Original Research

Status of Anthelmintic Resistance of Fenbendazole, Closantel and Levamisole against Gastrointestinal Nematodes in Sheep of Haryana

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Abstract

In the present study, 70 sheep naturally infected with gastro-intestinal nematodes and having at least 150 eggs per gram (EPG) count were selected and grouped into four groups i.e. T1, T2, T3 and T4. Group T1, T2 and T3 were treated with fenbendazole (@ 5 mg/kg b.wt. orally), closantel (@ 10 mg/ kg b.wt. orally) and levamisole (@ 7.5 mg/kg b.wt. subcutaneously), respectively while, Group T4 served as untreated control. Faecal samples were collected on 11th day post treatment from animals of all groups and individual faecal counts were done by modified McMaster technique. The faecal egg count reduction percentage in groups T1, T2, and T3 was 80.1, 96.02 and 72.39, respectively, indicating moderate resistance against fenbendazole and levamisole. Coprocultures from pre- and post-treatment faecal cultures revealed the predominance of Haemonchus contortus larvae.

Key words: Anthelmintic Resistance, Closantel, Fenbendazole, *Haemonchus Contortus*, Levamisole, Sheep

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Introduction

Sheep owing to their multi-facet utility in terms of wool, meat, milk, skin and manure, form an important component of rural economy in developing country like India. They provide a dependable source of income to the shepherds through sale of wool, live animals and their by-products, thus playing an important role in the livelihood of especially small and marginal farmers and landless labourers engaged in sheep rearing. The grazing habit of these animals predisposes them to various types of diseases especially parasitic gastroenteritis caused predominantly by gastro-intestinal (GI) nematodes like *Haemonchus contortus*, *Trichostrongylus axei*, *Nematodirus* spp. and *Strongyloides papillosus*. Among these GI nematodes, *H.*

contortus, is most pathogenic, widely prevalent and important worm in sheep in India responsible for high mortality and morbidity (Yadav, 1997).

Till date, the mostly used field method for the control of GI nematodes is by the use of anthelmintics. However, the frequent, indiscriminate use and under dosing of these drugs has resulted in widespread occurrence of anthelmintic resistance (Kumar and Singh, 2016). There are many reports of anthelmintic resistance available from different parts of India (Buttar *et al*., 2012; Singh *et al*., 2015) as well as from other countries (Balmer *et al*., 2015). Thus, regular monitoring of status of anthelmintic resistance is required as an integral part of worm control programme. The present study was planned to know the status of resistance against commonly used anthelmintics in GI nematodes of sheep under field conditions.

Materials and Methods

The study was conducted to assess the status of anthelmintic resistance of fenbendazole, closantel and levamisole against gastrointestinal nematodes in sheep at village Gajuwala, District Fatehabad (Haryana) by using faecal egg counts reduction test (FECRT). Seventy local sheep maintained by sheep farmers under free grazing system and naturally infected with GI nematodes with faecal eggs per gram (EPG) counts of $\geq$ 150 prior to treatment were selected for performing FECRT. The selected animals had not been administered any anthelmintic during the previous two months. These animals were divided into four groups i.e. T1, T2, T3 and T4 of 15, 15, 20 and 20 animals, respectively. Group T1, T2 and T3 were treated with fenbendazole (Fenazol-150®, Concept pharmaceutical Limited, Andheri, Mumbai) @ 5 mg/kg b.wt. orally, closantel (Zycloz®, Zydus Animal Health, Ahmedabad) @ 10 mg/ kg b.wt. orally and levamisole (Lemasol®-75, Zydus Animal Health, Ahmedabad) @ 7.5 mg/kg b.wt. subcutaneously, respectively while Group T4 served as untreated control. The faecal egg count of each animal was ascertained on day 0 and day 11 post treatment (DPT), by the modified Mc Master 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 third stage larvae (L_3) from each group on 0 day and 11 DPT. The infective larvae were identified as per criteria of Keith (1953). 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). Resistance was considered to be present in the worm population when the egg count reduction following treatment was less than 95% and the confidence limits were less than 90% (Coles *et al*., 1992). Worm populations were considered as moderately/slightly resistant when EPG was reduced between 60 to 95% (Sarika, 2012).

Results and Discussion

The faecal egg counts (Mean±S.E.) on 0 day and 11th DPT, percent reduction in faecal egg counts (FECR%), variance, upper and lower confidence limits (95%) of sheep naturally infected with GI

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nematodes and treated with different anthelmintics at village Gajuwala, Fatehabad (Haryana) are given in Table 1.

