



Zoonotic Risk Assessment of Hookworm and *Toxocara spp.* Infection in Soil Samples and Evaluation of Efficacy of Commonly Used Anthelmintics in Dogs

B. P. Kendre, J. G. Gudewar*, H.Y. Palampalle, R. P. Naringrekar A. E. Chopde and D. A. Pawalkar²

¹Department of Veterinary Parasitology, Mumbai veterinary College, Mumbai, Maharashtra Animal & Fishery Sciences University INDIA

²Department of Veterinary Medicine, Mumbai veterinary College, Mumbai, Maharashtra Animal & Fishery Sciences University INDIA

*Corresponding Author: jagdishgudewar@gmail.com

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Abstract

In the present study efficacy of pyrantel and fenbendazole were studied in canines against hookworm infections. Therapeutic evaluation of anthelmintic showed 98.32% ± 0.73 efficacy for Pyrantel pamoate and 92.28% ± 3.54 efficacy for Fenbendazole against hookworms and can be utilised for the effective treatment of hookworm disease in canines. In the present study soil/sand samples were collected from twenty different sites such as parks (9) and beaches (11) in the Mumbai region showed 45.0% prevalence of helminthic eggs such as hookworm and Toxocara eggs. The present study has added significance as some of the gastrointestinal parasites, recorded in the present study are of public health importance. Interventions, those are need of this hour should focus on health education provided to dog owners, strategic deworming of dogs using broad spectrum anthelmintic and proper sanitation and hygiene.

Keywords: Efficacy, Fenbendazole, Pyrantel, Hookworms, Zoonotic Risk.

Introduction

Gastrointestinal parasites of dog are of significant importance, not only because of their adverse effects on hosts but also due to their significant zoonotic potential. Intestinal parasites, helminths are most commonly encountered pathogenic agent and they constitute one of the main cause of mortality in dogs in India (Sengupta and Yadav, 2003). They infects dogs of all ages, including both kennel and free roam dogs (Overgaaauw and Boersema 1998, Endrias *et al.* 2010). Clinical symptoms of intestinal parasites in dogs include vomiting, diarrhoea, anaemia, anorexia, underweight, dermatitis, dehydration, and deterioration of health. Even though there are effective treatments available to treat parasites, the majority of parasites in dogs have highly complicated life cycles that make treatment hard (Dubie *et al.* 2023)

Hookworm disease, caused by *Ancylostoma caninum*, is prevalent in warm and moist climates, mainly affecting dogs. It poses significant health risks to both animals and humans, with symptoms ranging from diarrhoea in puppies to anaemia in adult dogs. The disease spreads through direct or indirect contact, including contaminated food and water. *A. caninum* is a zoonotic parasite, infecting millions of people globally each year, leading to iron-deficiency anemia and potentially severe consequences like mental retardation and growth issues, especially in children. (Kour *et al.* 2020). Toxocariasis is a parasitic disease that poses a significant health risk globally, causing considerable morbidity. Infections primarily affect children, usually occurring through the ingestion of *Toxocara* eggs. These eggs are often encountered via direct contact with puppies, contact with the dog's fur, or ingestion of contaminated vegetables or soil containing *Toxocara* eggs. The soil in public areas such as parks and playgrounds commonly harbors these eggs, which can remain infectious for many years, contributing to the spread of the disease. Hence the objective of the present study is to assess the zoonotic risk of helminthic eggs contaminating beaches / parks of Mumbai.

To control the infection among dogs regular monitoring and deworming with anthelmintic treatment is necessary. For many years, most of the anthelmintic preparation used in India contains Pyrantel pamoate and or Fenbendazole for treatment of hookworms and *Toxocara canis*. There are report of resistance against these two commonly used anthelmintics across many countries. Hence for monitoring the deworming for effective implementation of control strategy another objective of present study is to evaluate the Pyrantel from tetrahydropyrimidine class and Fenbendazole from benzimidazole class against gastrointestinal helminths in dogs.

Materials and Methods

Therapeutic Evaluation of Commonly Used Anthelmintics

The faecal samples were randomly collected from pet and stray dogs of urban and sub-urban region of Mumbai. The faecal samples which were positive for hookworm infection during qualitative examination were subjected to stoll's egg counting technique as per method described by (Soulsby, 1982) for therapeutic trials to evaluate anthelmintic drugs pyrantel and fenbendazole for their clinical efficacy. Dogs with faecal sample positive for helminth eggs were recruited for the comparative study (n =24) which were grouped into two groups consisting of 12 dogs in each group (group 1 and group 2). Group 1 involved 12 dogs were treated with single drug pyrantel and group 2 involved 12 dogs were treated with benzimidazole (fenbendazole) formulation once orally. EPG (Egg per gram) count before treatment (day 0) and thereafter on 7th and 14th day post treatment were recorded (Lefkaditis *et al.* 2004). The therapeutic efficacy of all the drugs calculated on the basis of number of animals found free of worm infection as determined by faecal sample examination and reduction in EPG count of the faeces of the group following formula.

