

COMPARATIVE EFFICACY OF ANCYLOL, IVOMEC, MEBENDAZOLE AND PIPERAZINE AGAINST *Ancylostoma caninum* IN EXPERIMENTALLY INFECTED PUPS

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ABSTRACT

The efficacy and side effects of single dose treatment at different dose regimen of four anthelmintics against Ancylostoma caninum in experimentally infected pups were evaluated and compared based on total worm count and egg per gram (epg) count. Ancylool at both normal (1 mg/kg/BW) and elevated dose level (1.5 ml/kg/BW) showed 93.15 % and 93.87% (based on worm count) and 93.13 and 93.75 % (based on epg count) respectively. Whereas Ivomec® (a brand of Ivermectin) at normal dose level (1 ml/50 kg) and elevated dose level (1.5 ml/50 kg) was found to be effective. The results were 79.48 % and 86.81% based on worm count and 89.44 % and 92.50 % based on epg count. Mebendazole and Piperazine even at elevated dose level was observed ineffective. Pups treated at normal and elevated dose level revealed acute toxicosis whereas those treated with Mebendazole showed cough and vomiting tendencies which later subsided and also, there was no risk involved in the administration of the drugs. Statistical analysis showed that there was statistical difference ($P < 0.05$) in the efficacy of the drugs. However there was significant difference ($P < 0.05$) between % efficacy and dosage. There was also significant difference between ($P < 0.5$) between epg count and drugs. The use of various compounds against ancylostomiasis in dogs has been discussed.

Keywords: Anthelmintics, Efficacy, *Ancylostoma caninum*

INTRODUCTION

Ancylostomiasis (hookworm disease) is a disease of worldwide distribution. The most widespread of all hookworm species is *A. caninum* and it parasitizes dog throughout the tropics and subtropics. Due to its high prevalence and its zoonotic significance, *Ancylostoma caninum* has gained major importance in the field of veterinary as well as public health research. In recent years the realization that *A. caninum* can cause human gut disease has sparked off renewed interest in its study.

In the normal canine host, infection with *A. caninum* usually follows skin contact with L3 larvae that have developed in the soil. Invasive L3 larvae undergo either tracheal migration to reach the gut (leading to obvious infection) or somatic migration to invade and stay as reservoirs mainly in the skeletal muscle fibres. Heterogenous infections in animals and amphixenous anthroponosis are common causing apthous ulceration, eosinophilic enteritis (Prociw, 1998)

Many highly effective anthelmintics are available but such drugs must be used correctly to obtain favourable clinical response. The ideal anthelmintics should have a wide margin of safety, considerable activity against immature stages, easy to administer, inhibit re-infection and be compatible with other compound not require long withholding periods because of residue and be cost effective. To overcome the problem of hookworm infestation, the

anthelmintics with good efficacy and least toxic effects is desired.

The present study deals with the anthelmintic efficacy of Ancylool, Ivomec, Mebendazole and Piperazine against *A. caninum* infection in pups.

MATERIALS AND METHODS

100 g of stool from each of 3 infected dogs selected was mixed with 300 g of sterilized sand (hot air oven sterilized). This was kept in a moist chamber at 25 °C (Sen *et al.*, 1965). The culture solution with which the filter paper was kept wet and protected consisted of 0.01 % HCl and 0.2 % NaCl (Bhai and Pande, 1981). The culture was sprayed with mycostatin (Bhai and Pande, 1981) ten days later, the dish was tilted and about 0.5 ml of water which percolated was aspirated into test tubes.

Bearnan's apparatus (Cheesbrough, 1987) was used to isolate larvae that may remain in the filter paper. Larvae collected were further cleaned in sucrose gradient (400g sucrose in a litre of distilled water). The larvae were kept at 4 °C for few days before use (Banerjee *et al.*, 1970; Ikeme, 1976). Finally active larvae were collected by using muslin impregnated with 1.5 % agar (Warren, 1965).

Ninety six pups (mongrel breed) of about 4 weeks age were dewormed with Ivomec at 1 ml/50 kg BW for 2 days and divided into four groups each subdivided into 3 subgroups with each subgroup containing 6 pups.

For each experiment a separate infected control group without treatment was maintained. All the pups were infected orally with 300 larvae of *A. caninum*. On day 24 post infection, each was treated with different anthelmintics. Each of the subgroup was treated with different dosage of the anthelmintics. The treatment schedule followed in this experiment is shown in Table 1.

Two methods were adopted for therapeutic evaluation of the drugs. One of the methods was based on total egg count by Stools dilution method (Soulsby, 1982) and the other was based on total worm count (Georgi, 1969). The eggs per gram values of 96 pups were determined at pre-treatment and day 7, 14, 28 and 30 days post treatment. For the total worm count, the animals were sacrificed. Necropsy was performed according to Sen *et al* (1965). The gastrointestinal system of each pup was removed. The stomach, small intestine and large intestine were cut open lengthwise with a fine scissors and the content scrapped and washed into the separated containers and later strained through sieves. The sieves were spread out with the aid of needles and searched for worms visually and with handlens.

