



Status of Anthelmintic Resistance against *Ancylostoma* spp. in Naturally Infected Dogs

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10.18805/IJAR.B-5447

ABSTRACT

Background: Anthelmintic efficacy field surveys in dogs are extremely sparse as compared to ruminants. Therefore the present study was planned with the objective to know the status of anthelmintic resistance against *Ancylostoma* spp. in naturally infected dogs.

Methods: A total of 754 faecal samples of dogs were collected to know the prevalence of gastrointestinal parasites. Saturated salt floatation technique was applied for the detection of nematode eggs. Out of which forty dogs, having egg per gram of faeces (epg) more than 300 for *Ancylostoma* spp., were selected and randomly divided into four study groups of 10 dogs per group. Group I served as untreated control. Dogs of Group II, III and IV were given fenbendazole @ 50 mg/kg BW, pyrantel @ 15 mg/kg BW and Ivermectin @ 0.6 mg/kg BW once orally respectively. Faecal samples were collected pre-treatment on day 0 and post-treatment on days 10 and 14 from naturally infected dogs in field-trial conditions and efficacy was calculated as per standard protocol of WAAVP.

Result: The overall prevalence of gastrointestinal parasites was 22.41% which was comparatively higher in stray dogs, female dogs and in monsoon season. Among different parasites *Ancylostoma* spp. had highest prevalence (12.33%). Efficacy of fenbendazole at day 10 post infection was 96% with lower 95% confidence interval of 88%. Efficacy of pyrantel at day 10 post infection was 97% with lower 95% confidence interval of 88%. Efficacy of Ivermectin at day 10 post infection was 99% with lower 95% confidence interval of 93%. As per WAAVP guidelines, both fenbendazole and pyrantel showed suspected resistance while *Ancylostoma* worms were susceptible for Ivermectin. All the three anthelmintic were 100% effective at day 14 post treatment.

Key words: *Ancylostoma caninum*, Anthelmintic resistance, Dogs, Efficacy.

INTRODUCTION

The most common infectious agents of dogs are gastrointestinal parasites. In India, the most common intestinal nematode parasite of dogs is *Ancylostoma caninum*, also known as the canine hookworm. The pathophysiology linked to hookworm infection includes iron-deficiency anaemia, hypoalbuminemia and enteritis. These conditions are followed by diarrhoea that may contain fresh or digested blood. *A. caninum* causes traumatic lesions by biting the intestinal mucosa. Feeding sites bleed continuously for a long time before the worm relocates and causes normocytic hypochromic anaemia (Qadir *et al.*, 2011). Furthermore, by producing cutaneous larva migrans, eosinophilic enteritis and patent infections in humans, these parasites may also represent a serious threat to public health (Jimenez Castro *et al.*, 2020).

Thus, to protect dogs from zoonotic illnesses and to guarantee their health, regular parasitological surveillance and suitable treatment approaches are needed (Miro *et al.*, 2007). In developing nations such as India, pyrantel, ivermectins and benzimidazoles are frequently used singly or in combination to treat various parasitic infections. Benzimidazoles work by preventing tubulin from polymerizing into microtubules. Pyrantel Pamoate produces an excitatory blockade by functioning as an agonist at the nicotinic acetylcholine receptor (nAChR), which depolarises neuromuscular blocking. This causes a massive overstimulation of the target nematode's muscle, which in turn causes persistent spastic contraction and paralysis. Ivermectin works by preventing the transmission of parasite nerves

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How to cite this article: Waskel, S.S., Dixit, P., Dixit, A.K. and Shrivastava, S. (2025). Status of Anthelmintic Resistance against *Ancylostoma* spp. in Naturally Infected Dogs. Indian Journal of Animal Research. 1-4. doi: 10.18805/IJAR.B-5447.

Submitted: 15-07-2024 **Accepted:** 28-01-2025 **Online:** 19-04-2025

linked to GABA, which paralyses the pharyngeal muscles and causes the worm to become flaccidly paralysed.

Multi-drug resistant (MDR) nematode populations are on the rise globally, as a result of total dependence on anthelmintic medications in ruminants and their heavy usage for worm management (Kalpan, 2004). In contrast, anthelmintic resistance in *A. caninum* has developed far more slowly, with only a few examples reported. Jackson *et al.* (1987) reported the first case of pyrantel resistance in a greyhound puppy imported from Australia; this was followed by many cases (Hopkins *et al.*, 1988; Hopkins and Gyr, 1991; Kopp *et al.*, 2007; Kopp *et al.*, 2008a and 2008b). According to recent reports, a greyhound dog's strain of *A. caninum* was resistant to benzimidazoles and

macrocyclic lactones (Kitchen *et al.*, 2019). Soon after, Jimnez Castro *et al.* (2019) reported multiple drug resistance to all three of the main drug classes that were most frequently used to treat the parasite in dogs.

There are fewer field surveys on the efficacy of anthelmintics in dogs and cats than in ruminants. Therefore the present study was planned with two objectives, to estimate the prevalence of gastrointestinal parasites in dogs in Rewa, India and to study the therapeutic efficacy of fenbendazole, pyrantel and ivermectin against gastrointestinal nematodes in dogs.

