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Evaluation of anthelmintic efficacy against gastrointestinal nematodosis in Cervids of Dehradun Zoo

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Abstract
Maintenance of wildlife population is an inevitable task. Endoparasitic diseases are one of the major problems in wildlife leading to morbidity and mortality. Indiscriminate use of anthelmintics for deworming of animals against gastrointestinal parasites has lead to development of anthelmintic resistance. Therefore, prompt decision on usage of right drug based on its efficacy is essential. The study to compare the efficacies of drugs namely Ivermectin and Fenbendazole was conducted in the Cervids of Dehradun Zoo. Animals were divided into an untreated, control group (I) and two treatment groups i.e. ivermectin (II) and fenbendazole (III). As per the routine Zoo regime, ivermectin was administered @0.2mg/kg body weight, orally and fenbendazole @7.5mg/kg body weight, orally. EPG (egg per gram) of collected faecal samples was determined on 0, 7th, 14th and 21st day post treatment, using the modified McMaster method to quantify nematode egg burdens. Faecal egg count reduction test (FECRT) showed 97 per cent efficacy for ivermectin, constantly reducing egg shedding by more than 95 per cent. Fenbendazole was also found 96.15% effective against nematodes, 14th day post-treatment. A greater than 95% reduction in faecal egg counts, in both the anthelmintic groups indicated that anthelmintics used are still beneficial. There was no significant (p>0.05) difference evident in efficacy of ivermectin and fenbendazole. Therefore, both the anthelmintics can be used in a strategic control program against gastrointestinal nematodosis in Cervids.

Keywords: Anthelmintic efficacy, fecal egg count, fenbendazole, ivermectin, Cervids

1. Introduction
Deer is a ruminant belonging to the family Cervidae and is included in the order Artiodactyla. The highest concentration of large deer species occurs in Southeast Asian countries like India and Nepal. Environmental changes and ecological disturbances, due to both natural phenomena and human intervention act like indiscriminate hunting and habitat destruction have contributed to the decline of this valuable creature of the country (Kanungo *et al*., 2011) [17]. Gastrointestinal (GI) nematodes have been shown to be a cause of morbidity and mortality in captive wild ungulates (Goossens *et al*., 2006) [12]. A major health issue in captive and wild deer is infection with helminthes (Thawait and Maiti, 2015) [27]. Abomasal damage comprised of varying degrees of hyperaemia, oedema, pitting and thickening of the abomasal mucosa. Banerjee *et al*. (2005) [2] in their study reported 17.7% samples of spotted deer, as positive for either single or mixed parasitic infection and Niranjan *et al*. (2022) [24] reported about 27.77% infection amongst captive herbivores in Uttarakhand. Strategic use of effective anthelmintics has been essential for the control of GI parasites in deers. Benzimidazoles are the most commonly used anthelmintics in captive wild ungulates owing to their wide margin of safety (Isaza *et al*., 1995) [14] and can be administered orally using different carriers such as feed or mineral licks. In the early 1980s ivermectin, a member of a new family of anthelmintics, the macrocyclic lactones (ML) or "mectins", was introduced and its efficacy in deers was investigated. The need for efficacy of anthelmintics arises from Agricultural Compounds and Veterinary Medicines (ACVM) Act, which provides for prevention or management of risks associated with the use of agricultural compounds including veterinary medicine (Geary *et al*., 2012)[10]. Anthelmintic efficacies against helminthes in captive deer have been reported by various workers from time to time in different zoos of the country (Mason, 1994; Islam *et al*., 2003; Singh *et al.,* 2006 and Castillo-Alcala *et al.,* 2007) [20, 15, 26, 3]. But such information is very limited in Cervids of Uttarakhand.

Considering these facts, the present study was undertaken to evaluate the therapeutic efficacy of in-feed administration of fenbendazole and ivermectin against gastrointestinal nematode infections in members of Cervidae family.

