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Priyanka
Department of Veterinary Parasitology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, Haryana, India

Sukhdeep Vohra
Department of Veterinary Parasitology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, Haryana, India

Satyavir Singh
Department of Veterinary Parasitology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, Haryana, India

Arun K. Sangwan
Department of Veterinary Parasitology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, Haryana, India

Correspondence
Priyanka
Department of Veterinary Parasitology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, Haryana, India

Moderate to severe anthelmintic resistance against commonly used anthelmintics in a village goat flock of Haryana

Priyanka, Sukhdeep Vohra, Satyavir Singh and Arun K Sangwan

Abstract
The present study was conducted to detect the status of anthelmintic resistance of commonly used anthelmintic drugs against gastrointestinal nematodes in goats of village Satnali, district Mahendergarh, Haryana. Sixty goats with eggs per gram of more than or equal to 150 were divided into four groups i.e. G1, G2, G3 and G4 of 15 animals each. Group G1, G2 and G3 were treated with fenbendazole @ 10 mg/kg b.wt. orally, morantel @ 20 mg/ kg b.wt. orally and ivermectin @ 0.4 mg/kg b.wt. subcutaneously, respectively. Group G4 served as untreated control. Faecal egg counts of goats were ascertained on day of treatment (0 day) and 12th day post treatment (PT) of all groups by the modified McMaster technique. Pooled faecal cultures were made to recover infective larvae on day 0 and 12 PT. Results revealed that fenbendazole reduced the faecal egg counts by 14.23% with upper and lower confidence levels as 39.10% and 20.29%, respectively indicating severe anthelmintic resistance. Morantel and ivermectin reduced the faecal egg counts by 60.87% and 89.72%, respectively on 12th day PT with upper and lower confidence levels as 83.20% and 8.86% and 97.11% and 63.51%, respectively indicating moderate anthelmintic resistance against both drugs. The post-treatment coproculture showed larvae of *Haemonchus contortus* and *Strongyloides* sp. Thus, the present study revealed presence of multiple anthelmintic resistance of fenbendazole, morantel and ivermectin against *H. contortus* in goats of unorganized sector in Mahendergarh, Haryana

Keywords: Anthelmintic resistance, fenbendazole, morantel, ivermectin, goat

Introduction
Small ruminants are important source of income for rural communities whose livelihood is largely based on livestock production (Sharma *et al*., 2015) [23]. These animals produce milk, wool, manure, leather and meat with minimum maintenance charges. They are also source of cash income for farmers at the time of need. As these animals are grazed on common land, which predispose them to various parasitic infections. Gastrointestinal (GI) parasites results in major production losses and represent an important animal welfare problem worldwide. Parasitic gastroenteritis is not only associated with direct harms like anaemia, diarrhoea, poor growth, reduced production but also associated with other complications like impaired immunity and secondary infections (Singh and Swarnkar, 2007) [24]. Gastrointestinal nematdes infection is of economic importance in goat farming worldwide. Further, goats can be significantly more heavily infected than sheep and both the acquisition and expression of immune responses against GI nematdes are less efficient in goats than sheep (Hoste *et al*., 2010) [10]. The control of GI nematdes infection in goats is still largely based on use of anthelmintics at regular intervals and this has become more complicated by the presence of resistant nematodes to one or more type of drugs. The phenomenon of anthelmintic resistance is spread in many countries with differences in prevalence and some data showed that GI nematodes develop anthelmintic resistance more rapidly in goats (Waller, 1994; Domke *et al*., 2011) [6, 31]. The growing importance of these anthelmintic-resistant nematodes and the need for reliable information on their occurrence and spread has increased to rule out their occurrence in a particular area. However, there is no information on the prevalence of anthelmintic resistance in goats from villages of Mahendergarh district of Haryana. So, the present study was planned with the objective to know the status of anthelmintic resistance against GI nematodes in unorganized goat sector.

Materials and Methods
During September, 2018, a study was conducted at village satnali, District Mahendergarh, Haryana to determine the efficacy of anthelmintics against gastrointestinal nematodes of goats

using faecal egg count reduction (FECR) test. Sixty goats naturally infected with gastrointestinal nematodes and having eggs per gram (EPG) of faeces ≥ 150 counts prior to treatment were used. The selected animals had not been administered any anthelmintic during the previous two months. These animals were weighed, identified, their eggs per gram (EPG) estimated and divided into four groups i.e. G1, G2, G3 and G4 of 15 animals each. Group G1, G2 and G3 were treated with fenbendazole (FENAZOL-150® tablets, Concept Pharmaceuticals Ltd., Animal Health Division, Mumbai) @ 10 mg/kg b.wt. orally, morantel (Banminth® Tab., Boehringer Ingelheim India Private Ltd. Mumbai) @ 20 mg/kg b.wt. orally and ivermectin (Trumectin®, Zydus Animal Health Limited, Ahmadabad) @ 0.4 mg/kg b.wt. subcutaneously, respectively. Group G4 served as untreated control.

