

MULTIPLE ANTHELMINTIC RESISTANCE IN GOAT FARMS FROM DRY SUB-HUMID ZONE OF HARYANA, INDIA

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ABSTRACT

Two unorganized goat farms from dry sub-humid 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 Kharukhera village, Ambala (KVA) and Mehmoodpur village, Ambala (MVA) with at least average 150 egg per gram (EPG) of faeces were selected. Goats were divided into four groups of 15 animals each in KVA (G1, G2, G3 and G4) and MVA (B1, B2, B3 and B4) farms. Group G1 and B1 were treated with fenbendazole (@ 10 mg/kg b.wt. orally, FBZ), group G2 and B2 were treated with closantel (@ 20 mg/kg b.wt. orally, CLS), group G3 and B3 were treated with ivermectin (0.4 mg/kg, subcutaneous injection, IVM) and group G4 and B4 served as untreated control. Fresh faecal samples from rectum were collected on zero and 14th day before and after treatment, respectively from all groups. Quantitative fecal egg count test were done by Modified Mc Master technique. Percent reduction in faecal egg counts FBZ (57.94), CLS (91.58) and IVM (82.24) in KVA and in MVA by FBZ (70.00), CLS (89.00) and IVM (78.00) indicating severe to moderate anthelmintic resistance. The pre and post-treatment coproculture of both KVA and MVA farms showed majority of *Haemonchus contortus* larvae in unorganized goat farms from dry sub-humid zone of Haryana.

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

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Goats are the main meat producing animals in India which require almost negligible maintenance. In Haryana state goat population is 0.335 million as per DAHD, Haryana (2020). Being grazing animals goats are exposed to many gastrointestinal nematodes like *Haemonchus contortus*, *Trichostrongylus axei*, *T. colubriformis*, *T. vitrinus*, *Nematodirus spathiger*, *N. battus*, *Chabertia ovina*, *Oesophagostomum columbianum*, *O. venulosum*, *Bunostomum trigonocephalum*, *Gaigeria pachyscelis*, *Cooperia curticei*, *Mecistocirrus digitatus* and *Strongyloides papillosus* causing parasitic gastroenteritis. Among these gastrointestinal nematodes, *H. contortus* is number one and most pathogenic, widely prevalent and important worm in India (Yadav, 1997). These gastrointestinal nematodes are controlled mainly by the use of anthelmintic drug. The frequent and indiscriminate use of these compounds on approximate body weight has resulted in underdosing of drugs, thus causing wide spread occurrence of anthelmintic resistance. There are many reports of anthelmintic resistance from different parts of India in goats viz. Godara *et al.* (2011) in Jammu, Rialch *et al.* (2013) in sub-Himalayan region of northern part of India, Chandra *et al.* (2015) in Uttar Pradesh, Kalkal *et al.* (2019) in Hisar and Bihaqi *et al.* (2020) in Kashmir. Thus, regular monitoring of efficacy of the available drugs and examination of anthelmintic resistance is required, at least once in two years (Rialch *et al.*, 2013). Therefore, the present study was aimed to investigate the status of

fenbendazole, closantel and ivermectin anthelmintics against gastrointestinal nematodes of goats reared in dry sub-humid zone of Haryana India.

MATERIALS AND METHODS

The present study was conducted in goats from dry sub-humid zone of Haryana. For this two unorganized goat farms from Kharukhera village, Ambala (KVA) and Mehmoodpur village, Ambala (MVA) 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 was not been administered any anthelmintics before two months of trail. 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 KVA and B1, B2, B3 and B4 of MVA. 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-

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treatment (PT) by modified McMaster technique to an accuracy of one egg counted representing 50 EPG. Pooled faecal cultures were kept at $27\pm 2^{\circ}\text{C}$ for 7 days to recover infective third stage larvae from each group. The infective larvae were identified as per the criteria describe by 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 \pm 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 fenbendazole, closantel and ivermectin in goat naturally infected with gastrointestinal nematodes at Kharukhera village, Ambala and Mehmoodpur village, Ambala are given in table 1 and 2, respectively. Results revealed that fenbendazole, closantel and ivermectin reduced the faecal egg counts by 57.94%, 91.58% and 82.24% in KVA and 70%, 89% and 78% in MVA, respectively. The result indicates severe and moderate resistance against fenbendazole in KVA and MVA, respectively. However, moderate resistance against closantel and ivermectin was observed in both MVA and KVA unorganized goat farms from dry sub-humid zone of Haryana.

The fenbendazole @ 10 mg/kg b. wt. reduced faecal

egg count by 57.94% and 70% in KVA and MVA, respectively indicating resistance. The reason for resistance may be due to repeated use of the fenbendazole by farmers as the drug is supplied in the veterinary hospitals. 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 gastrointestinal nematodes of goats has been reported in India by Priyanka *et al.* (2019) in Hisar (14.23%), Singh *et al.* (2017) in Amritsar (45.33%) and in Ludhiana (86.55%) of Punjab and Bihaji *et al.* (2020) in Kashmir Valley (62.5%) as well as aboard by Chartier *et al.* (2001) in France (64.3%) and Pena-Espinoza *et al.* (2014) in Denmark (56%).

Futher, closantel @ 20 mg/kg b. wt. reduced faecal egg count by 91.58% and 89% in KVA and MVA, respectively indicating resistance. Closantel act by inhibiting energy metabolism i.e. uncoupling of oxidative phosphorylation (Martin, 1997). Prolonged activity of closantel i.e. for weeks after administration (Hall *et al.*, 1981) may the reason for development of resistance. A similar observation was reported in goats by Zajac and Gipson (2000) in USA, Kumsa and Abebe (2009) in Ethiopia and Bihaji *et al.* (2020) in Kashmir Valley.