2. Materials and Methods

2.1 Study area and animals

After getting approval from Chief Wildlife Warden, Uttarakhand and Institutional Animal Ethical Committee, the investigations were made in the Dehradun Zoo. The Zoo is located at Dehradun Mussoorie Highway. It is a part of reserve forest of Malsi Range and is dominated by coppice crop of Sal along with other species like Haldu, Rohini, Jhingan etc. The total area under this Zoo is 25 Ha (62.5 Acre). Spotted deer, Sambar, Barking deer belonging to family Cervidae, having egg counts more than 150epg were selected. Animals were randomly distributed into an untreated, control group (I) and two treatment groups i.e. ivermectin (II) and fenbendazole (III).

2.2 Treatment

As a part of the routine three monthly deworming strategies, animals were treated with anthelmintics, according to the manufacturer's recommended dose. Ivermectin (Bolus Neomac, Intas Pharmaceuticals Ltd., Ahmedabad, India) was administered at the dose rate of 0.2mg/kg body weight orally, mixed with concentrate feed and fenbendazole (Bolus Panacur 1.5VET, Intervet India Pvt. Ltd., Thane, Maharashtra) at the dose rate of 7.5 mg/ kg body weight orally, mixed with concentrate feed.

2.3 Study design

The *in vivo* efficacy of the drugs was assessed by FECR tests. Fresh faecal samples were collected at 0, 7, 14, 21 days after treatment. About 5-10g of fresh faecal samples were collected randomly from the holding area and transported to the laboratory in separate sterile sealed containers. The faecal egg counts (FECs) were determined within 24 hours by a modified McMaster method, using a saturated solution of sodium chloride. The FECR was calculated using the formula by Young *et al*. (2000) [30]: {([pretreatment mean EPG] − [post-treatment mean EPG])/ (pretreatment mean EPG)} × 100 = % efficacy. For comparison, the FECR was also calculated as recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles *et al.*, 2006) [5], using the formula FECR% = $100\times(1-X_t/X_c)$, where X_t is the arithmetic mean EPG of the treated group and X_c is the arithmetic mean EPG of the control group.

2.4 Statistical analysis

Statistical analysis was made using Student's t test for comparing mean EPGs reduction between II (Ivermectin) and III (Fenbendazole) groups.

3. Results

Table 1: Average EPG, Geometric Mean, Median, Range, Variance of counts, Standard Deviation of Control (I Group)

Days	Arithmetic Mean/Average EPG*	Geometric Mean	Median	Range	Variance	Standard deviation
0	500	492.30	500	350-650	8333.34	91.29
7	505	498.92	500	400-650	6916.67	83.17
14	510	502.63	525	350-600	7666.67	87.56
21	515	512.10	500	450-600	3361.12	57.97

*Not significant at 5% level of probability (p>0.05)

Table 2: Efficacy of Ivermectin (II Group) against Gastrointestinal Nematodosis in Cervids of Dehradun Zoo

Days	Arithmetic mean / Average EPG*	Geometric Mean	Median	Range	FEC Reduction (%)	Drug efficacy (%)	Variance	Standard deviation
0	500	484.27	500	300-700	-	-	16666.67	129.10
7	25	0	0	0-100	95.04	95	1250.00	35.36
14	15	0	0	0-50	97.05	97	583.34	24.15
21	20	0	0	0-50	96.12	96	666.66	25.81

*Significant at 1% level of probability (p<0.01)

Table 3: Efficacy of Fenbendazole (III group) against Gastrointestinal Nematodosis in Cervids of Dehradun Zoo

Days	Arithmetic mean / Average EPG*	Geometric Mean	Median	Range	FEC Reduction (%)	Drug efficacy (%)	Variance	Standard deviation
0	520	512.67	525	350-650	-	-	7888.89	88.82
7	25	0	25	0-50	95.05	95.19	694.45	26.35
14	20	0	0	0-50	96.08	96.15	666.67	25.82
21	30	0	25	0-100	94.17	94.23	1222.22	34.96

*Significant at 1% level of probability (p<0.01)

