Haryana Vet. (June, 2022) **61(1),** 82-85

Research Article

DETECTION OF MULTIPLE ANTHELMINTIC RESISTANCE IN TWO UNORGANIZED SHEEP FARMS FROM SEMI-ARID ZONE OF HARYANA

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Received: 23.06.2021; Accepted: 21.12.2021

ABSTRACT

Faecal egg count reduction test (FECRT) was used to assess the status of anthelmintics against gastrointestinal nematodes at two unorganized sheep farms of semi-arid zones of Haryana state. A total of 120 sheep, 60 each from Sinsar village, Jind (SVJ) and Budhakhera village, Kaithal (BVK) with at least 150 egg per gram (EPG) of faeces were selected. Sheep were divided in four groups of 15 animals each in SVJ (S1, S2, S3 and S4) and BVK (B1, B2, B3 and B4) farms. Group S1 and B1 were treated with fenbendazole (@ 5 mg/kg b.wt. orally, FBZ), group S2 and B2 were treated with closantel (@ 10 mg/kg b.wt. orally, CLS), group S3 and B3 were treated with ivermectin (0.2 mg/kg, subcutaneous injection, IVM) and group S4 and B4 served as untreated control. Faecal samples were collected on zero and 14th day after treatment from all groups and egg counts were done by Modified Mc Master technique. Per cent reduction in faecal egg counts by FBZ, CLS and IVM in SVJ was 65.82, 82.27 and 74.68 and in BVK was 75.26, 92.47 and 82.79, respectively. The present study indicates the presence of moderate anthelmintic resistance against FBZ, CLS and IVM and predominance of *Haemonchus contortus* larvae in two unorganized sheep farms from semi-arid zones of Haryana.

Keywords: Anthelmintic resistance, Closantel, Fenbendazole, Haryana, Ivermectin, Sheep

How to cite: Kalkal, H. and Vohra, S. (2022). Detection of multiple anthelmintic resistance in two unorganized sheep farms from semi-arid zone of Haryana. *Haryana Vet.* **61(1)**: 82-85.

Sheep farming is important source of income for low earning and landless farmers of India. These animals contribute towards their livelihood by providing a rich source of protein, wool as well as milk. Haryana has 0.289 million sheep population as per DAHD, Haryana (2020) i.e. 0.08 % of total livestock. Gastrointestinal nematodosis is one of the major constraints in limiting the production which in terms lead heavy economic losses in terms of meat and wool production (Jacobson *et al.*, 2020). Among these gastrointestinal nematodes, *Haemochus contortus* is the prime pathogenic nematode of abomasum widely prevalent and important worm in sheep (Kotze and Prichard 2016). The prevalence and intensity of these nematodes has led to the widespread use of anthelmintics. The incorrect dosing and increased frequency of treatment by anthelmintics have often led to the development of anthelmintic resistance (Falzon *et al.*, 2013). There are many reports of anthelmintic resistance from different parts of India (Singh and Gupta, 2010; Rialch *et al.*, 2013 and Kumar and Singh 2016) as well as from other countries (Santiago-Figueroa *et al.*, 2019; Dey *et al.*, 2020). As anthelmintic status changes with time and year which makes the regular monitoring is important. Considering, these factor the present study was planned to elucidate the current status of anthelmintic in unorganized sheep farms of semi-arid zones of Haryana against gastrointestinal nematodes.

