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Multiple Anthelmintic Resistance in Goat Farms from Semi-Arid Zone of Haryana

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ABSTRACT

Two unorganized goat farms from dry semi-arid zone of Haryana were surveyed to assess the status of anthelmintics against gastrointestinal nematodes using faecal egg count reduction test (FECRT). A total of 120 goats, 60 each from Sighwal village, Jind (SVJ) and Andhli village, Kaithal (AVK) with at least 150 egg per gram (EPG) of faeces were selected. Goats were divided into four groups of 15 animals each in SVJ (G1, G2, G3 and G4) and AVK (B1, B2, B3 and B4) farms. Group G1 and B1 were treated with fenbendazole (@ 10 mg/kg b.wt. orally), group G2 and B2 were treated with closantel (@ 20 mg/kg b.wt. orally), group G3 and B3 were treated with ivermectin (0.4 mg/kg, subcutaneous injection) and group G4 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 fenbendazole, closantel and ivermectin in SVJ was 63.01, 86.01 and 72.60 and in AVK was 57.31, 80.48 and 78.04, respectively. The post-treatment coproculture of both SVJ and AVK farms showed only *Haemonchus contortus* larvae. Thus, the present study indicates the presence of resistance against fenbendazole, closantel and ivermectin i.e. multiple anthelmintic resistance in unorganized goat farms from semi-arid zone of Haryana.

HIGHLIGHTS

- Fenbendazole, closantel and ivermectin are commonly used anthelmintics.
- All the gastrointestinal parasite of goat farms showed multiple anthelmintic resistance.

Keywords: Anthelmintic resistance, Closantel, Fenbendazole, Haryana, Ivermectin and Goats.

The goat industry plays an important role in the economy of the country due to low initial input, less maintenance cost, minimum disease exposure and quick, high and profitable returns. In Haryana state (27°39' to 30°35' N and 74°28' to 77°36' E) goat population is 0.335 million as per DAHD, Haryana (2020). The gastrointestinal Nematodes (GINs) of small ruminants are effectively controlled by chemical based anthelmintics, however, continuous and improper use of anthelmintics has led to widespread selection of resistant nematodes within populations (Falzon *et al*., 2013). In India, *Haemonchus contortus* is the most prevalent and pathogenic GIN and is responsible for high morbidity and mortality. The control of this parasite is not only important but essential for profitable goat farming and it relies primarily on the use of anthelmintic drugs. There are many classes of anthelmintic like benzimidazole, *imidazothiazole*, pyrimidines, *salicylanilides*, organophosphates, piperazine and *avermectins* used for treatment and control of gastrointestinal nematodes. The frequent and indiscriminate use of these compounds on approximate body weight has resulted in underdosing of drugs, thus causing widespread occurrence of anthelmintic resistance. There are many reports of anthelmintic resistance from different parts of India in goat viz. Rialch *et al*. (2013) in

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sub-Himalyan region of northern India, Vohra *et al*. (2013) in Hiar and Bihaqi *et al*. (2020) in Kashmir. No new class of anthelmintics has been introduced in the market in last 25 years (Kaplan, 2004). Therefore, regular monitoring of status of anthelmintic efficacy for the existing drugs is required, at least once in two years in an unorganized flock for suitable worm control programme. Therefore, the present study was aimed to investigate the status of fenbendazole, closantel and ivermectin anthelmintics against gastrointestinal nematodes of goats reared in semi-arid zone of Haryana.