Drug efficacy was evaluated using the method described by USDVM thus: Efficacy = (Mean of the control - mean treated) x 100/ Mean control (Robinson *et al.*, 1976).

Data collected were analysed using descriptive statistics and analysis of variance (ANOVA) and F-LSD to indicate statistical significance ($P < 0.05$).

RESULTS

Efficacy of the drugs tested based on worm count and epg count were shown on Tables 1 and 2. Table 3 revealed that Ancylosol had the highest efficacy followed by Ivomec super especially at elevated and normal levels. Mebendazole and Piperazine were least efficacious even at elevated dose level. Ancylosol has the highest efficacy of 93.87 % and 93.15 % at elevated and normal dose levels respectively while at subdose level, efficacy of 84.31 % was obtained. Using Ivomec at elevated and normal dose levels, efficacy of 86.81 % and 79.48 % efficacy were obtained. At elevated and normal dose levels of Mebendazole efficacy of 67.39 % and 61.28 % were obtained. While at subdose level 44.44 % efficacy was obtained. Using piperazine, at elevated and normal levels 58.88 % and 52.58% were obtained. But at subdose level 42.51 % efficacy was obtained. Statistical analysis using ANOVA and F-LSD showed that there was statistical difference in the efficacy of the drugs ($P < 0.05$).

The result in Table 2, revealed efficacy of the based on epg count. From the result it could be seen that the highest efficacy were obtained at elevated dose level in the entire anthelmintic group as the case may be. Statistical analysis using ANOVA showed that there was significant difference ($P < 0.05$) between epg counts in Ancylosol treated group and control and also between Ivomec treated group

and control group. F-LSD for epg count and dose also showed significant difference ($P < 0.05$) between the treated groups and the control group; moreover, there was also a significant difference between epg count and days (periods) of the experiment.

DISCUSSION

From the result of the study, pups treated with Ancylosol had very few worms in their intestinal lumen showing efficacy of 93.2 % and 93.8 % for normal and elevated dose treatments respectively. Efficacy based on epg count was also high. However, faster respiration and depression were noticed immediately after drug was injected into the body. This lasted for about 30 minutes before normalcy returned. Tiwari and Bandopadhyay (1995) had earlier reported similar reactions in dogs treated with disophenol. Furthermore, in another study, Pneumarthy *et al.* (1995) reported acute toxicosis in pups injected disophenol subcutaneously at 0.8 ml/kg. Misraulia *et al.* (1989) reported Ancylosol poisoning in dog at a higher dose level. In their study, the symptoms of toxicity developed few minutes of post injection. Signs of acute toxicosis were not reported in an Irish wolf Hound breed given recommended dose of Ancylosol (Legendre, 1973). This may be attributed to the genetic difference among the different breeds of pups. Therefore, further research is needed in this area. Tiwari and Bandopadhyay (1995) reported a case of sub-acute/chronic toxicosis in a pedigree Doberman Picher dog aged 15 months suffering from acute hookworm infection and anaemia. According to their report, Ancylosol at 1 ml/kg subcutaneously produced after dew days oedematous swelling at the site of injection anorexia, vomiting, faster respiration and depression. The toxicosis observed in this study later subsided when a teaspoonful vitamin B12 was given orally.

Result from our study indicated that the group treated Ivomec at subdose level had efficacy of 53.5 % based on worm count and 88.3 % based on epg count. At a normal dose level, efficacy of 79.5 % based on worm count was obtained while at elevated dose level, 86.8 % efficacy was obtained. This implies that even at elevated dose level, efficacy with the normal dose are almost the same, therefore increasing the dose is merely a waste of resources as the difference was not statistically significant. In an earlier study, similar result in respect to efficacy of Ivomec at different dose levels has been reported by (Anderson and Robinson, 1982) for *Toxocara canis* infected Indian dogs. On the contrary, Ramiz (1984) obtained a lesser efficacy in Labrado breed infected with Ascarid worm. This really showed that efficacy of drugs can also depend on the breed of the animals and the brand of the drug used. From the result it could be seen that even Ivomec was not an effective drug against hookworm disease because a reasonable number of worms persist in the intestine after treatment. Incidentally it is sad to report that it is the common drug used in most Veterinary Clinics in Anambra State. No wonder why the disease persist despite the efforts made to eradicate it.