Faecal egg count of each animal was ascertained on 0 day and 12th day post treatment (PT), by the modified McMaster technique to an accuracy of one egg counted representing 50 EPG. Pooled faecal cultures at 27 ± 2 ˚C for 7 days were made to recover infective larvae (L_3), from each group on day 0 and 12th day PT. The infective larvae were identified as per criteria (Keith, 1953) [12]. Faecal egg count reduction percentage and confidence intervals (95%) were determined following the method of the World Association for the Advancement of Veterinary Parasitology using arithmetic mean egg counts (Coles *et al*., 1992) [4]. The drug was considered fully effective when it reduced the egg counts by more than 95% and lower confidence limits were higher than 90%. The drug was considered moderately resistant when it reduced the egg counts between 60% to 95% and considered severely resistant when the reduction in egg counts was below 60% along with lower confidence limits below 90%. All the recorded data was statistically analyzed by one way ANOVA test (SPSS software version 2.0).

Results

Faecal egg counts (Mean ± S.E.) on 0 and 12th day post-treatment (PT), percent reduction in faecal egg counts (FECR%), variance, upper and lower confidence limits (95%) of goats naturally infected with gastrointestinal nematodes and treated with different anthelmintics at Satnali village, Mahendergarh are given in table 1. Results revealed that fenbendazole @ 10 mg/kg b. wt. (G1) reduced the faecal egg counts by 14.23% on 12th day PT with upper and lower confidence levels as 39.10% and 20.29%, respectively indicating severe anthelmintic resistance. Further, morantel @ 20 mg/kg b. wt. (G2) caused 60.87% reduction in faecal egg counts with upper and lower confidence levels as 83.20% and 8.86%, respectively indicating moderate anthelmintic resistance. Ivermectin @ 0.4 mg/kg b. wt. (G3) caused 89.72% reduction in faecal egg counts with 95% upper and lower confidence levels as 97.11% and 63.51%, respectively indicating again moderate anthelmintic resistance. The infected untreated control (G4) had significantly ($P<0.05$) higher faecal egg counts (1686.67 ± 107.73) on day 12 than group G1, G2 and G3.

Table 1: Response to various anthelmintics in goats naturally infected with gastrointestinal nematodes at Satnali village, Mahendergarh

Group	Anthelmintic	Dose (mg/kg)	No. of goats treated	Route of administration	Faecal egg counts on days (Mean ± S.E.)		Faecal egg counts reduction on day 12 post treatment		Confidence limits at 95%	
					0	12	%	Variance	Upper	Lower
G1	Fenbendazole	10	15	Oral	2200.00^{a} ± 238.45	1446.67^{b} ± 223.58	14.23	0.03	39.10	20.29
G2	Morantel	20	15	Oral	2066.67^{a} ± 370.93	660.00^{b} ± 269.18	60.87	0.17	83.20	8.86
G3	Ivermectin	0.4	15	S/C	2000.00^{a} ± 358.7	173.33^{b} ± 106.67	89.72	0.38	97.11	63.51
G4	Control	---	15	---	1726.67^{a} ± 113.17	1686.67^{a} ± 107.73	0	---	---	---

Means with same superscripts in column are not significantly different ($p<0.05$)

The coproculture of pooled faecal cultures of infective third stage larvae in different groups and untreated control on day 0 and 12 (PT) are depicted in Table 2. A total of 100 infective larvae in each group (G1, G2, G3 and G4) were counted. The result showed different genera of GI nematodes of goats with the predominance of *H. contortus* (83-90%) followed by *Strongyloides* sp. (10-11%), *Trichostrongylus* sp. (4-5%) and only 1% *Oesophagostomum* spp. larvae in all the treated and untreated control groups on day 0. After 12 days of treatment, there was predominance of *H. contortus* larvae in fenbendazole and morantel treated animals while *Strongyloides* sp. larvae were predominant in ivermectin treated animals.

Table 2: Anthelmintic effect on different genera of gastrointestinal nematodes of goats at Satnali village, Mahendergarh

Group	Species	Goat	
		Per cent larval composition on day	
		0	12
G1-Fenbendazole	*Haemonchus* spp.	83	87
	Trichostrongylus spp.	5	0
	Oesophagostomum spp.	1	0
	Strongyloides sp.	11	13
G2- Morantel	*Haemonchus* spp.	84	90
	Trichostrongylus spp.	5	0
	Oesophagostomum spp.	1	0
	Strongyloides sp.	10	10
G3- Ivermectin	*Haemonchus* spp.	84	30
	Trichostrongylus spp.	4	0
	Oesophagostomum spp.	1	0
	Strongyloides sp.	11	70

