Comparative efficacy of ivermectin and fenbendazole against ancylostomiasis in dogs

Rojali Chandra Bhanjadeo
Orissa University of Agriculture and Technology: Odisha University of Agriculture and Technology
Ramesh Chandra Patra (rcpatra@gmail.com)
College of Veterinary Science and Animal Husbandry
Dibya Panda
Orissa University of Agriculture and Technology: Odisha University of Agriculture and Technology
Debiprasanna Das
Orissa University of Agriculture and Technology: Odisha University of Agriculture and Technology

Research Article

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Abstract

The present experiment was carried ut to assess the comparative efficacy of ivermectin and fenbendazole individually for anthelmintic therapy for the hookworm infested dogs. Dogs presented to the Department of Veterinary Clinical Medicine or Veterinary Clinical Complex, Bhubaneswar were randomly screened for *Ancylostoma caninum* infection and the positive dogs were selected for the therapeutic trial Faecal samples were collected randomly from presented dogs immediately after defaecation or from the rectum directly using a faecal scoop. The collected sample was examined by floatation technique to detect the positive cases of *Ancylostoma caninum* infection. The dogs with normal clinical parameters and no eggs or ova in the faeces were included in in group 1 (n=12). Dogs with faecal sample positive for *Ancylostoma caninum* ova were recruited for the comparative study (n=24) which were grouped into two groups consisting of 12 dogs in each (group 2 and 3). Group 2 dogs (n=12) were treated with ivermectin at 200 µg/kg body weight once orally repeated after 15 days with proper supportive therapy each time. Group 3 (n=12) were treated with fenbendazole at 50 mg/kg body weight once orally repeated after 15 days with proper supportive therapy each time.Haematological examinations and serum biochemical tests were carried out in all groups each time on day 0, 15 and 30 of the experiment. The therapeutic efficacy of both the drugs was calculated on the basis of number of animals found free of *Ancylostoma* infection as determined by faecal sample examination and reduction in EPG count of the faeces of the group following the treatment. The reduction in eggs per gram (EPG) count on day 15 and day 30 was more significant in group 2 than group 3. The mean EPG count in fenbendazole-treated dogs reduced significantly to 24.17 $\pm$ 11.44 on day 15 from day 0 level of 1650.00 $\pm$ 247.25. The value again reduced from day 0 to become nil on day 30 .The 15th day after treatment, mean ± SE values of protein, albumin and globulin were changed to 5.63 ± 0.12, 2.64 ± 0.12 and 2.99 ± 0.15 g/dl, respectively. The 30th day after treatment the values were 6.23 ± 0.14, 3.20 ± 0.18 and 3.03 ± 0.21 g/dl, respectively. The total protein and albumin values were significantly changed by 15th day and 30th day at 1% level of significance as compared to that of day 0 in group 2 and 3 respectively.Following treatment with ivermectin, the 15^{th} day haematological values increased significantly at 1% level ($P<0.01$) of significance. There was significant increase in the values at 1% level on the 30^{th} day compared to day 0. The mean values were non-significantly comparable to the healthy control group except PCV and TEC on the 30^{th} day.

Introduction

Tropical climate with high rainfall make the animals susceptible to diverse intestinal helminthes. Frequent infections by one or more species of endoparasites can cause high morbidity, weight loss, anaemia and lowered immune status. Among all helminthes infestations, *Ancylostoma caninum* is the most common and dangerous parasite in dogs which has global importance (Vaughan & Murphy, 1962). This hookworm affects dogs of almost all age groups. However, it is life threatening for very young pups (Soulsby, 1982). The worm gets entry into the pups through trans-mammary route (Miller, 1965) or into the adults through skin penetration. Ancylostomiasis causes severe haemorrhagic gastroenteritis leading

to severe iron deficient microcytic hypochromic anaemia (Layrisse, 1961). The adult worms attach to and feed on the intestinal mucosa causing intestinal haemorrhages (Georgi, 1968), presence of blood in faecal matter, severe anaemia and intestinal ulceration. The measured daily loss of blood is reported to be 0.013 ml per adult male worm and 0.043 ml per female adult worm (Wang et al., 1983). Hence, more severe blood loss is caused by the female adult worms. The blood picture reveals varying degree of anaemia and hypoproteinaemia (Miller, 1974). The incidence of this disease is more in the stray dogs (Arle et al., 1992). The disease has zoonotic importance too as hook worm of dog causes cutaneous larva migrans in humans.

