The Pharma Innovation Journal 2022; SP-11(5): 1080-1086

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(5): 1080-1086

www.thepharmajournal.com
Received: 07-03-2022
Accepted: 16-04-2022

Rahul Kumar Meena
Department of Veterinary Parasitology, PGIVER Rajasthan University of Veterinary and Animal Sciences, Jaipur, Rajasthan, India

C P Swarnkar
Division of Animal Health, ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India

Bhavana Rathore
Department of Veterinary Parasitology, PGIVER Rajasthan University of Veterinary and Animal Sciences, Jaipur, Rajasthan, India

Priyanka Saini
Department of Veterinary Parasitology, PGIVER Rajasthan University of Veterinary and Animal Sciences, Jaipur, Rajasthan, India

Abhishek Kumar Meena
Department of Veterinary Parasitology, PGIVER Rajasthan University of Veterinary and Animal Sciences, Jaipur, Rajasthan, India

Corresponding Author
C P Swarnkar
Division of Animal Health, ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India

Prevalence of anthelmintic resistance in gastrointestinal nematodes of goats of semi-arid Rajasthan

Rahul Kumar Meena, C P Swarnkar, Bhavana Rathore, Priyanka Saini and Abhishek Kumar Meena

Abstract
A study was conducted to assess the prevalence of anthelmintic resistance in gastrointestinal nematodes (GINs) of goats reared by farmers in semi-arid Rajasthan. From July to December 2021, a total of 28 flocks from five villages (Jaipur) were tested through *in-vivo* faecal egg count reduction test (FECRT) and *in-vitro* egg hatch assay (EHA). For FECRT, the test anthelmintics used were fenbendazole (@ 5.0 mg/kg body weight), levamisole (@ 7.5 mg/kg body weight) and closantel (@ 10.0 mg/kg body weight). On FECRT, the magnitude of reduction in the faecal egg counts exhibited prevalence of strains of GINs resistant to fenbendazole and levamisole in 100% of the flocks tested. Against closantel, 100% efficacy was observed against predominant *Haemonchus contortus*; however, for combined species of GINs 71.43% of flocks showed less than 95% efficacy. On EHA, among 28 flocks, benzimidazole resistant strains of strongyle worms were observed in 27 flocks (96.43%). A high agreement (96.42%) was observed between results from FECRT and EHA for benzimdazole resistance. It was inferred that small ruminant producers and veterinarians can no longer rely solely on benzimidazole and Imidazothiazole group of anthelmintics for effective parasite control and to preserve the drugs that are still effective, all must change their attitudes and approaches to parasite control.

Keywords: Anthelmintic resistance, egg hatch assay, faecal egg count reduction test, goat, Rajasthan, semi-arid

Introduction
The goats (*Poor man's Cow*) plays an important role in the economy of the country due to low initial input, less maintenance cost and quick, high and profitable returns. As per 20th livestock census, India has the second largest goat population (148.88 million, 27.8% of total livestock) in the world and Rajasthan stands first in the country with 20.84 million goats BAHS (2019). Throughout the World, gastrointestinal (GI) parasitism is one of the major health issue for grazing animals causing loss in body weight, impaired growth, decreased feed conversion ratio, reduced milk production and death in extreme situations (Torres-Acosta *et al.*, 2012) [39] with massive economic loss in tropical countries including India (Shah and Chaudhry, 1995; Singh *et al.*, 2015) [31, 33]. While farmers had effective antiparasitic drugs in the 1980s and 1990s, parasite resistance to anthelmintics developed quickly for all available drugs widely used in leading sheep and goat producing countries. The extensive and sole reliance on anthelmintics with their indiscriminate use (widespread use, incorrect dosing and increased frequency of treatment) have resulted in emergence of parasites, resistant to one or more of the widely used anthelmintics throughout world including India (Singh *et al.*, 2002; Yadav and Garg, 2007; Jaiswal *et al.*, 2013; Rialch *et al.* 2013; Chandra *et al.*, 2015; Rathod *et al.*, 2019; Bihaqi *et al.* 2020; Kalkal and Vohra, 2021) [35, 43, 21, 29, 7, 28, 4, 22]. Frequently, majority of field Veterinarians, Paravets and farmers from Jaipur region (Rajasthan) are reporting about poor response to anthelmintics in small ruminants. Therefore, regular monitoring of status of anthelmintic efficacy for the existing drugs is required for suitable and effective worm management programme. Hence, a study was conducted to assess the status of anthelmintic resistance inGI nematodes (GINs) in unorganized goat flocks reared in Jaipur district of Rajasthan using both *in vivo* Faecal Egg Count Reduction Test (FECRT) and *in vitro* Egg Hatch Assay (EHA).