Table 1: Efficacy of Ancylool, Ivomec, Mebendazole and Piperazine against *A. caninum* infections in pups

Sub group	No of animals	Dose of drug used	Av. Worm recovered	% Worm established	% Efficacy
Ancylool treatments (ml/kg BW)					
1	6	0.5	32.33	10.77	84.31 a
2	6	1	15.00	3.00	93.15 a
3	6	1.5	7.67	2.55	93.87 a
Control	6	Water	90	30.00	
Ivomec treatments (ml/50 kg BW)					
1	6	0.5	43.33	16.55	44.44 c
2	6	1	18.67	11.44	61.28 b
3	6	1.5	12.00	9.55	67.39 b
Control	6	Water	90	30.00	0
Mebendazole treatments (mg/10 kg BW)					
1	6	0.5	51.33	17.11	42.51 c
2	6	1	42.67	14.22	52.58 c
3	6	1.5	37.00	12.33	58.58 c
Control	6	Water	90	30.00	0 d
Piperazine treatments (ml/kg BW)					
1	6	125	51.33	17.11	42.51 c
2	6	150	42.67	14.22	52.58 c
3	6	175	37.00	12.33	58.58 c
Control	6	Water	90	30.00	0 d

Unsimilar letters on the same column= significantly different means.

Table 2: Efficacy of anthelmintics against *A. caninum* infections in pups based on epg count

Sub group	No of animals	Pre-treatment period	7 dpt	14 dpt	21 dpt	30 dpt	% Efficacy
Ancylool treatments (ml/kg BW)							
1	6	340	250	130	79	31	91.83 a
2	6	338	185	92	51	18	95.13 a
3	6	350	150	88	48	12	96.75 a
Control	6	350	374	405	396	363	1.89 e
Ivomec treatments (ml/50 kg BW)							
1	6	320	280	121	88	42	88.33 b
2	6	342	205	96	66	38	89.44 b
3	6	356	186	92	45	27	92.50 a
Control	6	350	372	375	362	356	1.11 e
Mebendazole treatments (mg/10 kg BW)							
1	6	345	310	290	155	142	61.07 c
2	6	340	226	181	152	137	59.65 c
3	6	348	280	210	140	118	66.47 c
Control	6	350	354	356	350	345	1.10 e
Piperazine treatments (ml/kg BW)							
1	6	340	333	321	305	202	42.28 d
2	6	346	321	309	303	191	45.42 d
3	6	352	312	301	290	182	48.00 d
Control	6	350	352	354	350	348	0.60 e

Unsimilar letters on the same column= significantly different means.

On the group treated with Mebendazole, it was observed that few minutes after the administration of the drug all the pups showed signs of cough and vomiting tendency for about 30 minutes and later subsided. At normal dose level and even at an elevated dose level, the efficacy was low. However, some studies have shown that this may be due to strain variation, as different strains of *A. caninum* have variable degree of infectivity (Zenkov, 1971). Melhorn (1998) had earlier reported that Mebendazole was effective against *Echinococcus granulosus* with survival of few worms in the intestinal lumen of dog. Anderson (1975) reported 98.8 % efficacy of Mebendazole against nematodes of dogs and ruminants. Gemmel *et al* (1975) has also reported effectiveness of Mebendazole against

Moniezia expansa in dogs. Sangeeva and Suryanaraya (1995) reported that two dose levels of Mebendazole given 24 hrs apart at the dose rate of 50 mg/kg was 100 % effective in treating dog infected with *Dipylidium caninum*.

Kates *et al.* (1972) recorded an outstanding efficacy of Mebendazole against most intestinal nematodes and lung worms of sheep. This was confirmed by Armour *et al.* (1981) who recorded 99 % reduction in worm and burdens over a grazing season with a mean improvement in weight gain.

On the group treated with Piperazine adipate, there was 52.5 % efficacy at normal dose level. Those that receive sub dose treatment showed efficacy of 42.5 % while those of elevated dose level showed 58.6 %.

On necropsy, many worms were seen in the intestinal lumen. It was also observed that the liver of the pups were grossly damaged and adversely inflamed especially those treated at sub dose level. Pulmonary damage in the form of haemorrhagic pneumonia was also observed.

Signs and symptoms such as diarrhoea, rough skin coat, falling of furs, paleness of mucous membrane and weakness persisted after treatment. Similar result in an earlier study of Piperazine efficacy has been reported for *Toxocara* infection in Australian pups (English and Sprent, 1965). Robinson *et al* (1976) had reported 68 % efficacy against immature *Toxocara canis* in Australian pups dogs when given 150 mg/kg, whereas Shearer and Gemmelt (1969) suggested higher dose of 200 mg/kg for complete removal of mature worms. It has also been found to be highly effective against *Moniezia expansa* and *Thysaniezia giardi* in sheep, goat and cattle at 65 mg/kg b. wt (English and Sprent, 1965). However, it has been reported to be ineffective against *Stilesia hepatica* in calves (Genealor and Reinacke, 1970, Medherkar *et al.*, 1987; Gruss *et al.*, 1988). The ineffectiveness reported in Piperazine might be due to the drug selectivity to helminth species because from result of this study, Piperazine even at elevated dose level did not give encouraging result.

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