MATERIALS AND METHODS

The present study was conducted in district Jind and Kaithal falling under semi-arid zones of Haryana. Two unorganized sheep farms from Sinsar village, Jind (SVJ) and Budhakhera village, Kaithal (BVK) were selected to determine the efficacy of anthelmintics against gastrointestinal nematodes using faecal egg count reduction test (FECRT) as described by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles *et al.*, 1992). A total of 60 animals from each village naturally infected with gastrointestinal nematodes having overall healthy status (with EPG of more than 150 prior to treatment) were selected. The selected animals had not been administered any anthelmintics during the previous three months. Four groups of 15 sheep (S1, S2, S3 and S4) of SVJ and (B1, B2, B3 and B4) of BVK were created. Group S1 and B1 was treated with fenbendazole (@ 5 mg/kg b.wt. orally), group S2 and B2 with closantel (10 mg/kg b.wt. orally), group S3 and B3 with ivermectin (0.2 mg/kg b.wt. subcutaneous injection) and group S4 and B4 served as untreated control. Faecal egg count of each animal was ascertained on zero and 14th day post treatment (PT) by modified McMaster technique (Singh and Gupta, 2010) to an accuracy of one egg counted representing 50 EPG. Pooled faecal cultures (Singh and Gupta, 2010) were kept at 27±2 °C for 7 days to recover infective third stage larvae from each group. 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 WAAVP using arithmetic mean egg counts. The drug was considered fully effective when they reduced the egg counts by more than 95% and lower confidence

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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 using SPSS software version 27.0.

RESULTS AND DISCUSSION

The faecal egg counts (Mean ± S.E.) on 0 and 14th day post treatment (PT), percent reduction in faecal egg counts (FECR%), variance, upper and lower confidence limits (95%) for fenbandazole, closantel and ivermectin in sheep naturally infected with gastrointestinal nematodes at SVJ and BVK are given in table 1 and 2, respectively. The 99% of egg observed were *Strongyles* spp. and 1% was *Trichuris* spp. Results revealed that FBZ, CLS and IVM reduced the faecal egg counts by 65.82%, 82.27% and 74.68% in SVJ and 75.26%, 92.47% and 82.79% in BVK,

Fig. 1. Infective third stage larvae of *Haemonchus contortus* kinked tail (40X)

Table 1

Pre and post anthelmintic treatment faecal egg counts in sheep naturally infected with gastrointestinal nematodes at Sinsar village, Jind

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 14 post treatment		Confidence limits at 95%	
					0	14	%	Variance	Upper	Lower
S1	Fenbendazole	5	15	Oral	$606.66^{a} \pm 1.1.68$	$180^{b} \pm 38.04$	65.82	0.06	79.68	42.51
S2	Closantel	10	15	Oral	$653.33^{a} \pm 103.21$	$93.33^{b} \pm 37.11$	82.27	0.17	92.63	57.36
S3	Ivermectin	0.2	15	S/C	$626.66^{a} \pm 117.70$	$133.33^{b} \pm 43.27$	74.68	0.12	87.84	47.28
S4	Control	—	15	—	$633.33^{a} \pm 80.27$	$526.66^{a} \pm 67.94$	0	—	—	—

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

Table 2

Pre and post anthelmintic treatment faecal egg counts in sheep naturally infected with gastrointestinal nematodes at Budhakhera village, Kaithal

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 14 post treatment		Confidence limits at 95%	
					0	14	%	Variance	Upper	Lower
B1	Fenbendazole	5	15	Oral	$693.33^{a} \pm 80.75$	$153.33^{b} \pm 60.05$	75.26	0.16	89.57	41.31
B2	Closantel	10	15	Oral	$700^{a} \pm 91.54$	$46.66^{b} \pm 35$	92.47	0.57	98.47	62.81
B3	Ivermectin	0.2	15	S/C	$706.66^{a} \pm 86.44$	$106.66^{b} \pm 53.86$	82.79	0.27	94.23	48.67
B4	Control	—	15	—	$726.66^{a} \pm 81.33$	$620^{a} \pm 78.19$	0	—	—	—

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

Table 3

Pre and post anthelmintic treatment coproculture results at Sinsar village, Jind (SVJ) and Budhakhera village, Kaithal (BVK)