Panigrahi et al. (2014) studied the presence of gastrointestinal helminthic parasites in clinically normal dogs in Bhubaneswar, Odisha and reported the incidence of ancylostomiasis to be the second highest after the mixed parasitic infection in stray dogs. However, routine deworming practice at regular intervals can minimize the ill health effects in dogs and reduce public health concerns.

Many dog owners are unaware of mode of transmission and severity of the disease both in dogs and humans. Hence, they don't commonly follow strict deworming protocol. Various drugs like ivermectin, pyrantel pamoate, praziquintal, fenbendazole and other combination of anthelmintics are being used in India for the treatment of endoparasitic infestations in dogs. Studies have revealed high level of pyrantel resistance in canine ancylostomiasis (Kopp et al., 2008). Therefore, the present study was aimed at assessing the comparative efficacy of ivermectin and fenbendazole individually to ascertain the best anthelmintic therapy for the hookworm infected dogs.

Materials And Methods

The present study was conducted on dogs presented to the Department of Veterinary Clinical Medicine or Veterinary Clinical Complex, Bhubaneswar. The presented dogs were randomly screened for *Ancylostoma caninum* infection and the positive dogs were selected for the present study.

Faecal sample

Faecal samples were collected randomly from presented dogs immediately after defaecation or from the rectum directly using a faecal scoop. The collected sample was examined by floatation technique to detect the positive cases of *Ancylostoma caninum* infection. Identification was made according to the morphological characters of the eggs.

Experimental protocol: The dogs with normal clinical parameters and no eggs or ova in the faeces were included in in group 1 (n=12). Dogs with faecal sample positive for *Ancylostoma caninum* ova were recruited for the comparative study (n=24) which were grouped into two groups consisting of 12 dogs in each (group 2 and 3). Group 2 dogs (n=12) were treated with ivermectin at the dose rate of 200 µg/kg body weight once orally repeated after 15 days with proper supportive therapy each time. Group 3 (n=12) were treated with fenbendazole at 50 mg/kg body weight once orally repeated after 15 days with proper supportive therapy each time.

Hookworm positive samples were then analyzed by Mc Master Technique for EPG (Eggs per Gram) count before treatment (day 0) and thereafter on 15^{th} and 30^{th} day post treatment.

Haematological examinations and serum biochemical tests were carried out in all groups each time on day 0, 15 and 30 of the experiment, and the dog owners were advised to keep the dogs indoors preferably under hygienic management conditions.

Qualitative analysis of faecal sample

Microscopic examination: The samples were examined microscopically adopting qualitative techniques using flotation method for detection of parasitic material (Soulsby, 1982).

Direct smear: A pinch of faecal sample was placed on one end of a slide and mixed with a drop of water. After mixing and spreading, smears were covered with cover slip and examined directly under microscope. At least 3 slides from different parts of the faecal sample were examined.

Flotation method: In this method, saturated salt or sugar solution was used to prepare the faecal suspension. Faecal sample (1-2 g) was taken in a mortar and pestle and triturated after adding a little quantity of saturated salt or sugar solution. Then the faecal suspension was allowed to settle for twenty minutes. After this from The superficial layer (10 – 15 ml) was collected in a test tube and a clean cover slip was placed on the top of the tube in such a way that the upper meniscus of the faecal suspension present in the test tube touches the lower surface of the cover slip. The test tube was then allowed to stand undisturbed for twenty minutes after which the cover slip was lifted gently and placed over a glass slide for microscopic examination under 10X and 40X.

Quantitative analysis

Positive faecal samples were examined by quantitative technique (McMaster counting method) to determine the parasitic load and the load was expressed as eggs per gram (EPG) of faeces.

McMaster counting method: In the McMaster egg counting technique, the eggs are floated up in a counting chamber. The counting chamber is made of two glass slides, separated by three or four narrow, transversely placed strips of glass 1.5mm thick, so that two or three spaces of 1.5mm depth are obtained between the two slides. On the under surface of the upper slide an area of 1cm square is ruled over each space. The volume underneath this ruled area will therefore be 0.15ml. From the faeces, 2 gm of sample was weighed and soaked in 30 ml of water until it was sufficiently soft. To the vessel, 30 ml of saturated salt solution was added. After thorough shaking, a small volume of sample was withdrawn by means of wide (8mm) pipette and run at the counting chamber, filling all the spaces. The eggs floated to the under surface of the upper slide, so they were all in focus under the ruled area. The number of eggs within each ruled area, multiplied by 200 represents the number per gram of the original sample.