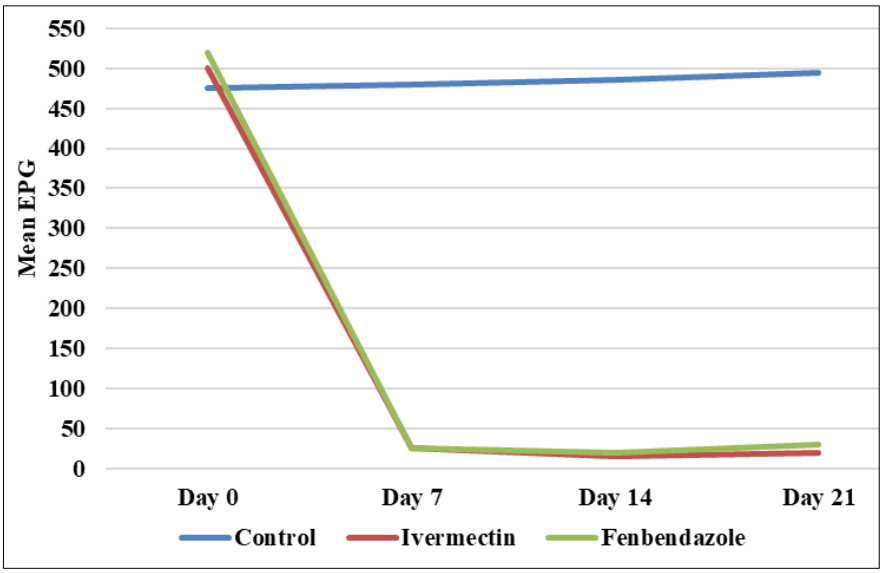

Fig 1: Comparative mean EPGs of Control (I), Ivermectin (II) and Fenbendazole (III) groups on 0, 7, 14, 21days

The average EPG, geometric mean, median, range, variance of counts and standard deviation of untreated Cervids, designated as control (I group) is presented in Table1. The average EPG, geometric mean, median, range, anthelmintic efficacy (using the formula of Young *et al*., 2000) [30], FEC percent reduction (using the formula of Coles *et al*., 2006) [6], variance of counts and standard deviation in egg counts of ivermectin is illustrated in Table 2. The Cervids (Group T1) naturally infected with G.I. helminths were treated with ivermectin at the dose rate of 0.2 mg /kg body weight orally. Percent efficacy of drug was recorded on days 7th, 14th and 21st. The average EPG counts done on 7th day post treatment indicated 95% efficacy of the drug whereas 97% efficacy was observed on 14th day with 97.05% FEC reduction, lastly on 21st day, 96% anthelmintic efficacy was observed with 96.12% FEC reduction. Another group of Cervids (T2) naturally infected with G.I. helminths was treated with fenbendazole at the dose rate of 7.5mg/kg body weight orally. The average EPG, geometric mean, median, range, anthelmintic efficacy, FEC percent reduction, variance of counts and standard deviation in egg counts of fenbendazole is presented in Table 3. The drug was found to be 95.19% efficacious post 7th day with 95.05% reduction in EPG counts. On 14th day 96.08% reduction in EPG counts was recorded and anthelmintic efficacy of 96.15% was found. Reduced efficacy of 94.23% was observed by day 21st.

Compared to the control group, there was sharp decline of mean EPG in both the treated groups up to14th day post treatment. However, the mean EPG rose, post 14th day in both the groups (Fig. 1). Statistical analysis using Student's t-test revealed that, since p-value>0.05 at 95% CI, there is evidence to accept null hypothesis (H0), which explains that mean EPG reduction of both the drugs is similar. Therefore, there is no significant difference between efficacy of ivermectin and fenbendazole. Hence, both the treatments are equally effective against gastro-intestinal nematodes in Cervids of Dehradun Zoo.

