



## Cross Anthelmintic Resistance in *Haemonchus contortus* in Sheep of Unorganized Sector in Haryana

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Received: 23 July, 2019

Revised: 10 Aug., 2019

Accepted: 14 Aug., 2019

### ABSTRACT

The present study was conducted to detect the status of anthelmintic resistance of commonly used anthelmintic drugs viz. fenbendazole and morantel against gastrointestinal nematodes in sheep of village Badhra, district Charkhi dadri, Haryana. Forty five sheep with egg per gram of more than or equal to 150 were divided into three groups i.e. S1, S2 and S3 of 15 animals each. Group S1 and S2 were treated with fenbendazole @ 5 mg/kg b.wt. orally and morantel @ 10 mg/ kg b.wt. orally, respectively. Group S3 served as untreated control. Faecal samples were collected on the day of treatment (zero day) and 12<sup>th</sup> day post treatment (PT), from sheep of all groups and individual FEC were determined by the modified McMaster technique. Pooled faecal cultures were made to recover infective larvae on day 0 and 12 PT. Results revealed that fenbendazole (S1) reduced the FEC by 74.77% on 12th day PT with upper and lower confidence levels as 86.41% and 53.15%, respectively indicating moderate anthelmintic resistance. Morantel (S2) caused 49.84% reduction in FEC with upper and lower confidence levels as 65.04% and 28.04%, respectively indicating severe anthelmintic resistance. Thus, the present study revealed presence of cross anthelmintic resistance against fenbendazole and morantel in *Haemonchus* population in sheep of unorganized sector in Haryana as the post-treatment coproculture showed only *Haemonchus contortus* larvae.

**Keywords:** Anthelmintic resistance, *Haemonchus contortus*, sheep

Sheep are mainly reared by small farmers and landless labourers in rural India. They contribute greatly to the farmers' economy by providing edible proteins, wool, leather, manure etc. However, sheep production is hindered by many factors including animal health constraints, inadequate nutrition and poor husbandry system. Parasitic gastroenteritis caused by many gastrointestinal (GI) nematodes like *Haemonchus contortus*, *Trichostrongylus* spp., *Oesophagostomum* spp., *Nematodirus* spp. and *Strongyloides papillosus* is the major constraint. Among these GI nematodes, *H. contortus*, is most pathogenic, widely prevalent and important worm in sheep in India which is responsible for high mortality and morbidity (Yadav, 1997). The GI nematodes are controlled by the use of anthelmintics. There are three broad spectrum anthelmintic drugs commonly used for treatment and control of nematodes viz. fenbendazole, morantel and

ivermectin. The frequent and indiscriminate use and under dosing of these drugs has resulted in widespread occurrence of anthelmintic resistance. There are many reports of anthelmintic resistance from different parts of India (Buttar *et al.*, 2012; Singh and Gupta, 2010) as well as from other countries (Verissimo *et al.*, 2012; Balmer *et al.*, 2015). Anthelmintic resistance should be identified as soon as possible under field conditions. Thus, regular monitoring of status of anthelmintic resistance is required, at least once in two years and it is as an integral part of worm control programme (Rialch *et al.*, 2013). Further, to prolong the useful life of anthelmintics early detection of resistance may be greatly beneficial, as withdrawal of an anthelmintic from a flock where a low level of resistance

**How to cite this article:** Priyanka, Vohra, S., Singh, S. and Sangwan, A.K. (2019). Cross anthelmintic resistance in *Haemonchus contortus* in sheep of unorganized sector in Haryana. *J. Anim. Res.*, 9(5): 653-656.

is detected could lead to reversion to susceptibility by diluting the resistant population with susceptible or hybrid phenotype (Le Jambre *et al.*, 1982).

## MATERIALS AND METHODS

During September, 2018, a study was conducted at village Badhra, District Charkhi Dadri, Haryana to determine the efficacy of anthelmintics against gastrointestinal nematodes of sheep using faecal egg count reduction (FECR) test. Forty five sheep 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 EPG estimated and divided into three groups i.e. S1, S2 and S3 of 15 animals each. Group S1 and S2 were treated with fenbendazole @ 5 mg/kg b.wt. orally and morantel @ 10 mg/kg b.wt. orally, respectively. Group S3 served as untreated control.

Faecal egg count of each animal was ascertained on 0 day and 12<sup>th</sup> day post treatment (PT), by the modified McMaster technique to an accuracy of one egg counted representing 50 EPG. Pooled faecal cultures at  $27 \pm 2^\circ\text{C}$  for 7 days were made to recover infective larvae ( $L_3$ ), from each group on day 0 and 12<sup>th</sup> day PT. 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). The drug was considered fully effective when they reduced the egg counts by more than 95% and lower confidence limits were higher than 90%. The drug was considered moderately resistant when they 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 were below 90%. All the recorded data was statistically analyzed by one way ANOVA test (SPSS software version 2.0).