$$\% \text{ Efficacy of drug} = \frac{\text{Mean EPG before Treatment} - \text{Mean EPG after Treatment}}{\text{Mean EPG before Treatment}} \times 100$$

Egg Detection from Soil for Zoonotic Risk Assessment

The present study was carried out in the Department of Veterinary Parasitology Mumbai Veterinary College, Mumbai. In order to assess zoonotic risk of canine gastrointestinal helminths, soil samples were collected from beaches and dog parks in Mumbai (dog walking /playing area). 5-10gm soil samples were collected in plastic ziplock bag and sealed and brought to laboratory for further processing to check zoonotic helminthic eggs. For egg detection,

soil samples were dried overnight at room temperature and sifted through a 150 µm mesh sieve. About 2 gm of powdery soil was placed in a test tube and suspended in about 8 ml of 0.05% Tween- 20 solution. After centrifugation of the test tube at 1500 rpm for 10 min. the supernatant was removed and sucrose solution with specific gravity of 1.2 was added up to a level of 1 cm from the top of the tube. The contents of the tube were mixed well and centrifuged at 1500 rpm for 10 minutes. The tube was then filled to the top with the sucrose solution so that a small convex bubble formed and a coverslip could be placed on the tube. After 20minutes, the coverslip from the tube was put on a microscopic slide and examined at a magnification of 100 X for Helminthic eggs (Zibaei *et al.* 2010).

Results and Discussion

Therapeutic Evaluation of Commonly Used Anthelmintics

Therapeutic trials were conducted to evaluate anthelmintic efficacy of Pyrantel and Fenbendazole in infected dogs. Dogs with faecal sample positive for hookworm eggs with high intensity were recruited for the faecal egg count reduction test (FECRT) study (n =24) which were grouped into two groups consisting of 12 dogs in each group (group 1 and group 2). Group 1 involved 12 dogs were treated with single drug pyrantel pamoate @ 5 mg/kg bw and group 2 involved 12 dogs were treated with fenbendazole @ 50 mg/kg bw formulation once orally. Positive samples were then analysed by Stoll's egg counting technique for EPG (Egg per gram) count before treatment (day 0) and thereafter on 7th, 14th and 21st day post treatment and results were depicted in table 1 and 2.

The Mean ±SE of EPG in pyrantel pamoate and fenbendazole treated dogs was 2033.33 ±87.32 and 1858.33 ± 76.33 respectively on day 0. The percent efficacy on day 21 in pyrantel pamoate in treated group was observed as 98.32 ±0.73. When data of 0 day (before treatment) and 21 days (after treatment) of pyrantel pamoate analysed statistically by randomised block design it was found statistically significant (p < 0.01) at both 5% and 1% level. Similarly, the percent efficacy in fenbendazole in treated group was found as 92.28 ± 3.54. Statistical analysis of data on 0 day (before treatment) and 21 days (after treatment) of fenbendazole by randomised block design showed significant (p < 0.01) at both 5% and 1% level. The observation revealed that both the drugs pyrantel and fenbendazole were able to reduce the egg count significantly after the treatment and found efficacious. Further data of percent efficacy of 21 days post treatment of both the drugs pyrantel and fenbendazole compared statistically and it was found that there were no statistical difference (p > 0.05) between the percent efficacy of both these drugs (Table 3). According to WAAVP guidelines for evaluation of anthelmintic efficacy in companion animals in a field study a calculated percent efficacy (reduction of faecal egg counts of treated group) of 90% or more is desirable (Geurden *et al.* 2022) which indicate that even pyrantel pamoate numerically performed better than fenbendazole in percent efficacy both the drugs can be utilised for the effective treatment of hookworm disease in canines.

Table 1: Efficacy of Pyrantel pamoate in hookworm infection in dogs

Cases	0th Day	7 th Day	14 th Day	21 Day	% Efficacy
1	2300	0	0	0	100
2	2000	400	200	0	100
3	2200	300	100	100	95.45454545
4	2100	0	0	0	100
5	1900	0	0	0	100
6	2500	0	0	0	100
7	2400	200	100	100	95.83333333
8	2000	0	0	0	100
9	2100	200	100	100	95.23809524
10	1800	0	0	0	100
11	1500	700	200	100	93.33333333
12	1600	800	0	0	100
Mean± SE	2033.33±87.3				98.322±0.735

*Treatments found significant at 1% and 5% level of significance

Table 2: Efficacy of Fenbendazole in hookworm infection in dogs

Cases	0th Day	7 th Day	14 th Day	21 Day	% Efficacy
1	1800	0	0	0	100
2	1700	200	400	600	64.70588235
3	1900	0	0	0	100
4	1800	0	0	0	100
5	1600	0	0	0	100
6	1700	200	300	400	76.47058824
7	1800	0	0	0	100
8	1700	0	0	0	100
9	1600	0	100	300	81.25
10	2000	0	100	300	85
11	2500	0	0	0	100
12	2200	200	200	0	100
Mean± SE	1858.33±76.33				92.286