4. Discussion

In the present study, efficacy of the two most commonly available and frequently used anthelmintics against gastrointestinal parasites of deer was evaluated. The infected untreated control group continued to discharge the eggs during the entire treatment period, these observations were in accordance with Kanungo *et al*. (2011) and Islam *et al*. (2003) [17,15]. The percentage reduction in faecal egg count on day 7th and 14th for ivermectin was recorded 95.04%, 97.05% respectively and for fenbendazole 95.05% and 96.08%, respectively. As per Coles *et al.* (2006) [6] these values suggest that these two drugs are effective against gastrointestinal nematodes, as the percentage reduction in egg counts is more than 95.0%. In our study, ivermectin was found to be 95% effective on 7th day post treatment which is in close agreement with the observations made by Aman (2015) [1], who also found ivermectin to be 96.2% effective against naturally occurring GIN in deers of Zoological Park, Punjab. Moreover, efficacy of the drug increased to 97.0% on 14th day post treatment which is in agreement with Fathima *et al*. (2018) [9], who also reported 97.0% efficacy of ivermectin in spotted deer of Arignar Anna Zoological Park, Chennai. Studies of Jena *et al*. (2018) [16] showed ivermectin to be 100.0% effective on 14th day post treatment against gastrointestinal helminths of sheep in Jharkhand, whereas Mylrea *et al*. (1991) [22] suspected ivermectin resistance against gastrointestinal helminthosis in fallow deer. The faecal egg count reduction remained around 96.12% at day 21 post treatment, demonstrating higher efficacy of ivermectin. This is attributed to the usage of combination of anthelmintics, which aided in expansion of the spectrum of efficacy of drugs (Leathwick and Miller, 2013) [18]. Fenbendazole was found to be 95.19%, 96.15% effective against gastro-intestinal parasitism on 7th and 14th day post treatment, respectively. Our results were in agreement with Goossens *et al*. (2005) [11] who reported 94.0 to 98.0% reduction in faecal egg counts in captive wild ruminant after treatment with fenbendazole @ 7.5 mg/kg body weight in Belgium Zoo. These findings were not in agreement with Chintoan *et al*., (2014) [4] who reported resistance to benzimidazole and susceptible to ivermectin in wild roe deer at New Forest, UK using FECRT. However on the 21st day of post treatment there was rise in EPG leading to reduction in anthelmintic efficacy to 94.23%. These findings were in agreement with Das *et al*. (2009) [8] who in their study experienced parasitic build-up in enclosure, thus repeated exposure to infection. Nalubamba and Mudenda (2012) [23]

witnessed increase in EPG post 21 day of treatment. These observations were attributed to the rapid re-infection of the impala due to high pasture larval burdens and a large proportion of parasitically naïve young. The suckling impala, did not took in as much of the anthelmintic impregnated salt lick as the older ones but have increased EPGs until they attain 'self-cure' rendering the treatment to be less efficacious. A similar phenomenon has been shown by other researchers in Soay sheep in Belgium (Goossens *et al*., 2006) [12]. Arithmetic means may be more reliable when applied to small groups such as those involved in this study (Craven *et al*., 1998) [7], whereas geometric means may be more suitable in large herds (Vercruysse *et al*., 2001) [28]. Results from two of the studies in red deer (Mackintosh *et al.*, 1985; Watson and Charleston, 1985) [19, 29] suggested that deer are able to metabolise and excrete benzimidazole compounds more quickly as compared to sheep and cattle, it is therefore proposed that anthelmintics should be administered to these captive Cervids for a longer period of 3 days using various delivery methods including feed and mineral licks.

5. Conclusion

It can be concluded that treatment with both ivermectin and fenbendazole against gastrointestinal nematodosis in Cervids was found to be equally effective. The finding of this research will be helpful to know about the anthelmintic therapy by the veterinarian and zoo keepers. As is apparent from our study, it was not possible to entirely eliminate the parasitic burden in animals which was evident by the reoccurrence of infection, which mostly occurs due to stress and close proximity of animals in captive conditions as well as mass deworming, where it becomes tough to ensure whether each animal has consumed the drug. In order to combat existing infection suitable measures like alternating of the anthelmintic class annually between a benzimidazole and macro-cyclic lactones should be employed along with pasture hygiene for small establishments, reducing stocking densities (Isaza *et al*.,1995) [14] and dietary modulation for increased immunity to GI nematodes (McClure, 2008) [21].

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