G4- Control	*Haemonchus* spp.	85	87
	Trichostrongylus spp.	4	3
	Oesophagostomum spp.	1	1
	Strongyloides sp.	10	9

Discussion

The present study was planned to assess the status of anthelmintic resistance of fenbendazole, morantel and ivermectin against GI nematodes of goats in Mahendergarh district of Haryana by faecal egg counts reduction (FECR) test. The per cent FECR and confidence intervals (95%) were determined following the method of the World Association for the Advancement of Veterinary Parasitology (WAAVP) using arithmetic mean egg counts. The result of pre-treatment faecal egg counts indicated high prevalence of gastrointestinal nematodes with strongyles as the predominant gastrointestinal nematodes affecting goats (table 1). The finding is similar to that reported by other workers (Singh and Yadav, 1997; Chaudhari and Singh, 2003; Das and Singh, 2005; Kumar *et al*., 2008; Sarika, 2012; Ruchi, 2015; Vohra *et al.*, 2018) [1, 5, 14, 20, 21, 27, 30]. Identification of infective larvae from pre-treatment faecal culture revealed predominance of *H. contortus* (Table 2). The other genera observed were *Strongyloides* sp., *Trichostrongylus* spp. and *Oesophagostomum* spp. This finding is in agreement with the findings of earlier workers (Garg *et al*., 2004; Das and Singh, 2005; Chaudhri *et al*., 2007; Kumar *et al*., 2008; Sharma *et al*., 2009; Kumar and Singh, 2016) [2, 5, 8, 14, 15, 22]. Previously, It had also been reported that *H. contortus* is the most prevalent and pathogenic species among various GI nematodes which is responsible for high mortality and morbidity in India (Yadav, 1997) [32]. The results of faecal egg counts (Mean ± S.E.) on 12th day post-treatment (PT) of gastrointestinal nematodes of goats against fenbendazole reduced the egg count by 14.237% indicating severe anthelmintic resistance. Fenbendazole belongs to benzimidazole class and its resistance to gastrointestinal nematodes in goats had been reported by many workers from our country (Uppal *et al*., 1992; Singh, *et al*., 2012; Kumar *et al*., 2017; Singh *et al*., 2017) [13, 25, 26, 29] and as well as abroad (Howell *et al*., 2008; Holm *et al*., 2014) [9, 11]. The repeated administration of the compound predisposes the nematodes to develop resistance. History of use of anthelmintic and government supply in veterinary hospitals revealed that this is the most commonly used and supplied drug.

Further, morantel caused 60.87% reduction indicating moderate anthelmintic resistance. The resistance of morantel against GI nematodes has also been reported by many workers (Elliott, 1987; Uppal *et al*., 1992; Singh and Yadav, 1997) [7, 27, 29]. History revealed that morantel was frequently used after fenbendazole depending upon availability and convenience of owner.

Ivermectin caused 89.72% reduction in faecal egg counts indicating moderate anthelmintic resistance against gastrointestinal nematodes. The anthelmintic resistance of ivermectin has earlier been reported by other workers (Kumsa and Abebe, 2009; Priyanka, 2012; Holm *et al*., 2014; Pena-Espinoza *et al*., 2014) [9, 16, 17, 18]. This drug has been used sometimes in the flock. Coles *et al.* (1999) have reported the development of anthelmintic resistance even when two or three treatment are given annually. The infected untreated control (G4) had significantly ($P<0.05$) higher faecal egg counts (1686.67 ± 107.73) than group G1, G2 and G3 on day 12 (Table 1).

The coproculture of pooled faecal cultures of infective third stage larvae on day 0 indicated the predominance of *H. contortus* (83-90%) followed by *Strongyloides* sp. (10-11%), *Trichostrongylus* sp. (4-5%) and *Oesophagostomum* spp (1%) larvae in all the treated and untreated control groups. After 12 days of treatment, there was predominance of *H. contortus* in fenbendazole and morantel treated animals and *Strongyloides* sp. was predominant in ivermectin treated animals. The presence of *H. contortus* and *Strongyloides* sp. larvae was also reported by Singh *et al*., 2012 and Rialch *et al*., 2013 [19, 25]. In the present study predominance of *Strongyloides* sp. larvae might be due to its reinfection in experimental animals because of its short prepatent period (Soulsby, 1965) [28].

Conclusion

The results of the present study revealed severe resistance of fenbendazole in goats of Mahendergarh district. The drug should be immediately withdrawn from this area as the treatment is not cost effective and will result in increase in resistant worm burden in the area. Moderate resistance of morantel and ivermectin against gastrointestinal nematodes was also observed from this area. Therefore, it may be concluded that the choice of anthelmintic in a flock should be based on the previous history of use of drug, frequency of use of drug, dose of drug and status of anthelmintic resistance.

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