MATERIALS AND METHODS

The present study was conducted in goats (Beetal) from semi-arid zone of Haryana. For this two unorganized goat farms from Sighwal village, Jind (SVJ) and Andhli village, Kaithal (AVK) 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). Sixty animals from each village naturally infected with gastrointestinal nematodes and having EPG of faeces more than 150 counts prior to treatment were selected. The selected animals had not been administered any anthelmintics during the previous two months. These animals were identified, weighed and their EPG was estimated. Goats from each village were divided into four groups of 15 animals i.e. G1, G2, G3 and G4 of SVJ and B1, B2, B3 and B4 of AVK. Group G1 and B1 were treated with fenbendazole (@ 10 mg/kg b.wt. orally, Panacur®, MSD), group G2 and B2 with closantel (@ 20 mg/kg b.wt. orally, Zycloz®, Zydus), group G3 and B3 with ivermectin (@ 0.4 mg/kg b.wt. subcutaneous injection, Zenvet®, INTAS) while group G4 and B4 served as untreated control. The faecal egg count of each animal was ascertained on zero and 14th day post-treatment (PT) by modified McMaster technique to an accuracy of one egg counted representing 50 EPG. Pooled faecal cultures 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 the 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 it 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 using SPSS software version 27.0.

RESULTS AND DISCUSSION

The faecal egg counts (Mean ± S.E.) on 0 and 14th day PT, percent reduction in faecal egg counts (FECR%), variance, upper and lower confidence limits (95%) for fenbendazole, closantel and ivermectin in goat naturally infected with gastrointestinal nematodes at Sighwal village, Jind and Andhli village, Kaithal are given in table 1 and 2, respectively. Results revealed that fenbendazole, closantel and ivermectin reduced the faecal egg counts by 63.01%, 86.30% and 72.60% in SVJ and 57.31%, 80.48% and 78.04% in AVK, respectively. The result indicates severe and moderate resistance against fenbendazole in AVK and SVJ, respectively. However, moderate resistance against closantel and ivermectin was observed in both SVJ and AVK unorganized goat farms from semi-arid zone of Haryana.

The fenbendazole @ 10 mg/kg b. wt. reduced faecal egg count by 57.31% and 63.01% in AVK and SVJ, respectively indicating resistance. The resistance against fenbendazole may be due to the following factors: treating animals with fenbendazole without knowing the appropriate bodyweight of animals which lead to under-overdosing, frequent and prolonged use of fenbendazole. Also, the farmers are unaware of the usage of fenbendazole at twice the recommended dose in goats. Once the benzimidazole resistant population of nematodes develop, it continues to persist in the absence of any benzimidazole use over years in the field (Webb *et al*. 1979 and McKenna, 1990). The resistance to fenbendazole in GINs of goats has been reported in India by Vohra *et al.* (2013) in Hisar (79.04% efficacy) and Bihaqi *et al*. (2020) in Kashmir Valley (62.5% efficacy) as well as aboard by Chartier *et al*. (2001) in France (64.3% efficacy) and Pena-Espinoza *et al*. (2014) in Denmark (56% efficacy).

Futher, closantel @ 20 mg/kg b. wt. reduced faecal egg count by 86.30% and 80.48% in SVJ and AVK, respectively indicating resistance. The resistance against closantel

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

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 14post-treatment		Confidence limits at 95%	
					0	14	%	Variance	Upper	Lower
I	Fenbendazole	10	15	Oral	600^{a}±112.54	180^{b}±39.27	63.01	0.06	78.15	37.38
II	Closantel	20	15	Oral	600^{a}±100.47	66.66^{b}±25.19	86.30	0.15	94.05	68.42
III	Ivermectin	0.4	15	S/C	613.33^{a}±124.33	133.33^{b}±37.37	72.60	0.09	85.59	47.87
IV	Control	—	15	—	606.66^{a}±86.44	486.66^{a}±60.05	0	—	—	—

Table 2: Response to various anthelmintics in goats naturally infected with gastrointestinal nematodes at Andhli village, Kaithal

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 14post-treatment		Confidence limits at 95%	
					0	14	%	Variance	Upper	Lower
I	Fenbendazole	10	15	Oral	706.66^{a}±83.64	233.33^{b}±82.61	57.31	0.14	80.78	51.18
II	Closantel	20	15	Oral	706.66^{a}±90.74	106.66^{b}±37.11	80.48	0.28	91.11	57.16
III	Ivermectin	0.4	15	S/C	713.33^{a}±94.54	120^{b}±47	78.04	0.17	90.82	47.48
IV	Control	—	15	—	700^{a}±92.58	546.66^{a}±75.50	0	—	—	—