Haematological studies

Blood sample (2 ml) was collected from saphenous or cephalic vein of dogs in EDTA coated vial for haematological examinations. Haemoglobin was estimated by Sahli's acid haematin method using N/10 HCl using Haemoglobinometer. The haemoglobin concentration was expressed in g/dl.PCV (Packed cell volume) was estimated by Microhematocrit method. The Wintrobe haematocrit tube was filled with EDTA containing blood and centrifuged for 40 min at 2500 rpm. Total Leukocyte Count (TLC) was estimated using hemocytometer method and the values were expressed /µl of blood. Similarly, Total Erythrocyte Count (TEC) was done by Hemocytometer method and the values were expressed in 10^6/ mm^3. Differential Leukocyte Count (DLC) was estimated after staining thin blood smear on a glass slide with diluted Giemsa stain. Hundred cells were counted in high power oil immersion and different white blood cells were calculated basing on their morphology. The values were expressed as percentage of cells. The erythrocytic indices such as MCV (Mean Corpuscular volume), MCH ((Mean Corpuscular Haemoglobin) and MCHC (Mean Corpuscular Haemoglobin Concentration) were calculated.

Biochemical studies

Another 3 ml of blood sample was collected in clot activator vials on day 0 and thereafter on day 15 and 30 post treatment for harvesting serum. Total serum protein levels were determined by Biuret method using colorimeter and Biuret kit manufactured by Coral clinical systems. Serum albumin was determined by BCG (Bromo Cresol Green) method using colorimeter and BCG kit manufactured by Coral Clinical Systems. The serum globulin was estimated by subtracting serum albumin from serum total protein.

Therapeutic efficacy

The therapeutic efficacy of both the drugs was calculated on the basis of number of animals found free of *Ancylostoma* infection as determined by faecal sample examination and reduction in EPG count of the faeces of the group following the treatment by applying the following formula:

$$\% \text{ efficacy of drug} = \frac{\text{Mean EPG before treatment - Mean EPG after treatment}}{\text{Mean EPG of the group before treatment}} \times 100$$

Statistical analysis

The data collected during the present study in respect of different parameters were statistically analysed using SPSS.

Results

The common symptoms recorded in the dogs positive for hookworms were diarrhoea, red or black colour faeces, pale mucous membrane, vomition, inappetence, anorexia, dermatitis. The mean (±SE) haematological values of the healthy control and both the therapeutic groups on day 0, 15 and 30 of the experiment are given in table 1. The hematological parameters (mean ± S.E) in ivermectin-treated dogs (Group 2; n=12) are depicted in table 2. The day 0 mean ± S.E of Hb (g/dl), PCV (%), TEC (×10^6/µl) were

7.32 ± 0.37, 27.33 ± 0.98, and 4.13 ±0.18, respectively. However following treatment, the 15th day values increased significantly at 1% level (P<0.01) of significance to reach 10.00 ± 0.37, 32.75 ± 1.06 and 4.95± 0.22, respectively. There was significant increase in the mean values at 1% level in group 2 on the 30th day compared to day 0. The mean values were non-significantly comparable to the healthy control group except PCV and TEC on the 30th day. The day 0 mean (±S.E.) level of Hb (g/dl), PCV (%) and TEC ($\times 10^6$/µl) in group 3 dogs treated with fenbendazole were 9.68 ± 0.28, 31.92 ± 0.87 and 4.82 ± 0.13, respectively. The MCV, MCH, and MCHC value on day 0 were 66.38 ± 1.70, 20.13 ± 0.60 and 30.55 ± 1.17, respectively. However the treatment with fenbendazole on first day, the values of PCV and TEC increased significantly at 5% level to reach 35.25 ± 0.93 and 5.21 ± 0.14 on day 15 but the Hb, MCV, MCH, MCHC values did not increase significantly. The values of Hb, PCV, TEC on day 30 increased significantly from day 0 at 5% level. The TLC values were 11625 ± 962.25 on day 0, 11466 ± 586.76 on day 15 and 11466 ± 586.76 on day 30. This did not show significant variation statistically over the periods.