98Group	Species	SVJ Per cent larval composition on day		BVK Per cent larval composition on day	
		0	14	0	14
I-Fenbendazole	*Haemonchus* spp.	71	98	73	91
	Trichostrongylus spp.	9	2	7	9
	Oesophagostomum spp.	9	0	2	0
	Strongyloides spp.	11	0	8	0
II-Closantel	*Haemonchus* spp.	81	88	91	83
	Trichostrongylus spp.	10	12	2	17
	Oesophagostomum spp.	2	0	2	0
	Strongyloides spp.	7	0	5	0
III-Ivermectin	*Haemonchus* spp.	77	86	95	98
	Trichostrongylus spp.	9	14	2	2
	Oesophagostomum spp.	7	0	2	0
	Strongyloides spp.	7	0	1	0
IV-Control	*Haemonchus* spp.	74	80	79	84
	Trichostrongylus spp.	6	10	11	6
	Oesophagostomum spp.	1	5	4	2
	Strongyloides spp.	9	5	6	8

respectively indicating moderate resistance against all anthelminthics. While, *H. contortus* larvae showed highest resistance followed by *Trichostrongylus* spp. at these unorganized sheep farms. Sheep of farms were healthy but some sheep showed signs of anemia were treated accordingly their symptoms.

Reduction of fecal egg count by fenbendazole indicates resistance in SVJ and BVK farms. This may be due to continual use of this drug along with other anthelmintics, as per availability, convenience and supply from government in veterinary hospitals. Once the benzimidazole resistant population of nematodes has developed, it continues to persist in the absence of any benzimidazole use over years in the field (Kotze and Prichard 2016). The resistance to fenbendazole in gastrointestinal nematodes of sheep has been reported in India by Kumar and Singh (2016) in Haryana (44.49% resistance) and Rialch *et al.* (2013) in sub-Himalyan region of northern India (92.40% resistance) as well as abroad by Falzon *et al.* (2013) in Canada (28.6% resistance).

Further, closantel @ 10 mg/kg b.wt. reduced faecal egg count by 82.27% and 92.47% in SVJ and BVK, respectively indicating resistance. The reason for moderate resistance against closantel may be due to reduced accumulation of drug in parasite body by mechanism such as reduced feeding, failure to dissociate the drug-albumin complex in the gut or increased efflux of drugs from resistant worms. Similar observation was reported by Gupta *et al.* (2003) in sheep of western Haryana (89.2% resistance) and Flavia da Silva *et al.* (2018) in Brazil (84.4% resistance).

Ivermectin belongs to avermectins group of drugs which is effective against gastrointestinal nematodes as well as ecto-parasite therefore termed as endectocites (El-Saber Batiha *et al.*, 2020). The ivermectin reduced faecal egg count by 74.68% and 82.79% in SVJ and BVK, respectively indicating moderate resistance. Ivermectin is widely and commonly used compound by the veterinarian without estimating body weight of animals well as frequency of treatment. Resistant, against gastrointestinal nematodes has been also reported by Singh and Gupta (2010) in Hisar (92% resistance), Ploegera and Everts (2018) in Netherlands (84.9% resistance) and Kumar and Singh (2016) in Haryana (68.02% resistance). So, there is a need to be vigilant while using anthelmintic against gastrointestinal nematodes of small ruminants.

The pooled faecal cultures of infective third stage larvae in different groups and untreated control on day zero and 14 PT are depicted in Table 3. A total of 100 infective larvae in each group (S1, S2, S3 and S4) of SVJ and (B1, B2, B3 and B4) of BVK were counted. The result showed different genera of gastrointestinal nematodes of sheep with the predominance of *H. contortus* (71 to 95%) followed by *Trichostrongylus* spp. (10-11%), *Strongyloides* spp. (9-11%), and *Oesophagostomum* spp. (9-7%) larvae in all the treated and untreated control groups on day zero in both

villages. After 14 days of treatment, there was predominance of *H. contortus* (Fig.1) resistance larvae in fenbendazole, closantel and ivermectin treated animals also *Strongyloides* spp. and *Oesophagostomum* spp. did not any resistance. The presence of *H. contortus* as predominant larvae after treatment with fenbendazole, closantel and ivermectin was also reported by, Kumar and Singh (2016).

CONCLUSION

The anthelmintic should be administrated as per the body weight, history of use of drug, frequency of use of drug and status of anthelmintic resistance in the farm. So, according to the report multiple anthelmintic resistance is present in these unorganized sheep farms from semi-arid zones of Haryana.

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