The Pharma Innovation Journal 2019; 8(8): 174-177

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(8): 174-177

www.thepharmajournal.com
Received: 22-06-2019
Accepted: 24-07-2019

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Multiple anthelmintic resistance in a village sheep flock in Mahendergarh district (Haryana)

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Abstract
Sixty sheep with egg per gram of more than or equal to 150 were divided into four groups i.e. S1, S2, S3 and S4 of 15 animals each to assess the prevalence of anthelmintic resistance in gastrointestinal nematodes against commonly used anthelmintics in Mahendergarh district (Haryana). Group S1, S2 and S3 were treated with fenbendazole @ 5 mg/kg b.wt. orally, morantel @ 10 mg/ kg b.wt. orally and ivermectin @ 0.2 mg/kg b.wt. subcutaneously, respectively. Group S4 served as untreated control. Faecal egg count was ascertained on day of treatment (0 day) and 12th day post treatment (PT), from sheep of all groups and individual faecal egg counts 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 reduced the faecal egg counts by 67.19% on 12th day PT with upper and lower confidence levels as 83.26% and 35.69%, respectively, morantel caused 92.11% reduction in faecal egg counts with upper and lower confidence levels as 96.77% and 80.74%, respectively and ivermectin caused 80.44% reduction in faecal egg counts with upper and lower confidence levels as 90.82% and 58.32%, respectively indicating moderate anthelmintic resistance for all three drugs. The post-treatment coproculture showed *Haemonchus contortus* and *Strongyloides* spp. larvae. Thus, the present study revealed presence of multiple anthelmintic resistance against fenbendazole, morantel and ivermectin in sheep of Mahendergarh district in Haryana.

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

Introduction
Sheep and goat rearing has been a major source of income especially to the marginal farmers of the country (Pathak and Pal, 2008) [12]. Gastrointestinal parasitic infection is a serious threat to small ruminant production systems. In fact, most of the economic losses caused by internal parasites are due to associated production losses in terms of decreased milk / wool production, poor hair coat or fleece growth, cost of prevention, cost of treatment and the death of infected animals (Gwaze *et al*., 2009) [7]. Parasitic gastroenteritis caused by many gastrointestinal (GI) nematodes like *Haemonchus contortus*, *Trichostrongylus* spp., *Oesophagostomum* spp., *Nematodirus* spp. and *Strongyloides papillosus* and is the major constraint in profitable animal husbandry practice. 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) [26]. The degree of parasitism or worm burden greatly depends on the management and hygienic conditions of the area (Singla, 1995) [23]. Control of GIT parasites is mainly achieved by the use of anthelmintic drugs and it will continue to remain, as there seems to be no other alternative for helminth control in small ruminants (Sanyal, 2004) [17]. The extensive use of anthelmintics for control of gastrointestinal nematodes has resulted in development of resistance to one or more of the widely used anthelmintics in many countries. (Maingi *et al*., 1998) [11]. In addition, multiple resistance to most of the anthelmintics against gastrointestinal nematodes have also been detected in many countries (Paraud *et al*., 2009) [14]. Thus, regular monitoring of status of anthelmintic resistance is required, atleast once in two years and it is as an integral part of worm control programme (Rialch *et al*., 2013) [15]. The present study was envisaged to detect the status of anthelmintic resistance to the most commonly used anthelmintic *viz*. fenbendazole, morantel and ivermectin against gastrointestinal nematodes of sheep in Mahendergarh district of Haryana.

Materials and Methods
During August, 2018, a study was conducted at village Satnali, District Mahendergarh, Haryana to determine the efficacy of anthelmintics against gastrointestinal nematodes of sheep using faecal egg count reduction (FECR) test.

Sixty 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 four groups i.e. S1, S2, S3 and S4 of 15 animals each. Group S1, S2 and S3 were treated with fenbendazole (FENAZOL-150® tablets, Concept Pharmaceuticals Ltd., Animal Health Division, Mumbai) @ 5 mg/kg b.wt. orally, morantel (Banminth® Tab., Boehringer Ingelheim India Private Ltd. Mumbai) @ 10 mg/kg b.wt. orally and ivermectin (Trumectin®, Zydus Animal Health Limited, Ahmadabad) @ 0.2 mg/kg b.wt. subcutaneously, respectively. Group S4 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 was incubated in petridish at 27 ± 2˚C for 7 days and the infective larvae (L_3) were recovered from each group on day 0 and 12th day PT. The infective larvae were identified as per criteria of (Keith, 1953)[9]. Faecal egg count reduction percentage 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 (Coles *et al*., 1992) [3]. 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 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 sheep naturally infected with gastrointestinal nematodes and treated with different anthelmintics at Satnali village, Mahendergarh are given in table 1. Results revealed that fenbendazole @ 5 mg/kg b. wt. (Group S1) reduced the faecal egg counts by 67.19% on 12th day PT with upper and lower confidence levels as 83.26% and 35.69%, respectively indicating moderate anthelmintic resistance. Further, morantel @ 10 mg/kg b. wt. (Group S2) caused 92.11% reduction in faecal egg counts with upper and lower confidence levels as 96.77% and 80.74%, respectively, again indicating moderate anthelmintic resistance. Ivermectin @ 0.2 mg/kg b. wt. (Group S3) caused 80.44% reduction in faecal egg counts with 95% upper and lower confidence levels as 90.82% and 58.32%, respectively, also indicating moderate anthemintic resistance.