Table 1: Response to various anthelmintics in sheep naturally infected with gastro-intestinal nematodes

Group	No. of Sheep	Faecal Egg Count (Mean ± SE)		FECR on 11th DPT		Confidence Limits at 95%	
		0 day	11 day	%	Variance	Upper	Lower
T1	15	553.33 ± 78.24	133.33 ± 34.85	80.1	0.074	88.6	65.27
T2	15	673.33 ± 115.65	26.67 ± 14.81	96.02	0.314	98.74	87.46
T3	20	600 ± 47.96	185 ± 33.37	72.39	0.03814	81.49	58.81
T4	20	640 ± 54.95	670 ± 50.05	0	-----	------	------

Group T1 *= Treated with fenbendazole @ 5 mg/kg b. wt. orally;* ***Group T2*** *= Treated with closantel @ 10 mg/kg b. wt. orally;* ***Group T3*** *= Treated with levamisole @ 7.5 mg/kg b. wt. subcutaneously;* ***Group T4*** *= Untreated control*

Results revealed that fenbendazole @ 5 mg/kg b. wt. (T1) reduced the faecal egg counts by 80.1% on 11th DPT with 95% upper and lower confidence levels as 88.60 and 65.27, respectively indicating moderate resistance. Fenbendazole belongs to benzimidazole class of drug and is effective against roundworms and, depending on the dose, against some tapeworms (e.g. *Moniezia* spp.). The repeated administration of the compound predisposes the nematodes to develop resistance and this has been reported in sheep by many workers from India (Yadav *et al*., 1995; Sarika, 2012; Sharma *et al*., 2015) as well as abroad (Dolinská *et al*., 2014).

Closantel @ 10 mg/kg b. wt. (T2) caused 96.02% reduction in faecal egg counts with 95% upper and lower confidence levels as 98.74% and 87.46%, respectively indicating that drug is likely to become resistant. The drug has persistent anthelmintic effect, which however is variable, depending on the dose rate and the route of administration and also on the host species (Guerrero, 1984). In the past, Gupta *et al.* (2003) reported *H. contortus* resistance to closantel in sheep of western Haryana. Further, Vohra *et al*. (2013) during a study in goats at Goat Breeding Farm, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar also reported slight resistance of closantel against *H. contortus*. But, owing to very scanty use of drugs under field conditions, no resistance was found in the present study. Levamisole @ 7.5 mg/kg b. wt. (T3) caused 72.39% reduction in faecal egg counts with 95% upper and lower confidence levels as 81.49% and 58.81%, respectively indicating moderate resistance. Previously, Singh and Yadav (1997) reported 100% efficacy of levamisole in Government Sheep Breeding Farm, Hisar, but there is emergence of resistance as a result of continuous use of levamisole/tetramisole for years under field conditions. Furthermore, Sarika (2012) and Singh *et al*. (2015) reported 84.25% and 92.50% efficacy of levamisole in University's Sheep Breeding Farm, Hisar, respectively. Reduced levamisole field efficacy has also previously been reported in sheep (Cezar *et al*., 2010; Sargison *et al*., 2010). The coproculture

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results of infective third stage larvae recovered in treated and untreated control on day 0 and 11 are depicted in Table 2.

Table 2: Anthelmintic effect on different genera of gastro-intestinal nematodes of sheep

Group	Species	Percent Larval Composition on Day	
		0	**11**
T1- Fenbendazole	*Haemonchus contortus*	93	97
	Trichostrongylus sp.	1	1
	Oesophagostomum sp.	1	0
	Bunostomum sp.	--	--
	Strongyloides papillosus	5	2
T2- Closantel	*Haemonchus contortus*	95	0
	Trichostrongylus sp.	1	29
	Oesophagostomum sp.	1	10
	Bunostomum sp.	1	0
	Strongyloides papillosus	2	61
T3- Levamisole	*Haemonchus contortus*	96	98
	Trichostrongylus sp.	1	0
	Oesophagostomum sp.	1	0
	Bunostomum sp.	1	1
	Strongyloides papillosus	1	1
T4- Control	*Haemonchus contortus*	95	94
	Trichostrongylus sp.	--	--
	Oesophagostomum sp.	1	1
	Bunostomum sp.	1	1
	Strongyloides papillosus	3	4

The result showed presence of five genera of GI nematodes of sheep i.e. *H. contortus*, *Trichostrongylus* sp., *Oesophagostomum* sp., *Bunostomum* sp. and *Strongyloides papillosus* with the predominance of *H. contortus* on day 0 (pre-treatment) and on day 11 (post-treatment) in fenbendazole, levamisole and untreated control groups. However, the animals treated with closantel showed the predominance of *S. papillosus* and *Trichostrongylus* sp. indicating the efficacy of drug against *H. contortus*. The strains of *H. contortus* resistant to various anthelmintics in small ruminants/sheep have already been reported by Fleming *et al.* (2006) and Singh *et al.* (2013).

Conclusion

Based on the results of the present study, 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 and status of anthelmintic resistance. It should always be considered primarily to use an anthelmintic judiciously and the anthelmintic efficacy must be estimated at least once in two years. The drugs which show moderate resistance should be changed immediately and discontinued for some years so that the larval population resistant to the drug is

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diluted and the portion of susceptible larval population is increased in the sheep flocks. Due to frequent use of fenbendazole and levamisole/tetramisole, resistance against these anthelmintics has developed in this flock. However, no anthelmintic resistance was detected against closantel. This is the first report of cross anthelmintic resistance in sheep under field condition of Haryana.

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