Materials and Methods
Thestudy was conducted during July to December, 2021 in 28 goat flocks reared by farmers from five villages (Booj, Chawandiya, Sarjoli, Sindoli and Jhar) of Bassi and Jamwa Ramgarh Tehsil (Jaipur) in Semi-arid Rajasthan. Flocks having history of not using any anthelmintics for the last three months were selected.

All the other variables, such as breed, age, sex and history of nematodiosis were not considered. The flocks were managed under semi-intensive system by grazing during day hours on forest/community grazing land and rest in corrals at night. For worm management, in general, anthelmintics were used as per their knowledge / convenience at frequency of 1 to 4 times a year.

In-vivo FECRT was performed as per guidelines of World Association for Advancement of Veterinary Parasitology (Coles *et al.*, 1992) [10]. Animals within a flock were randomly divided into three treatment and one control groups (10 animals in each group). Before treatment, faecal samples were collected *per rectum* from each animal in separate polythene bags. The samples were evaluated for pre-treatment faecal egg counts by modified McMaster technique (MAFF, 1986). After pre-treatment sampling, animals of 1st, 2nd and 3rd groups were drenched with fenbendazole (Panacur®, Intervet @ 5.0 mg/kg body weight), levamisole (Nilverm®, AgriVet @ 7.5 mg/kg body weight) and closantel (Zycloz™, Zydus @ 10.0 mg/kg body weight), respectively. The animals in control group were left untreated for the natural changes in egg counts during the test period. All the animals were allowed to graze on same pasture. Post-treatment faecal samples from each animal were collected on day 14th and estimated faecal egg counts by modified McMaster technique. On both occasion left over faeces was pooled (flock-wise on pre-treatment and group-wise on post-treatment) and subjected to coproculture at 27 °C for 5-7 days in BOD incubator. The infective larvae were identified based on morphological features (Soulsby, 1982; Wyk and Mayhew, 2013) [36, 42].

In vitro EHA was performed for benzimidazole group of anthelmintics using pure thiabendazole (TBZ) as a reference drug in a dilution series of 0.0625 to 1.00 µg TBZ/ml in 24 multi-well plates as per Le Jambre (1976) and WAAVP guidelines (Coles *et al.*, 1992) [10].

The per cent faecal egg count reduction (%FECR) was calculated by using RESO computer programme (Martin and Wursthom 1991) [25] for strongyle worms as a whole and for different species of strongyle worms (*H. contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp.) based on generic composition of larvae. The results were interpreted as resistance if (i) the percentage reduction in egg counts was less than 95% and (ii) the 95% confidence level was less than 90. If only one of the two criteria was met, resistance was suspected (Coles *et al.*, 1992) [10]. Probit analysis was performed on EHA data to obtain TBZ concentration which on average would prevent 50% of eggs from hatching (ED_{50}) at 95% confidence limit (Finney, 1965) [15] and ED_{50}value in excess of 0.1 µg TBZ/ml was consideredas benzimidazole resistance (Coles *et al.*, 1992) [10].