MATERIALS AND METHODS

Study area

Rewa is situated at 24°31'57"N latitude and 81°17'32" E longitudinal at 309 MSL (Mean sea level), having mild generally subtropical climate with average rainfall of 1128 mm.

Faecal sample collection and examination

Faecal samples of 754 dogs of different age, sex and breed were randomly collected. Signalment of these dogs and consistency of these faecal samples were also recorded. These samples were examined for parasitic infections by standard qualitative and quantitative methods (Zajac *et al.*, 2021). The prevalence data were analysed using the chi square test (Snedecor and Cochran, 1994).

Efficacy of anthelmintics

40 dogs with more than 300 eggs per gram of faeces were chosen based on the prevalence study and placed into four groups, each with 10 dogs. Group G1 was kept as undewormed control and Group G2 was given fenbendazole @ 50 mg/kg BW, Group G3 was given pyrantel @ 15 mg/kg BW and group G4 was given ivermectin @ 0.6 mg/kg BW orally. Each animal rectum was sampled for faeces on day 0 (pre-treatment), day 10 and day 14 after treatment. Using the RESO (version 2.0) computer program (Martin and Wursthorn, 1991), the EPG data were statistically analysed in accordance with the recommendations provided by the World Association for the Advancement of Veterinary Parasitology for the detection of anthelmintic resistance (Coles *et al.*, 1992; Geurden *et al.*, 2022). If the FECR was less than 95% and the lower 95% confidence limit was less than 90%, resistance to anthelmintics was taken into consideration. Resistance was suspected if only one of these two conditions was satisfied.

RESULTS AND DISCUSSION

169 of the 754 faecal samples were positive *i.e.* the overall prevalence of gastrointestinal parasites was 22.41% (169/754). This value is in agreement with 27.08% prevalence reported previously by Qadir *et al.* (2012) in dogs in Jabalpur (M.P.). This prevalence was comparatively lower than 65.5% reported by Shahat *et al.* (2022). According to Kumar *et al.* (2015), dogs from Junagadh, Gujarat, have an overall

prevalence of gastrointestinal parasites of 21.97%. In Palampur, Himachal Pradesh, Moudgil *et al.* (2016) also found that the general prevalence of gastrointestinal parasites in dogs was 28.04%. One possible explanation for the lesser prevalence of gastrointestinal parasites in the present study could be the higher number of domestic dogs (n =611) compared to stray dogs (n =143). The prevalence was comparatively higher in stray dogs (32.87%) as compared to that of pet dogs (19.97%). Pet owners might be more aware of the deworming of their pets.

Parasite wise prevalence was significantly highest for *Ancylostoma* spp. 12.33 per cent (93/754) followed by that of *Toxascaris leonina* 6.23 per cent (47/754), *Dipylidium caninum* 5.44 per cent (41/754), *Toxocara canis* 2.79 per cent (21/754), *Isospora* spp. 2.12 per cent (16/754) and *Spirometra* spp. 0.13 per cent (1/754). Kurumadas *et al.* (2020), Duncan *et al.* (2020) and Sukupayo and Tamang (2023) also reported highest prevalence of *Ancylostoma* spp. in dogs. Out of 169 positive samples, 45 samples (26.63%) were positive for mixed infection while 124 samples (73.37%) were positive for mono infection *i.e.* only one parasite species was present (Table 1). The results corroborate those of Mukaratirwa and Singh (2010) and Palmer *et al.* (2008), who found that the percentage prevalence of mono infection was higher than that of mixed infection. The prevalence was only slightly higher in adult (23.43%) than young dogs (21.94%) contrary to other studies (Merga and Sibhat, 2015; Moudgil *et al.*, 2016) that found puppies to be more infected than adults. There was no discernible difference in the prevalence of gastrointestinal parasites between the two age groups in the present study. The findings, however, are consistent with those of Stafford *et al.* (2020), who found that young adult dogs (45.6%) had a greater prevalence of gastrointestinal parasites than immature adults (20.4%) and puppies (2.4%). In females, the prevalence was non-significantly higher (22.97%) as compared to that of male dogs (22.08). The increased frequency of gastrointestinal parasites in female dogs may be caused by a stress factor linked to their physiology, which lowers their immunity to parasite infection. Mirzaei and Fooladi (2012) reported non-significant difference in prevalence percentage between male and female dogs *i.e.* 7.7 and 6.5 respectively, while Satyal *et al.* (2013) reported non-significantly higher prevalence of gastrointestinal parasites in female dogs (51.6%) as compared to that of male dogs (39.8%). Merga and Sibhat (2015) reported that sex has no significant association with parasite infection in dogs. The monsoon season's prevalence (23.02%) was not statistically different from that of the summer season (21.66%), (Table 1). Lower prevalence in summer season might be due to adverse climate conditions resulting in delay in infectiveness of parasite eggs. In a retrospective study, Vatsya *et al.* (2010) also found that dogs had a higher frequency of gastrointestinal parasites during the rainy season than during the summer.