*Treatments found significant at 1% and 5% level of significance

Table 3: Statistical analysis of efficacy of Pyrantel pamoate and Fenbendazole

Treatment Means					
Anova Table					
Treatment			Average		
Pyrantal pamoate			1858.33		
Fenbendazole			2033.33		
Source of variation	Degrees of freedom	Sum of squares	Mean of sum of squares	F cal	F prob
Replications	11	800.438	72.767	0.858	0.598
Treatments	1	218.605	218.605	2.578	0.137
Error	11	932.616	84.783	-	-
Total	23	-	-	-	-
Coefficient of variation:9.667					

*Treatments found to be non-significant

In the present study Pyrantel pamoate was found effective against hookworm infection in dogs and this observation is in complete harmony with the findings of Clark *et al.* (1992), Nolan *et al.* (1992), Payghan *et al.* (2023). However, in contrast to present study Castro *et al.* (2020) reported only 23% efficacy against hookworms in dogs for Pyrantel indicating developing resistance.

In the present study Fenbendazole was found effective against hookworm infection in dogs and these findings are in accordance with the findings of Arle *et al.* (1992), Thangjam *et al.* (2018), Sathe, (2019) and Bhanjadeo *et al.* (2023). However in contrast to present study Singh *et al.* (1977) reported only 70 % efficacy against hookworms in dogs for Fenbendazole. Castro *et al.* (2020) reported only 26 % efficacy against hookworms in dogs for Fenbendazole indicating resistance. In the present study in few cases (n=4) (Table 2) there was reduction in the egg count by day 7 after fenbendazole treatment but FEC gradually increased by day 14 and day 21. This data demonstrate that fenbendazole causes temporary suppression in egg shedding in case of hookworms. This finding is also supported by Castro *et al.* (2020) who demonstrated after Fenbendazole treatment there is significant rise in egg count after initial suppression.

Zoonotic Risk Assessment of Gastro-Intestinal Parasites of Dogs in Mumbai

In present study soil/sand sample were collected from twenty different sites such as parks (9) and beaches (11) in the Mumbai region. Out of 20 soil/sand samples nine samples found positive for different helminthic eggs. From table 4 it is observed that out of nine sample sites from parks 5 were positive (55.55%) for GI helminths eggs such as strongyle type eggs assumed to be *Ancylostoma* spp. and *Toxocara* spp. eggs. out of 11 sample sites from beaches

4 were positive (36.36%) for GI helminths. Out of total 20 sample sites from parks or beaches of all over Mumbai region 9 were positive (45.0%) for GI helminth parasite eggs (table 4).

The objective of the discussed study was not just to observe the presence of gastrointestinal parasites in soil of parks and beaches of Mumbai, but also to assess the potential risk they pose to humans. One of most important finding of the present study was that the helminth such as Hookworm spp. and *Toxocara* spp. observed at many places which are the major zoonotic species pose threat to public health in the form of cutaneous larva migrans and visceral larva migrans respectively. The findings of the present study are in general agreement with Belsare (2011) who also reported presence of *Ancylostoma* spp. and *Toxocara canis* eggs in the soil samples collected from parks and beaches of Mumbai. Utaaker *et al.* (2018) reported moderate prevalence of *Ancylostoma* spp. and low prevalence of *Toxocara canis* and taeniid eggs in dogs roaming recreational parks in Chandigarh. Andresiuk *et al.* (2007) found prevalence of *Ancylostoma* spp. and *Toxocara canis* eggs in public squares of Mar del Plata city of Argentina. *Toxocara* spp. eggs have been found in sand or soil samples in Iran (22.2%), Brazil (53.0%), Turkey (63.0%), Italy (64.0%), Spain (67.0%), Japan (92.0%) and Thailand (6.0%) prevalence (Zibaei, *et al.* 2010; Ozkayhan, 2006; Giacometti *et al.* 2000; Ruiz *et al.*, 2000; Uga, 1993; Wiwanitkit and Waenlor, 2004).

The presences of hookworm and *Toxocara* spp. eggs in soil samples in the study area warrants immediate intervention measures are necessary to reduce the risk of transmission of parasites from dogs to humans. Interventions, those are need of this hour should focus on health education provided to dog owners, strategic deworming of dogs using broad spectrum anthelmintic and proper sanitation and hygiene. It is concluded from this study that pyrantel and fenbendazole are effective dewormers for dogs and can be used for periodic deworming of domestic as well as stray dogs to formulate comprehensive disease control program.

Table 4: Detection of helminth eggs from soil samples

Soil Samples collection	Total sample examined	Positive for hookworm/ <i>Toxocara</i> spp.eggs	Percent (%)
Parks	9	5	55.55
Beach	11	4	36.36
Total	20	9	45

Contribution by Authors

All the authors contributed equally to writing the manuscript. The final manuscript was read by all authors and consented to publication.

Conflict of Interests

There is no conflict of interest.

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