Results and Discussion

On FECRT for combined *Strongyle* species, the overall mean post-treatment FEC was 519.64±47.03 epg in flocks treated with FBZ as compared to 1786.43±106.07 epg in control flocks with 69.04±2.95% FECR indicating resistance to benzimidaole group of anthelmintics (Table 1). Village-wise profile exhibited that in flocks, FECR with FBZ varied from 44 to 93% in Booj, 15 to 70% in Chawandiya, 59 to 79% in Sarjoli, 74 to 84% in Sindoli and 45 to 83% in Jhar (lower 95% confidence limits less than 90), suggesting prevalence of benzimidazole resistance in *Strongyle* worm population in all the flocks. In flocks treated with levamisole, the overall mean post-treatment FEC was 360.71±32.07 epg as compared to 1786.43±106.07 epg in control flocks with 78.36±2.10% FECR, suggesting resistance to imidazothiazole group of anthelmintcs (Table 1). Village-wise profile exhibited that in flocks, FECR with LEV varied from 48 to 89% in Booj, 52 to 86% in Chawandiya, 73 to 84% in Sarjoli, 74 to 90% in Sindoli and 68 to 89% in Jhar (lower 95% confidence limits less than 90), suggesting prevalence of imidazothiazole resistance in *Strongyle* worm population in all the flocks.

Table 1: Extent of anthelmintic resistance in *Strongyle* worms from goat on *in-vivo* faecal egg count reduction test

Village	Control mean FEC	Treated			
		Mean post-treatment FEC	% FECR	95% Confidence limit	
				Lower	Upper
A. Benzimidazole (Fenbendazole @ 5mg/kg B.W.)					
Overall	1786.43±106.07	519.64±47.03	69.04±2.95	48.1±4.4	82.1±1.6
Booj	1728.0±236.28	354.00±65.59	75.60±8.28	0-78	75-98
Chawandiya	1272.00±145.10	586.00±101.57	52.80±9.97	0-26	55-88
Sarjoli	2326.67±252.11	775.00±147.78	67.83±2.72	36-85	74-87
Sindoli	1950.00±120.03	420.00±34.16	78.33±1.69	60-79	81-89
Jhar	1560.00±188.41	446.67±40.80	69.00±5.30	7-69	68-91
B. Imidazothiazole (Levamisole @ 7.5mg/kg B.W.)					
Overall	1786.43±106.07	360.71±32.07	78.36±2.10	59.7±4.9	88.5±1.0
Booj	1728.0±236.28	364.00±61.67	74.20±7.97	0-80	78-96
Chawandiya	1272.00±145.10	382.00±87.77	69.00±5.72	0-66	79-95
Sarjoli	2326.67±252.11	511.67±92.50	79.33±1.89	59-83	82-90
Sindoli	1950.00±120.03	310.00±37.86	84.00±2.21	61-82	82-95
Jhar	1560.00±188.41	240.00±13.42	83.00±3.11	42-82	82-94

Based on generic composition of *Strongyle* larvae on coproculture, the overall mean post treatment FECs for *H. contortus* in FBZ treated flocks were 433.71±38.54 epg as compared to 1472.46±90.22 epg in control flocks with %FECR of 69.43±2.82 (Table 2). Village-wise profile exhibited that in flocks, FECR with FBZ varied from 45 to 93% in Booj, 15 to 70% in Chawandiya, 60 to 77% in Sarjoli, 69 to 85% in Sindoli and 65 to 84% in Jhar (lower 95% confidence limits less than 90), suggesting prevalence of benzimidazole resistance in *H. contortus* population in all the flocks (Table 2). Like-wise, the overall mean post treatment FECs for *H. contortus* in LEV treated and control groups were 272.43±26.50 and 1472.46±90.22 epg, respectively with %FECR of 80.07±2.01. Village-wise, FECR with LEV varied from 47 to 90% in Booj, 63 to 89% in Chawandiya, 73 to 86% in Sarjoli, 77 to 91% in Sindoli and 74 to 90% in Jhar (lower 95% confidence limits less than 90), suggesting prevalence of LEV resistance in *H. contortus* population in all

the flocks. The overall mean post treatment FECs for *H. contortus* were 0.00±0.00 and 1472.46±90.22 epg in CLS treated and control groups with 100.00% FECR. Flock-wise, analysis exhibited that % FECR for closantel against *H. contortus* was 100.00% in all the flocks indicating existence of full susceptibility to closantel and absence of emergence of CLS resistant *H. contortus* in goat flocks of studied area (Table 2).