Ivermectin belongs to avermectins group of drugs is effective against gastrointestinal nematodes as well as ectoparasite (El-Saber Batiha *et al.*, 2020). In the ivermectin treated group reduced faecal egg count by 82.24% and 78% in KVA and MVA, respectively. 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. Gastrointestinal nematodes

Table 1

Anthelmintics reponse in goats against gastrointestinal nematodes at Kharukhera village, Ambala

Group	Anthelmintic	Dose (mg/kg)	No. of goats treated	Route of Administration	Faecal egg counts on days (Mean \pm S.E.)		Faecal egg counts reduction on day 14 post treatment		Confidence limits at 95%	
					0	14	%	Variance	Upper	Lower
I	Fenbendazole	10	15	Oral	680 ^a \pm 79.40	300 ^b \pm 325.13	57.94	0.31	89.83	24.23
II	Closantel	20	15	Oral	653.33 ^a \pm 88.83	60 ^b \pm 129.83	91.58	0.32	98.76	53.72
III	Ivermectin	0.4	15	S/C	686.66 ^a \pm 90.95	126.66 ^b \pm 284.01	82.24	0.34	96.14	21.08
IV	Control	—	15	—	653.33 ^a \pm 897.58	713.33 ^a \pm 261.49	0	—	—	—

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

Table 2
Anthelmintics reponse in goats against gastrointestinal nematodes at Mehmoodpur village, Ambala

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 14 post treatment		Confidence limits at 95%	
					0	14	%	Variance	Upper	Lower
B1	Fenbendazole	5	15	Oral	693.33 ^a ± 80.75	153.33 ^b ± 60.05	75.26	0.16	89.57	41.31
I	Fenbendazole	10	15	Oral	653.33 ^a ± 96.04	200 ^b ± 62.48	70	0.13	85.98	35.77
II	Closantel	20	15	Oral	666.66 ^a ± 93.43	73.33 ^b ± 52.06	89	0.53	97.64	48.67
III	Ivermectin	0.4	15	S/C	653.33 ^a ± 89.90	146.66 ^b ± 60.05	78	0.20	91.42	43.53
IV	Control	—	15	—	706.66 ^a ± 133.97	666.66 ^a ± 122.53	0	—	—	—

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

Table 3
Effect of anthelmintics on different genera of gastrointestinal nematodes of goats at Kharukhera village, Ambala (KVA) and Mehmoodpur village, Ambala (MVA)

Group	Species	KVA		MVA	
		Per cent larval composition on day		Per cent larval composition on day	
		0	14	0	14
I-Fenbendazole	<i>Haemonchus</i> spp.	85	100	83	100
	<i>Trichostrongylus</i> spp.	5	0	7	0
	<i>Oesophagostomum</i> spp.	3	0	5	0
	<i>Strongyloides</i> sp.	7	0	5	0
II-Closantel	<i>Haemonchus</i> spp.	73	100	80	100
	<i>Trichostrongylus</i> spp.	7	0	10	0
	<i>Oesophagostomum</i> spp.	5	0	1	0
	<i>Strongyloides</i> sp.	15	0	9	0
III-Ivermectin	<i>Haemonchus</i> spp.	86	100	90	100
	<i>Trichostrongylus</i> spp.	4	0	5	0
	<i>Oesophagostomum</i> spp.	3	0	2	0
	<i>Strongyloides</i> sp.	7	0	3	0
IV-Control	<i>Haemonchus</i> spp.	88	94	93	91
	<i>Trichostrongylus</i> spp.	2	2	5	3
	<i>Oesophagostomum</i> spp.	5	2	1	5
	<i>Strongyloides</i> sp.	5	2	1	1

showed resistant in goats. It has been also reported by Zajac and Gipson (2000) in USA, Kumsa and Abebe (2009) in Ethiopia, Bihagi *et al.* (2020) in Kashmir Valley and Priyanka *et al.* (2019) in Hisar. So, there is a need to be vigilant while using these anthelmintics against gastrointestinal nematodes of small ruminants.

The pooled faecal cultures of positive samples showed infective third stage larvae in different groups and untreated control on day zero and fourteen post treatment are depicted in table 3. A total of 100 infective larvae in

each group (G1, G2, G3 and G4) of KVA and (B1, B2, B3 and B4) of MVA were counted. The result showed different genera of gastrointestinal nematodes of goats with the predominance of *H. contortus* (83-93%) followed by *Trichostrongylus* spp. (4-10%), *Strongyloides* spp. (3-15%), and *Oesophagostomum* spp. (1-5%) 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* (Fig. 1) in fenbendazole, closantel and ivermectin treated animals in both villages. The presence



Fig. 1. Infective third stage larvae of *Haemonchus contortus* (10X) of only *H. contortus* larvae after treatment with fenbendazole, closantel and ivermectin was also reported by Priyanka *et al.* (2019) in Hisar, Singh *et al.* (2017) in Punjab and Flavia da Silva *et al.* (2018) in Brazil.

CONCLUSION

The present study concluded the presence of anthelmintic resistance against fenbendazole, closantel and ivermectin in goats from Kharukhera village, Ambala and Mehmoodpur village, Ambala. The presence of *H. contortus* larvae in PT culture revealed the parasite responsible for resistance. Therefore the choice of anthelmintic in a flock should be based on the status of anthelmintic resistance and occurrence of gastrointestinal nematodes in the area. Report showing multiple anthelmintic resistance against all commonly used anthelmintics in unorganized goat farms from dry sub-humid zone of Haryana.

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