The erythrocytic indices such as MCV (fl), MCH (pg), MCHC (g/dl) on day 0 were 66.87± 2.49, 18.11± 1.18 and 27.03±1.37, respectively in group 2 dogs. The mean values were changed to 66.92 ± 2.18, 20.58 ± 1.08 and 30.70 ± 1.07, respectively on 15th day after treatment with ivermectin. On 30th day, the values increased to 67.09± 1.99, 21.59 ± 0.93 and 32.12 ± 0.84, respectively. There was no significant change in MCV noted on 15th and 30th day after treatment compared to day 0 mean value. The MCH on day 0 was below reference range and on 15th and 30th day after treatment it was improved significantly at 5% level of significance. The MCHC values also increased significantly from day 0 to day 15 and day 30. The mean ± SE of TLC (/µl) on 0 day, 15th and 30th day after treatment were 11791 ± 918.80, 11550 ± 667.02 and 11725 ± 482.75, respectively in group 2 dogs. There was apparent decrease in TLC from day 0 by day 15 and apparent increase from day 15 to day 30. The change was not significant at 5% level of significance. The neutrophil (/µl) count on day 0, 15 and 30 were 61.83 ± 1.99, 62.42 ± 1.50 and 62.75 ± 0.96, respectively in group 2 dogs. There was absolute neutrophilia throughout the treatment but it was not significant statistically at 5% level of significance. The lymphocyte count did not change significantly. The values remained almost in a constant range throughout the periods. The eosinophil count reduced significantly at 1% level from 6.83 ± 0.53 on day 0 to 3.58 ± 0.48 on day 15 and 3.07 ± 0.26 on day 30.

The biochemical parameters of healthy control group and therapeutic groups are given in Table 3. On day 0, the mean (± SE) of total protein (g/dl), albumin (g/dl) and globulin (g/dl) in negative control group were 6.39 ± 0.20, 3.21 ± 0.17 and 2.93 ± 0.27, respectively. Non-significantly (P<0.05) similar values for all the parameters were observed on 15 and 30 day of the experiment. The 0 day values of total protein (g/dl), albumin (g/dl) and globulin (g/dl) in hook worm positive group 2 dogs were 5.01 ± 0.12, 2.25 ± 0.15 and 2.75 ± 0.25 g/dl, respectively. The 15th day after treatment with ivermectin, mean ± SE values changed to 5.63 ± 0.12, 2.64 ± 0.12 and 2.99 ± 0.15 g/dl, respectively. The 30th day after treatment, the values were 6.23 ± 0.14, 3.20 ± 0.18 and 3.03 ± 0.21 g/dl, respectively. The total protein and albumin values were significantly changed by 15th day and 30th day at 1% level of significance as compared to that of day 0. On day 0, the mean total protein, albumin, globulin in group 3 dogs were 4.94 ± 0.27, 2.48 ± 0.17 and 2.45 ± 0.21, respectively (Table 3). On 15th day after treatment with fenbendazole, the values increased to 5.00

± 0.26, 2.51 ± 0.19 and 2.48 ± 0.21, respectively. On 30th day, the values varied to reach 5.05 ± 0.28, 2.50 ± 0.20 and 2.55 ± 0.20, respectively.

The mean EPG counts in infected dogs were observed to be high (1725 ± 331.23 and 1650 ± 247.25 in group 2 and 3, respectively) before treatment which drastically fell down following treatment. The reduction in EPG count on day 15 and day 30 was more significant in group 2 than group 3. Eggs per gram of faeces (EPG) after treatment with ivermectin significantly reduced to 0 on day 15 and day 30 from the mean count of 1725 ± 331.23 on day 0 (Table 4).The mean EPG count in fenbendazole-treated dogs reduced significantly to 24.17 ± 11.44 on day 15 from day 0 level of 1650 ± 247.25. The value further reduced to nil on day 30 (Table 4).