Table 2: Extent of anthelmintic resistance in *Haemonchus contortus* from goat on *in-vivo* faecal egg count reduction test

Village	Control mean FEC	Treated			
		Mean post-treatment FEC	% FECR	95% Confidence limit	
				Lower	Upper
A. Benzimidazole (Fenbendazole @ 5mg/kg B.W.)					
Overall	1472.46±90.22	433.71±38.54	69.43±2.82	47.4±4.2	82.1±1.6
Booj	1435.80±195.68	286.00±56.79	76.20±8.14	0-79	75-98
Chawandiya	1054.40±128.49	474.00±83.21	53.40±10.13	0-31	55-88
Sarjoli	1957.33±235.40	652.83±113.51	66.17±2.55	38-71	71-86
Sindoli	1547.50±71.78	359.83±33.57	76.50±2.43	56-78	78-90
Jhar	1291.50±154.60	378.00±37.83	73.33±3.00	6-70	75-91
B. Imidazothiazole (Levamisole @ 7.5mg/kg B.W.)					
Overall	1472.46±90.22	272.43±26.50	80.07±2.01	62.1±4.5	89.3±0.9
Booj	1435.80±195.68	307.80±57.97	73.60±8.86	0-80	78-96
Chawandiya	1054.40±128.49	264.00±54.15	74.40±4.40	15-57	84-95
Sarjoli	1957.33±235.40	391.67±86.38	80.17±2.12	59-78	82-91
Sindoli	1547.50±71.78	230.00±23.47	84.83±1.87	66-82	84-95
Jhar	1291.50±154.60	137.17±9.39	85.33±2.36	53-84	85-95
C. Salicylanilide (Closantel @ 10mg/kg B.W.)					
Overall	1472.46±90.22	0.00±0.00	100.00±0.00	100.0±0.00	100.0±0.0
Booj	1435.80±195.68	0.00±0.00	100.00±0.00	100	100
Chawandiya	1054.40±128.49	0.00±0.00	100.00±0.00	100	100
Sarjoli	1957.33±235.40	0.00±0.00	100.00±0.00	100	100
Sindoli	1547.50±71.78	0.00±0.00	100.00±0.00	100	100
Jhar	1291.50±154.60	0.00±0.00	100.00±0.00	100	100

The overall mean post treatment FECs for *Tricostrongylus spp.* in FBZ treated flocks were 62.71±6.79 epg as compared to 250.75±19.96 epg in control flocks with %FECR of 70.18±3.62 (Table 3). Village wise analysis revealed prevalence of BZ resistance in *Trichostrongylus spp* in 100% flocks from all the five villages under study. The flock-wise %FECR ranged from 40 to 91, 0 to 68, 62 to 85, 71 to 89 and from 62 to 87 in Booj, Chawandiya, Sarjoli, Sindoli and Jhar village, respectively with lower 95% confidence limits <90. Like-wise, the overall mean post treatment FECs for *Tricostrongylus*spp in LEV treated and control group were 59.68±4.91 and 250.75±19.96 epg, respectively with %FECR of 72.39±2.95. Similar to BZ resistance in *Trichostrongylus spp*, village wise analysis also revealed prevalence of LEV resistance in *Trichostrongylus spp* in 100% flocks from all the five villages with %FECR ranging from 60 to 87, 31 to 84, 66 to 82, 61 to 91 and from 36 to 87 in Booj, Chawandiya, Sarjoli, Sindoli and Jhar village, respectively with lower 95% confidence limits <90.