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

Group	Anthelmintic	Dose (mg/kg)	No. of sheep 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
S1	Fenbendazole	5	15	Oral	$1866.67^{a} \pm 137.55$	$693.33^{b} \pm 217.66$	67.19	0.11	83.26	35.69
S2	Morantel	10	15	Oral	$1560.00^{a} \pm 98.46$	$166.67^{b} \pm 70.82$	92.11	0.19	96.77	80.74
S3	Ivermectin	0.2	15	S/C	$1660.00^{a} \pm 136.56$	$413.33^{b} \pm 147.32$	80.44	0.14	90.82	58.32
S4	Control	---	15	---	$2226.67^{a} \pm 238.54$	$2113.33^{a} \pm 205.37$	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 (S1, S2, S3 and S4) were counted. The result showed different genera of GI nematodes of sheep with the predominance of *H. contortus* (83-86%) followed by *Strongyloides* sp. (10-12%), *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 sheep at Satnali village, Mahendergarh

Group	Species	Sheep	
		Per cent larval composition on day	
		0	12
S1-Fenbendazole	*Haemonchus* spp.	86	90
	Trichostrongylus spp.	4	0
	Oesophagostomum spp.	1	0
	Strongyloides sp.	11	10
S2- Morantel	*Haemonchus* spp.	83	91
	Trichostrongylus spp.	5	0
	Oesophagostomum spp.	0	0
	Strongyloides sp.	12	9
S3- Ivermectin	*Haemonchus* spp.	85	34
	Trichostrongylus spp.	4	0
	Oesophagostomum spp.	1	0
	Strongyloides sp.	10	66
S4- Control	*Haemonchus* spp.	83	85
	Trichostrongylus spp.	5	4

	Oesophagostomum spp.	1	1
	Strongyloides sp.	11	10

Discussion

The faecal egg counts on 0 and 12th day post-treatment (PT) and upper and lower confidence limits in sheep of Satnali village, Mahendergarh revealed presence of multiple anthelmintic resistance against fenbendazole, morantel and ivermectin. Fenbendazole belongs to benzimidazole class and its resistance to gastrointestinal nematodes in sheep had been reported by many workers (Vohra *et al*., 2013; Dolinska *et al*., 2014; Sharma *et al*., 2015; Singh *et al*., 2017) [4, 19, 20, 25]. 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. The resistance of morantel against GI nematodes has also been reported by other workers (Singh and Yadav, 1997; Kumar and Singh, 2016) [10, 22]. History revealed that morantel was frequently used after fenbendazole depending upon availability and convenience of owner. The resistance of ivermectin has also been reported by many workers (Howell *et al*., 2008; Falzon *et al*., 2013; Pena-Espinoza *et al*., 2014; Sharma *et al*., 2015; Kumar and Singh, 2016) [5, 8, 10, 13, 19]. This drug has been used sometimes in the flock. Coles *et al.* (1999) [2] have reported the development of anthelmintic resistance even when two or three treatment are given annually. The multiple anthelmintic resistance against fenbendazole, morantel and ivermectin was also reported by other workers (Singh and Gupta, 2010; Butter *et al*., 2012, Garcia *et al*. 2016) [1, 6, 21].

The coproculture of pooled faecal cultures of infective third stage larvae in different groups on day 12 (PT) showed the predominance of *H. contortus* larvae in fenbendazole and morantel treated animals while *Strongyloides* sp. larvae was predominant in ivermectin treated animals. The presence of *H. contortus* and *Strongyloides* sp. larvae was also reported by workers (Sangwan *et al*., 2006; Sarika, 2012; Sharma *et al*., 2015) [16, 18, 19]. 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) [24] and unhygienic management conditions of the farm.

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.

Conclusion

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. This is the first report of multiple anthelmintic resistance against all commonly used anthelmintics in a sheep flock from Mahendergarh in Haryana.

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