Discussion

Ancylostomiasis is a very serious nematode disease in the dogs which exerts deleterious effects and cause very serious health hazards resulting in generalized ill health due to loss of blood and body tissues, lowered body resistance to other infectious diseases, reduced efficiency etc. The haematological parameters like haemoglobin, PCV and TEC in the infected groups 2 and 3 were significantly lower compared to day 0 mean level in group 1. This may be due to the progression of infection by *Ancylostoma caninum.* The worms attach to the small intestinal tissues and suck blood voraciously causing ulcerations and bleeding into intestinal cavity. Hookworms are capable of removing as much as 0.097 ml of blood from patients infested with hookworms (Areekul *et al*., 1970) resulting in anaemic state with lowered levels of haemoglobin, PCV and TEC on day 0 and indicating hemorrhagic anaemia. In group 1, there was no significant change in haematological values between day 0 and day 15 or 30. However, in group 2 and 3, the haemoglobin, PCV and TEC were seen to be below normal level on day 0. There was improvement in mean levels in subsequent observations in these two therapeutic groups. Certain degree of anaemia has earlier been reported in ancylostomiasis as evidenced by decreased RBC (3.95 + 1.24 m/c.mm), Hb (6.3 ± 2.5 g/dl) and PCV (20.3 ± 8.8%) (Ramakrishnan et al., 1972) In very severe cases, haemoglobin was as low as 2.5 g/dl in pups. Mitra and Sasmal (1985) also demonstrated haematological changes in pups experimentally infected with 500 infective larva of *Ancylostoma caninum* by different routes on day 21, and all the erythrocytic values reduced correspondingly with the age of infection. Srivastava et al. (1988) reported that hookworm infestation leads to reduction in blood Hb, PCV and TEC levels, and these parameters return to normal after deparasitization. Udonsi and Agunama (1991) reported progressive decreases in both PCV and Hb in 20 puppies of 8 week age with negative correlation noted between these parameters and faecal egg counts. Kalkofen (1987) reported Hb levels commonly below 10 mg/dl and TEC lower than 4 million/cu mm from 14th D.P.I onwards and concluded hook worm infection to be synonymous with iron deficiency anaemia. The parasitized animals had significantly ($P < 0.01$) lower levels of haemoglobin, packed cell volume and total erythrocyte counts than non-parasitized animals (Qadir et al., 2011). In the present study, the values of haemoglobin, PCV and TEC gradually increased significantly from day 0 mean level to day 15 and 30 subsequent to treatment with anthelmintic and haematinics.

The erythrocytic indices such as MCV, MCH, and MCHC in group 1 on day 0, 15 and 30 were within normal range as described for dogs i.e 30–36 g/dl. The mean values were significantly ($P < 0.01$) lower on day 0 in group 2 and 3.. The drastic reduction in MCH and MCHC values in hook worm infected dogs indicates the decrease in mean Hb content. On day 15 and 30, the MCH and MCHC values increased significantly from day 0 in treatment group 2 whereas they did not vary significantly in treatment group 3. The MCV value also changed significantly ($P < 0.01$) in group 2 dogs treated with ivermectin but the changes were apparent in group 3. There was moderate eosinophilia in both the infected groups. Migasena et al. (1971) reported eosinophilia in all hookworm infected dogs irrespective of the severity of infection. Meeusen and Balic (2000) also documented eosinophilia as a prominent leukocytic response to hookworm infection, because of the reason that they attack and kill the infective larvae. Ogunkoya et al. (2006) demonstrated eosinophilia as the most common in dogs infected with gastrointestinal parasites. The increase in eosinophil count in hookworm infection may be due to allergic reactions to proteins or to metabolic products of the worm. The hookworm infested patients are repeatedly being sensitized by antigens present in the metabolic products or arising from the bone marrow parenchyma into the sinusoids, thereby inducing eosinophilia in the peripheral blood (Hiraki and Inoue, 1959). There was significant fall in eosinophil count in group 2 and group 3 after treatment on day 15 and 30.

The mean level of protein, albumin and globulin was significantly lower in the infected population on day 0 compared to group 1. In group 2, the total protein and albumin values increased significantly after treatment at 5% level of significance compared to 0 day value but globulin level did not improve significantly. Sushma Rachel and Suryanarayana (2001) recorded the total serum protein in dogs with Ancylostomiasis as 5.47 ± 0.09 and 6.89 ± 0.07 g/dl before and after therapy respectively. Nwoha et al. (2013) reported decreased levels of albumin (hypoalbuminaemia) in dogs experimentally infected with *Ancylostoma caninum.* The reduction in total serum protein might be due to chronic internal haemorrhage during worm infestation and loss of serum via exudation or leakage in to the lumen of gut causing enteropathy. The altered rate of intestinal absorption of nutrients especially protein from the ulcerated intestinal tract during infection might have also contributed to the drop in the total serum protein (Dargie and Allonby, 1975). Hypoproteinemia may also be attributed to the interference with the efficacy of digestion and absorption by damaged intestinal mucosa and diarrhoea due to mechanical obstruction and irritation (Vanbeers et al. 1983) Miller (1974) revealed that *A. caninum* infection in dogs caused varying degree of anaemia and hypoproteinemia.