Table 3: Extent of anthelmintic resistance in *Trichostrongylus spp*. from goat on *in-vivo* faecal egg count reduction test

Village	Control mean FEC	Treated			
		Mean post-treatment FEC	% FECR	95% Confidence limit	
				Lower	Upper
A. Benzimidazole (Fenbendazole @ 5mg/kg B.W.)					
Overall	250.75±19.96	62.71±6.79	70.18±3.62	49.5±5.2	82.4±2.2
Booj	268.80±44.04	53.00±11.68	70.00±8.65	0-74	73-97
Chawandiya	168.40±22.11	89.60±15.77	46.00±11.97	0-30	40-87
Sarjoli	297.17±48.31	81.83±23.35	72.67±4.40	40-79	74-91
Sindoli	292.67±51.01	42.83±4.98	83.67±2.81	56-87	80-93
Jhar	216.00±35.62	49.17±7.37	74.50±4.39	35-81	68-92
B. Imidazothiazole (Levamisole @ 7.5mg/kg B.W.)					
Overall	250.75±19.96	59.68±4.91	72.39±2.95	50.6±5.2	85.0±1.6
Booj	268.80±44.04	50.80±6.87	78.20±5.18	5-75	83-93
Chawandiya	168.40±22.11	69.60±19.19	57.40±9.34	0-58	70-94
Sarjoli	297.17±48.31	68.00±8.28	76.00±2.35	45-68	79-87
Sindoli	292.67±51.01	59.67±12.55	77.50±4.86	48-88	73-94
Jhar	216.00±35.62	50.50±6.68	71.33±7.90	0-87	64-93

The overall mean post treatment FECs for *Oesophagostomum spp.* in FBZ treated flocks were 22.32±4.00 and 84.21±12.52 epg, respectively with %FECR of 61.93±6.58 (Table 4). In flocks from Booj village, the flock-wise %FECR ranged from 44 to 100 with lower 95% confidence limit of 100 in two flocks and < 90 in rest of the three flocks, suggesting

prevalence of benzimidazole resistance in *Oesophagostomum* spp in 60% of the flocks tested. Like-wise, in Sindoli village, 83.33% of flocks (5/6) exhibited presence of of BZ resistance in *Oesophagostomum* spp. In rest of three villages, prevalence of BZ resistance in *Oesophagostomum* spp. was found in 100% of the flocks under study. The overall mean post treatment FECs for *Oesophagostomum* spp in LEV treated and control group were 25.61±4.08 and 84.21±12.52 epg, respectively with %FECR of 60.78±5.78 (Table 4). In flocks from Booj village, flock-wise %FECR ranged from 0 to 100 with lower 95% confidence limit of 100 in one flocks and <90 in rest of the four flocks, suggesting prevalence of LEV resistance in *Oesophagostomum* spp in 80% of the flocks tested. In rest of four villages, prevalence of LEV resistance in *Oesophagostomum spp* was foundin 100% of the flocks under study.

Thus, it was evident that 100% of flocks were found to possess strongyle worms resistant to FBZ and LEV with an overall mean efficacy of 69.04±2.95 and 78.36±2.10%, respectively. Among strongyle nematodes, both population of *H. contortus* and *Trichostrongylus* spp. were found resistant to FBZ and LEV in 100% of the flocks. On the other hand, *H. contortus* population was found susceptible to CLS with 100% efficacy (Table 5).

On *in-vitro* EHA, among 28 flocks, benzimidazole resistant strain of strongyle worms were observed in 27 flocks (96.43%) of all the 5 villages. The flock-wise ED_{50} value (µg TBZ ml^{-1}) varied from 0.136±0.017 to 0.331±0.085 in Booj, 0.282±0.042 to 0.484±0.009 in Chawandiya, 0.206±0.015 to 0.466±0.063 in Sarjoli, 0.269±0.045 to 0.590±0.020 in Sindoliand from 0.095±0.014 to 0.306±0.037 in Jhar, respectively. Out of 28 flocks, only one flock from Jhar was found to harbour benzimidazole susceptible strongyle worms with ED_{50} value of 0.095±0.014 µg TBZ ml^{-1} (Table 6).The results for benzimidazole resistance are in conformity on both the assays for 96.43% (27/28) occasions. On comparison of results for benzimidazole resistance through FECRT and EHA in the field flocks, it was observed that overall prevalence for benzimidazole resistance in strongyle worms was 100% on FECRT and 96.43% on EHA.