In the current investigation, *Ancylostoma* spp. was the most common parasite (12.33%). *Ancylostoma* has a high pathogenicity for pups, which can lead to oxidative stress, anaemia, hypoalbuminaemia, impaired immunity and a diminished response to vaccinations, in addition to its several modes of transmission. Young puppies' limited iron reserves make them particularly vulnerable. When this parasite causes an acute infection, puppies may die. Given that benzimidazole is frequently used either alone or in conjunction with pyrantel and/or praziquantel for canine deworming. During the first three months of their lives, puppies were routinely dewormed every 2-3 weeks and after that, on a regular basis (Ridley *et al.*, 1994). Regular deworming can put more pressure on the parasite to select for anthelmintic resistance. The pharmaceutical companies create and promote use of endo-parasiticides that can combat a wide range of intestinal parasites in dogs. However some researchers have shown in a few different parts of the world, routine, metaphylactic use of broad-spectrum anthelmintic combinations may foster the emergence of anthelmintic resistance (AR) as compared to deworming for a specific parasite (Jackson *et al.*, 1987; Kopp *et al.*, 2007, 2008a).

According to Kopp *et al.* (2007), the FECRT has limited usefulness as a quantitative measure of anthelmintic efficacy against *A. caninum* isolates of intermediate resistance status, but it may help identify highly resistant or susceptible

isolates. FECRT is a screening tool that can be used to find resistant isolates that require further investigation. In the present study, efficacy of fenbendazole at day 10th post treatment was 96% with lower 95% confidence interval of 88% (Table 2). Efficacy of pyrantel at day 10 post treatment was 97% with lower 95% confidence interval of 88%. Efficacy of Ivermectin at day 10 post treatment was 99% with lower 95% confidence interval of 93%. All the three anthelmintic were 100% effective at day 14 post treatment (Table 2). These results showed that *Ancylostoma* worms were susceptible for ivermectin while suspected resistance was shown against fenbendazole and pyrantel.

Studies on livestock nematodes have revealed that SNPs in the β -tubulin isotype 1 gene produce benzimidazole resistance. Tyrosine is substituted for phenylalanine (TTC→TAC) by SNPs in codons 200 and 167, whereas alanine is substituted for glutamate (GAG→GCG) by an SNP in codon 198. It has been discovered that all three of these SNPs independently confer resistance, despite the fact that the 200 and 167 SNPs are more frequently linked to resistance (Dixit *et al.*, 2017). The β -tubulin protein undergoes structural alterations due to these amino acid mutations, which prevents the BZs from binding. However, the canine nematode, *Ancylostoma caninum*, seems to have a distinct mechanism (Albonico *et al.*, 2004b; Schwenkenbecher *et al.*, 2007; Diawara *et al.*, 2013a). The

Table 1: Prevalence of canine gastrointestinal parasites in Rewa.

Factor	Level	Examined	Prevalence (%)	Degree of freedom	χ^2 value
Age	Young	515	113 (21.94%)	1	0.21
	Adult	239	56 (23.43%)		
Sex	Female	283	65 (22.97%)	1	0.08
	Male	471	104 (22.08%)		
Season	Monsoon	417	96 (23.02%)	1	0.20
	Summer	337	73 (21.66%)		
Type of infection	Mono	169	124 (73.37%)	1	73.85**
	Mixed	169	45 (26.63%)		

Figures in parentheses indicate percentage.

** χ^2 values were considered significant at $p < 0.01$ level.

* χ^2 values were considered significant at $p < 0.05$ level.

Table 2: Faecal egg count (FEC) and faecal egg count reduction (%) in dogs treated with three common anthelmintics.

Parameter	Control (G ₁)	Treated groups		
		Fenbendazole (G ₂)	Pyrantel (G ₃)	Ivermectin (G ₄)
No. of dogs	10	10	10	10
Mean FEC on day 0	800	1100	945	1030
Mean FEC on day 10 post treatment	1375	55	35	20
Mean FEC on day 14 post treatment	2145	5	0	0
FECR % on day 10	-	96	97	99
FECR % on day 14	-	100	100	100
95% confidence interval on day 10	-	88	88	93
Resistance	-	Suspected	Suspected	Susceptible

canine hookworm *A. caninum* strain Aca-KGR is resistant to multiple drugs (Kitchen *et al.*, 2019). The β -tubulin isotype 1 gene's codon 167 in this strain contains an SNP that converts the amino acid phenylalanine to tyrosine. This first multidrug-resistant strain of *A. caninum* promises anthelmintic resistance can develop in natural populations of hookworms as well. Even while widespread AR in canine intestinal helminths is not present at this time, further scientific data is clearly needed immediately.

CONCLUSION

In dogs, *Ancylostoma* spp. was the most common parasite. The *Ancylostoma* worms were found susceptible to Ivermectin but the status of resistance in fenbendazole and pyrantel was found to be suspected. Consequently, molecular research is needed to verify the resistance status of hook worms in the dogs residing in the area. Additionally, it will aid in comprehending the mechanism of resistance to anthelmintics.

ACKNOWLEDGEMENT

The authors thank the Dean of the College of Veterinary Science and A.H., Rewa, for providing the resources needed for this study.

Conflict of interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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