In the present study, observing the significant changes in haematological and serological parameters, ivermectin was seen to be 100% effective as anthelmintic at oral doses of 200 µg/kg body weight. Ivermectin is a derivative of avermectin B1, one of the naturally occurring substances produced by *Actinomycetes*, *Streptomyces avermectins* (Wang et al., 1989). It removed all the adult worms in its primary dose bringing down the faecal count to 0 on day 15th and 30th day from a mean of 1725.00 ± 331.23 on day 0. Ramisz (1984) recorded ivermectin as 97–100% effective against *Ancylostoma* spp. when given at a dose rate of 200 µg/kg body weight in canines. Bagherwal (1992) in a study on the efficacy of single dose of ivermectin against *A. caninum* at the rate of 0.1 mg/kg body weight subcutaneously in 48 domesticated dogs of either sex aged between 3 month to six years reported that

out of 48 treated dogs, 29 (60.5%) were found negative for *A. caninum* eggs on 3rd day post treatment while 13 (27%) and 6 (12.5%) dogs got cured by 5th and 7th day post treatment, respectively and concluded that ivermectin was 100% effective against *A. caninum* in dogs without any side effects. Chhabra et al. (2001) reported complete elimination of *Ancylostoma caninum* in dogs treated with single dose of ivermectin at 200 µg/kg body weight one week after its administration. Daurio et al. (1993) reported the efficacy of chewable formulation of ivermectin to be 52%, 98%, 95% and 97% at 6, 12, 18 and 24 µg/kg body weights respectively in 35 young dogs with induced infections of *A. caninum* and *U. stenocephala* .

The Fenbendazole treated group was not able to remove all the parasites in one oral dose of 50 mg/kg body weight. But as observed, treatment with fenbendazole at the dose rate of 50mg/kg body weight was efficacious only on day 30. In group 3, the EPG count was 1650.00 ± 247.25 on day 0 which significantly reduced at 5% level of significance to reach 24.17 ± 11.44 on day 15 and further reduced to reach nil on day 30. Singh et al. (1977) conducted postmortem of 22 dogs 14 days after treatment with different anthelmintics and reported that 6 of those treated with 50 mg/kg fenbendazole eliminated 71% of hookworms. Bruke *et al.* (1978) used 10% suspension of fenbendazole against experimental infections of *Toxocara canis* and *Ancylostoma caninum* in Beagle pups at a dose level of 50 mg/kg Body weight for 3 days and obtained 93% efficacy against hookworms. The use of fenbendazole @ 50 mg/kg body weight led to disappearance of previously diagnosed *Ancylostoma caninum* eggs at 2 weeks post treatment (Dakhly and Soliman, 2008). The mean EPG count on day15th in this study became 24.17 ± 11.44. It may be due to the entrance of parasites from the dormant tissue to the intestinal lumen and/or the production of eggs by the female worms may be erratic in moderate to low worm burden (Kopp et al., 2008).

Ivermectin is a chemical derivative of naturally occurring fermentation product, avermectin B, which now bears the non-proprietary name abamectin. This substance is one of a series of compound avermitilis, which was originally isolated from soil in Japan. It kills the parasite by opening glutamate gated chloride channels and increasing Cl- conductance, by binding to allosteric site on the acetylcholine nicotinic receptor to cause an increase in transmission, leading to motor paralysis or by binding to aminobutyric acid receptor. It is similar to the macrolide antibiotic, but is virtually devoid of antibacterial activity. The ivermectin is active against arrested and developing larvae and adults of important nematode, including those showing resistance to existing anthelmintic families. In nematodes, the neurotransmitter that sends inhibitory signals from inter neurons to motor neurons is Gama aminobutyric acid (GABA). A number of studies have shown that avermectin potentiates the inhibitory effect of GABA. Signals from CNS are therefore not received by motor neurons and a state of flaccid paralysis of parasite develops (Jones, 1984).

Fenbendazole is a broad spectrum antiparasitic drug. It belongs to benzimidazole group which acts by binding to tubulin, an essential structural protein of the microtubules. This blocks the microtubules in the worms so that the uptake of glucose is blocked which eventually depletes the glycogen reserves. This disrupts the energy management system of the worms resulting in paralysis and leading to death of the worms.

Declarations

Ethical Approval

The research work was conducted in clinical cases presented to Veterinary Clinical Complex with the consent of the owners to publish the research outcome after use of anthelmentic and supportive therapy in affected dogs.

Competing interests : No conflicts of interest

(always applicable and includes interests of a financial or personal nature)

Authors' contributions (applicable for submissions with multiple authors)

Rojali Bhanjadeo: Project execution, Data analysis, writing

Patra, R. C: Planning of project, supervision, data analysis and editing of manuscript

Dibya Panda: Writing

Das, D. P: Planning of project, Co-supervision, data analysis

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If any of these declarations listed are not relevant to the content of your submission please state that this declaration is “not applicable”.