## RESULTS AND DISCUSSION

Faecal egg counts (Mean  $\pm$  S.E.) on 0 and 12<sup>th</sup> day post-treatment (PT), percent reduction in faecal egg counts (FECR%), variance, upper and lower confidence limits (95%) of sheep naturally infected with gastrointestinal

nematodes and treated with different anthelmintics at Badhra village, Charkhi Dadri are given in table 1. Results revealed that fenbendazole @ 5 mg/kg b. wt. (Group S1) reduced the faecal egg counts by 74.77% on 12<sup>th</sup> day PT with upper and lower confidence levels as 86.41% and 53.15%, respectively indicating moderate anthelmintic resistance. Fenbendazole belongs to benzimidazole class and its resistance to gastrointestinal nematodes in sheep had been reported by many workers from our country (Sharma *et al.*, 2015; Kumari *et al.*, 2017) as well as abroad (Dolinska *et al.*, 2014). The repeated administration of the compound predisposes the nematodes to develop resistance. History revealed that fenbendazole was frequently used after morantel depending upon availability and convenience of owner.

Further, morantel @ 10 mg/kg b. wt. (Group S2) caused 49.84% reduction in faecal egg counts with upper and lower confidence levels as 65.04% and 28.04%, respectively indicating severe anthelmintic resistance. The resistance of morantel against GI nematodes has also been reported by Singh and Gupta (2010) at Badhra village of Haryana, Buttar *et al.* (2012) at Government Sheep Breeding Farm, Ludhiana, Punjab and Kumar and Singh (2016) at Central Sheep Breeding Farm, Hisar. History of use of anthelmintic and government supply in veterinary hospitals revealed that this is the most commonly used and supplied drug.

The coproculture of pooled faecal cultures of infective third stage larvae in different groups and untreated control on day 0 and 12 (PT) was depicted in Table 2. A total of 100 infective larvae in each group (S1, S2 and S3) were counted. The result showed different genera of GI nematodes of sheep with the predominance of *H. contortus* (88-90%) followed by *Strongyloides* sp. (4-5%), *Trichostrongylus* sp. (5%) and only 1-2% *Oesophagostomum* spp. larvae in all the treatment and untreated control groups on day 0. After 12 days of treatment, *H. contortus* was the only species found to survive in all treatment groups. The presence of only *H. contortus* larvae was also reported by Vadlejch *et al.* (2014); Sharma *et al.* (2015) and Kumar and Singh, (2016). The strain of *H. contortus* resistant to various anthelmintics in sheep have already been reported by Fleming *et al.* (2006) and Singh *et al.* (2013).

It may be concluded that the choice of anthelmintic in a flock should be based on the previous history of use of

**Table 1:** Mean faecal egg counts and percent faecal egg count reduction (FECR) in sheep after treatment with different anthelmintics

Group	Anthelmintic	Dose (mg/kg)	No. of sheep treated	Route of administration	Faecal egg counts on days (Mean $\pm$ S.E.)		Faecal egg counts reduction on day 12 post treatment		Confidence limits at 95%	
					0	12	%	Variance	Upper	Lower
S 1	Fenbendazole	5	15	Oral	2440.00 <sup>a</sup> $\pm$ 401.52	540.00 <sup>b</sup> $\pm$ 160.3	74.77	0.09	86.41	53.15
S 2	Morantel	10	15	Oral	2793.33 <sup>a</sup> $\pm$ 476.14	1073.33 <sup>b</sup> $\pm$ 179.28	49.84	0.03	65.04	28.04
S 3	Control	—	15	—	2360.00 <sup>a</sup> $\pm$ 120.63	2140.00 <sup>a</sup> $\pm$ 120.63	0	---	---	---

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

**Table 2:** Pre and post treatment larval culture results in sheep treated with different anthelmintics

Group	Species	Sheep	
		Percent larval composition on day (%)	
		0	12
S1- Fenbendazole	<i>Haemonchus</i> spp.	88	100
	<i>Trichostrongylus</i> spp.	5	0
	<i>Oesophagostomum</i> spp.	2	0
	<i>Strongyloides</i> sp.	5	0
S2- Morantel	<i>Haemonchus</i> spp.	90	100
	<i>Trichostrongylus</i> spp.	5	0
	<i>Oesophagostomum</i> spp.	1	0
	<i>Strongyloides</i> sp.	4	0
S3- Control	<i>Haemonchus</i> spp.	89	89
	<i>Trichostrongylus</i> spp.	5	4
	<i>Oesophagostomum</i> spp.	1	1
	<i>Strongyloides</i> sp.	5	6

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 resistance may be estimated at least once in two years. The drugs which show partial resistance should be changed immediately and discontinued for some years so that the larval population resistant to the drug is diluted and the portion of susceptible larval population is increased in the sheep flocks. Due to frequent use of all classes of anthelmintics in sheep of this flock, resistance against all the classes of anthelmintics has developed. This is the first report of cross anthelmintic resistance in a single sheep flock from village conditions of aeolian plains of Haryana.

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