Table 3: Effect of anthelmintics on different genera of gastrointestinal nematodes of goats at Sighwal village, Jind and Andhli village, Kaithal in semi-arid zone of Haryana

Group	Species	SVJ		AVK	
		Per cent larval composition on day		Per cent larval composition on day	
		0	14	0	14
I-Fenbendazole	*Haemonchus* spp.	84	100	86	100
	Trichostrongylus spp.	6	0	9	0
	Oesophagostomum spp.	5	0	3	0
	Strongyloides sp.	5	0	2	0
II-Closantel	*Haemonchus* spp.	85	100	83	100
	Trichostrongylus spp.	6	0	5	0
	Oesophagostomum spp.	4	0	7	0
	Strongyloides sp.	5	0	5	0
III-Ivermectin	*Haemonchus* spp.	79	100	92	100
	Trichostrongylus spp.	10	0	3	0
	Oesophagostomum spp.	3	0	3	0
	Strongyloides sp.	8	0	2	0
IV-Control	*Haemonchus* spp.	73	88	86	96
	Trichostrongylus spp.	7	2	5	2
	Oesophagostomum spp.	5	1	6	1
	Strongyloides sp.	15	9	3	1

may be due to the following factors: Prolonged activity of closantel i.e. for weeks after administration (Hall *et al*., 1981), indiscriminate usage, under-overdosing due to inappropriate dosing which differ from recommendations given by the manufacturer of drug. Further, the 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 closantel from resistant worms attribute to anthelmintic resistance (Rothwell and Sangster, 1997). A similar observation was reported in goats by Zajac and Gipson (2000) in USA (90%), Vohra *et al.* (2013) in Hisar (89.35% efficacy) and Bihaqi *et al*. (2020) in Kashmir Valley (90%).

The ivermectin @ 0.4 mg/kg b. wt. reduced faecal egg count by 72.06% and 78.04% in SVJ and AVK, respectively indicating resistance. Ivermectin belongs to avermectins group of drugs also known as endectocides and is effective against gastrointestinal nematodes as well as ectoparasite (El-Saber Batiha *et al*., 2020). Ivermectin is a safe compound widely used by the veterinarian against gastrointestinal nematodes and ectoparasite without proper body weight estimation. This practice of use of anthelmintics without proper examination of infection and on estimated bodyweight basis exposes the worms to drug and add to the resistant character. Resistant against gastrointestinal nematodes in goats has been also reported by Zajac and Gipson (2000) in USA (90% efficacy), Bihaqi *et al*. (2020) in Kashmir Valley (83.5% efficacy) and Vohra *et al.* (2015) in Hisar (87.01% efficacy). So, there is a need to be vigilant while using these anthelmintics 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 (G1, G2, G3 and G4) of SVJ and (B1, B2, B3 and B4) of AVK were counted. The result showed different genera of gastrointestinal nematodes of goats with the predominance of *H. contortus* (73-92%) followed by *Strongyloides* spp. (2-15%), *Trichostrongylus* spp. (5-9%) and *Oesophagostomum* spp. (3-7%) larvae in all the treated and untreated control groups on day zero in both villages. After 14th days of PT, there were 100% larvae of *H. contortus* in fenbendazole, closantel and ivermectin treated animals in both villages. The presence of only *H. contortus* larvae after treatment with fenbendazole, closantel and ivermectin was also reported by Vohra *et al.* (2013) in Hisar and Flavia da Silva *et al.* (2018) in Brazil.

CONCLUSION

The present study revealed the presence of anthelmintic resistance against fenbendazole, closantel and ivermectin in goats from Sighwal village, Jind and Andhli village, Kaithal. The presence of *H. contortus* larvae in PT culture revealed the parasite responsible for resistance. Therefore, it may be concluded that the choice of anthelmintic in a flock should be based on the previous history of use of the drug, frequency of use of the drug, dose of drug and status of anthelmintic resistance. This is the first report of multiple anthelmintic resistance against all commonly used anthelmintics in unorganized goat farms from semi-arid zone of Haryana.

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