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Tables

Table 1: Clinical signs of hookworm infection in presented dogs

Sl. No.	History, Clinical signs and symptoms
1	Vomition, soft reddish stool, History of fever, Temperature-102^0 F mm-normal
2	bloody stool, anorexia, Depression, ticks over body, Stunted growth, MM-Pale
3	Anorexia, Constipation, History of fever, No deworming and vaccination done, Temperature-101.5^0 F, MM- Pale.
4	Anorexia, Vomition, Pica, Depression, Temperature- 103.3^0F, MM- Pale.
5	Animal brought for vaccination, MM- slightly Pale,Temperature- 100^0F
6	Vomition, History of Fever, Black colour feces, Temperature- 1040F
7	Worms in feces, Inappetance, Pica, Temperature- 102.6^0F, MM- Slightly Pale
8	Anorexia, Vomition, Bloody Diarrhoea, Temperature- 102.3^0F, MM- Slightly Pale
9	Vomition, Diarrhoea, Depression, Inappetance, MM- Slightly Pale.
10	Vaccination request by owner
11	Black colour Feces, Inappetance, Tick infestation, Temperature- 100.8^0 F, MM- Slightly Pale.
12	Pica, Black colour feces, Temperature- 102.2^0F, MM- Slightly Pale.
13	Black colour Feces, Inappetance, No Vaccination and Deworming, Temperature- 101.2^0F, MM-Pale
14	Pica, faecal consistency hard, Temperature- 101.7^0 F, MM- Normal.
15	Inappetence, itching skin lesions over body, patchy hair loss, MM- pale
16	Owner Came for Vaccination, Black colour Feces, MM- Slightly Pale.
17	Inappetance, Reduced Water intake, Defecation Normal, No Vomition, Temperature- 102^0F, MM- Pale.
18	Inappetance, Vomition, Black Colour Feces, Deworming not Done, Temperature- 98.40F
19	Inappetance, Vomition, Black colour feces, No Deworming, Temperature- 1020F, MM- Slightly Pale.
20	Hair fall, No deworming and vaccination, Pica, Black colour feces, Temperature- 1000F, MM-Slightly Pale.
21	Vomition, Diarrhoea, No Vaccination and Deworming, Temperature- 1010F. MM- Normal.
22	Inappetance, Ascietes, Black Scanty Feces, Vomition, Temperature-1020F, MM- Slightly Pale.
23	Vomitiom, Lethargy, Black Colour Feces, Temperature- 1020F, MM- Congested.
24	Pica, diarrhoea, No deworming, Temperature- 1020F, MM- Normal

Table 2: Haematological parameters (Mean ± S.E.) in healthy controls (N=12) and treatment groups before treatment and thereafter on day 15 and 30

Parameters	Group	Observation Day		
		0	15	30
Hb (g/dl)	1	12.26 ± 0.42^{b}	12.22 ± 0.39^{b}	12.26 ± 0.40^{b}
	2	7.32 ± 0.37^{a}	10.00 ± 0.37*^{a}	12.06 ± 0.43*^{b}
	3	9.68 ± 0.28^{a}	10.43 ± 0.^{34ab}	10.92 ± 0.25*^{a}
PCV (%)	1	38.25 ± 1.34^{b}	38.00 ± 1.00^{b}	39.42 ± 1.50b
	2	27.33 ± 0.98^{a}	32.75 ± 1.06*a	37.58 ± 1.07*^{a}
	3	31.92 ± 0.87^{a}	35.25 ± 0.93*^{ab}	37.33 ± 1.32*^{a}
TEC (x10^{6}/µl)	1	5.92 ± 0.12^{b}	5.84 ± 0.13b	5.94 ± 0.18^{b}
	2	4.13 ± 0.18^{a}	4.95 ± 0.22*^{a}	5.64 ± 0.19*^{a}
	3	4.82 ± 0.13a	5.21 ± 0.14*^{ab}	5.69 ± 0.19*^{ab}
MCV (fl)	1	64.63 ± 2.03a	65.76 ± 2.32^{a}	66.49 ± 1.95^{a}
	2	66.87 ± 2.49^{a}	66.92 ± 2.18^{a}	67.09 ± 1.99^{a}
	3	66.38 ± 1.70^{a}	65.49 ± 1.64^{a}	65.83 ± 1.79^{a}
MCH (pg)	1	20.72 ± 0.64^{a}	21.05 ± 0.84^{b}	20.73 ± 0.63^{a}
	2	18.11 ± 1.18^{a}	20.58 ± 1.08*^{ab}	21.59 ± 0.93*^{b}
	3	20.13 ± 0.60^{a}	20.03 ± 0.60^{a}	19.32 ± 0.46^{a}
MCHC (g/dl)	1	32.07 ± 0.21^{b}	32.12 ± 0.34^{b}	31.20 ± 0.45^{ab}

