrophil (%)	71.40 ± 2.50^{aA}	66.37 ± 1.99^{bA}	73.65 ± 3.50^{aB}	68.64 ± 1.87^{bB}	79.19 ± 2.18^{aC}	70.48 ± 2.17^{bC}
Lymphocyte (%)	19.91 ± 2.22^{a}	24.98 ± 1.82^{b}	17.94 ± 3.04^{a}	23.48 ± 1.56^{b}	14.54 ± 1.99^{a}	21.13 ± 1.79^{b}
Monocyte (%)	7.23±0.97	8.30±0.68	7.09±0.98	7.13±0.65	5.14±0.58	7.72±0.64

Eosinophil (%)	4.25±0.53^{a}	2.59±0.28^{b}	4.58±0.56^{a}	2.21±0.25^{b}	5.48±0.64^{a}	2.50±0.26^{b}
MCH (pg)	19.85±0.33^{a}	21.99±0.16^{b}	19.90±0.41^{a}	22.36±0.41^{b}	19.68±0.57^{a}	21.95±0.43^{b}
MCV (fl)	61.42±0.87^{a}	66.42±0.57^{b}	61.08±0.83^{a}	66.92±0.90^{b}	60.75±1.52^{a}	66.88±1.42^{b}
MCHC (g/dl)	31.93±0.21^{a}	33.33±0.19^{b}	32.57±0.54^{a}	33.38±0.29^{b}	31.48±0.34^{a}	32.81±0.29^{b}
Platelet (10^3/ml)	386.67±46.01	316.83±40.01	320.92±57.32	354.08±38.94	321.67±43.48	372.25±34.43
Biochemical						
Total Proteins (g/dl)	4.98±0.25^{a}	5.56±0.24^{b}	4.52±0.16^{a}	5.28±0.20^{b}	4.87±0.15^{a}	5.70±0.10^{b}
Albumin (g/dl)	1.90±0.12^{a}	2.34±0.14^{b}	1.85±0.10^{a}	2.26±0.08^{b}	1.99±0.08^{a}	2.33±0.08^{b}
Globulin (g/dl)	3.08±0.17^{a}	3.22±0.13^{b}	2.67±0.11^{a}	3.11±0.14^{b}	2.88±0.13^{a}	3.37±0.10^{b}
BUN (mg/dl)	10.78±0.69^{aA}	12.97±0.98^{bA}	8.25±0.99^{aB}	11.66±0.75^{bB}	11.71±1.47^{aC}	14.23±0.66^{bC}

The biochemical changes associated with ancylostomosis with respect to Total Protein (TP), Albumin (Alb.), Globulin (Glob.), and Blood Urea Nitrogen (BUN) were studied. Total Protein, Albumin and Globulin showed lower mean values on Day 0th in all three treatment groups. There was a significant difference observed in Total Protein, Albumin and Globulin on day 14th post-treatment (Table 1), showing an increase in mean values after treatment. The findings are similar to Jasmin *et al.* (2020) [10], Schmidt *et al.* (2016) [17], and Nwoha *et al.* (2012) [15] who reported similar changes in serum proteins. The lower values of serum total protein in the present study can be explained by the serum seepage around the site of attachment of *Ancylostoma* spp. in the intestine leading to the loss of blood proteins in the faecal matter. These findings could also be attributed to malnutrition caused by the presence of worms in the intestinal lumen.

Lower mean BUN values were observed in infected dogs on Day 0th which showed increasing trend post-treatment on day 14th. The finding in the present study could be explained by the low dietary intake of proteins due to anorexia and reduced absorption or malabsorption of proteins from the intestines, resulting in low protein metabolism in the body and thus low BUN production. Additionally, the decreased BUN could be attributed to lower production in the damaged liver, due to the damage caused by the migrating *Ancylostoma* spp. larvae in the liver.

Out of 51 *Ancylostoma* spp. positive dogs 36 dogs were randomly divided and studied in three treatment groups to investigate the therapeutic response of Pyrantel Pamoate, Fenbendazole and Doramectin in Group I, Group II and Group III, respectively. The treatment efficacy was studied in terms of reduction in EPG of faeces before and after treatment. In group I the mean ± S.E. EPG on day 0th was 1564.25±184.94 which became zero on day 7th and remained nil till day 15th post-treatment with Pyrantel Pamoate @ 10mg/kg body weight per-os once only (Table 2). The findings were similar to Ashraf *et al.*, 2008 [2] and Klein *et al.*, 1978, who reported 90-100% efficacy against *Ancylostoma caninum* in dogs. In group II the mean ± S.E. EPG on day 0th was 2283.67± 357.07 which became 0 on day 7th and 15th post-treatment with Fenbendazole @ 50mg/kg body weight per-os for three consecutive day (Table 2). The present study's findings are in accordance with Arle *et al.* (1992) [1] who reported 69.71%, 96.90% and 100% efficacy of fenbendazole on day 3rd, day 5th and day 7th post-treatment, respectively. Moreover, in group III the mean ± S.E. EPG on day 0th was 2097.42±309.34 which became 0 on day 7th and remained nil till day 15th post-treatment (Table 2.) with Doramectin @ 0.04mg/kg body weight subcutaneously, once only. Similar to Shakya *et al.* (2013) who reported 100% efficacy of the Doramectin as 1% doramectin (dose @ 0.2mg per kg body weight) 2 injection at weekly intervals.

Table 2: Eggs per gram of faeces in different groups on 0th, 7th and 15th day

	Day 0	Day 7	Day 15
Group-I (Pyrantel Pamoate)	1564.25±184.94^{a}	0±0^{b}	0±0^{b}
Group-II (Fenbendazole)	2283.67±357.07^{a}	0±0^{b}	0±0^{b}
Group-III (Doramectin)	2097.42±309.34^{a}	0±0^{b}	0±0^{b}

Conclusion

In the present study, major haematobiochemical changes observed in dogs with ancylostomosis are anaemia, hypoproteinaemia, or hypalbuminaemia. The study revealed that Pyrantel pamoate, Fenbendazole, and Doramectin, have 100 percent therapeutic efficacy while treating cases of ancylostomosis. Hence, these drugs should be used alternatively to treat ancylostomosis to prevent the development of anthelmintic resistance.

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