	2	27.03 ± 1.37^{a}	30.70 ± 1.07*^{a}	32.12 ± 0.84*^{b}
	3	30.55 ± 1.17^{ab}	30.02 ± 1.29^{a}	29.57 ± 1.03^{a}
TLC (/µl)	1	11308 ± 444.06^{a}	11325 ± 376.61^{a}	11233 ± 349.74^{a}
	2	11791 ± 918.80^{a}	11550 ± 667.02^{a}	11725 ± 482.75^{a}
	3	11625 ± 962.25^{a}	11466 ± 586.76^{a}	11466 ± 586.76^{a}
Neutrophils (%)	1	63.08 ± 1.24^{a}	63.00 ± 1.08^{a}	63.17 ± 1.06^{a}
	2	61.83 ± 1.99^{a}	62.42 ± 1.50^{a}	62.75 ± 0.96^{a}
	3	64.00 ± 1.79^{a}	61.92 ± 1.28^{a}	62.25 ± 1.21^{a}
Eosinophils (%)	1	3.75 ± 0.53^{a}	4.00 ± 0.40^{a}	4.50 ± 0.48^{b}
	2	6.83± 0.53^{b}	3.58± 0.48*^{b}	3.07± 0.26*^{b}
	3	5.42 ± 0.63^{b}	3.75 ± 0.39*^{b}	2.83 ± 0.29*^{a}
Lymphocytes (%)	1	31.00 ± 1.51^{a}	30.83 ± 0.99^{a}	30.75 ± 0.88^{a}
	2	32.92 ± 2.36^{a}	33.92 ± 1.69^{a}	1. ± 1.36^{a}
	3	29.33 ± 1.94^{a}	32.25 ± 1.42^{a}	32.33 ± 1.79^{a}

*Significant compared to day 0 in a row. Mean value with similar superscript in a column within a parameter does not differ significantly (P≤0.05)

Table 3: Serum biochemical parameters (Mean ± S.E.) in healthy controls (n=12) and treatment groups before treatment and thereafter on day 15 and 30

Parameters	Group	Observation Day		
		0	15	30
Total protein (g/dl)	1	6.39 ± 0.20^{b}	6.29 ± 0.21^{b}	6.30 ± 0.20^{b}
	2	5.01 ± 0.12^{a}	$5.63 \pm 0.12^{*a}$	$6.23 \pm 0.14^{*b}$
	3	4.94 ± 0.27^{a}	5.00 ± 0.26^{a}	5.05 ± 0.28^{a}
Albumin (g/dl)	1	3.21 ± 0.17^{b}	3.13 ± 0.17^{b}	3.17 ± 0.20^{b}
	2	2.25 ± 0.15^{a}	$2.64 \pm 0.12^{*a}$	$3.20 \pm 0.18^{*b}$
	3	2.48 ± 0.17^{a}	2.51 ± 0.19^{a}	2.50 ± 0.20^{a}
Globulin (g/dl)	1	2.93 ± 0.27^{a}	3.07 ± 0.26^{a}	3.13 ± 0.33^{b}
	2	2.75 ± 0.25^{a}	2.99 ± 0.15^{a}	3.03 ± 0.21^{b}
	3	2.45 ± 0.21^{a}	2.48 ± 0.21^{a}	2.55 ± 0.20^{a}

*Significant at 1% level of significance compared to day 0 in a row. Mean value with similar superscript in a column within a parameter does not differ significantly ($P \leq 0.05$)

Table 4: EPG (mean ± SE) in different groups on day 0, 15 and 30th of treatment

Groups	EPG Day 0	EPG Day 15	EPG Day 30
Group 1	0^{a}	0^{a}	0^{a}
Group 2	1725 ± 331.23^{b}	0^{a}	0^{a}
Group 3	1650 ± 247.25^{b}	24.17 ± 11.44^{b}	0^{a}

*values with different superscripts are significantly different