Table 4: Extent of anthelmintic resistance in *Oesophagostomum spp*. from goat on *in-vivo* faecal egg count reduction test

Village	Control mean FEC	Treated			
		Mean post-treatment FEC	% FECR	95% Confidence limit	
				Lower	Upper
A. Benzimidazole (Fenbendazole @ 5mg/kg B.W.)					
Overall	84.21±12.52	22.32±4.00	61.93±6.58	47.8±7.5	75.9±5.0
Booj	32.00±15.54	2.60±1.12	83.00±13.30	0-100	75-100
Chawandiya	48.80±13.75	22.20±4.32	48.00±14.35	0-63	0-89
Sarjoli	152.67±20.39	40.33±14.26	68.33±14.40	0-89	39-95
Sindoli	110.00±27.77	17.33±5.73	75.00±12.73	0-100	81-100
Jhar	63.00±25.55	25.83±4.06	40.00±14.33	0-66	31-88
B. Imidazothiazole (Levamisole @ 7.5mg/kg B.W.)					
Overall	84.21±12.52	25.61±4.08	60.78±5.78	45.4±6.2	77.1±4.2
Booj	32.00±15.54	5.40±1.63	64.50±23.01	0-100	57-100
Chawandiya	48.80±13.75	40.20±15.20	27.20±11.34	0-58	0-94
Sarjoli	152.67±20.39	37.17±5.75	72.83±6.14	26-81	71-91
Sindoli	110.00±27.77	20.33±4.71	79.33±3.78	40-82	77-92
Jhar	63.00±25.55	17.33±2.91	55.67±11.85	0-66	22-93

Table 5: Prevalence (%) of anthelmintic resistance in *Strongyle* worms on *in–vivo* faecal egg count reduction test in goat flocks from semi-arid Rajasthan

	Overall	*Haemonchus contortus*	*Trichostrongylus* spp	*Oesophagostomum* spp.
No. of flocks tested	28	28	28	27
% Flocks with BZ-resistance	100.00 (69.04±2.95)	100.00 (69.43±2.82)	100.00 (70.18±3.62)	88.89 (61.93±6.58)
% Flock with TEM-resistance	100.00 (78.36±2.10)	100.00 (80.07±2.01)	100.00 (72.39±2.95)	92.59 (60.78±5.78)
% Flock with CLS-resistance	71.43 (83.79±3.49)	0.00 (100.00±0.00)	-	-

(Figure in parenthesis indicates efficacy on FECRT)

Table 6: Prevalence of benzimidazole resistance in *Strongyle* worms of goats insemi-arid region of Rajasthan by *in – vitro* egg hatch assay

Village	No. of flocks	Range of ED_{50} µg TBZ/ml	% flock having BZ-resistant worm
Booj	5	0.136±0.017 - 0.331±0.085	100.0
Chawandiya	5	0.282±0.042 - 0.484±0.009	100.0
Sarjoli	6	0.206±0.015 - 0.466±0.063	100.0
Sindoli	6	0.269±0.045 - 0.590±0.020	100.0
Jhar	6	0.095±0.014 - 0.306±0.037	83.33

The flock wise paired data obtained for %FECR with benzimidazole on *in vivo* FECRT and ED_{50} value for thiabendazole on *in vitro* EHA were correlated using linear regression analysis. The correlation coefficient (r^2) between %FECR and ED_{50} value was low (0.0364) indicating poor linear correlation among them (Fig. 1).

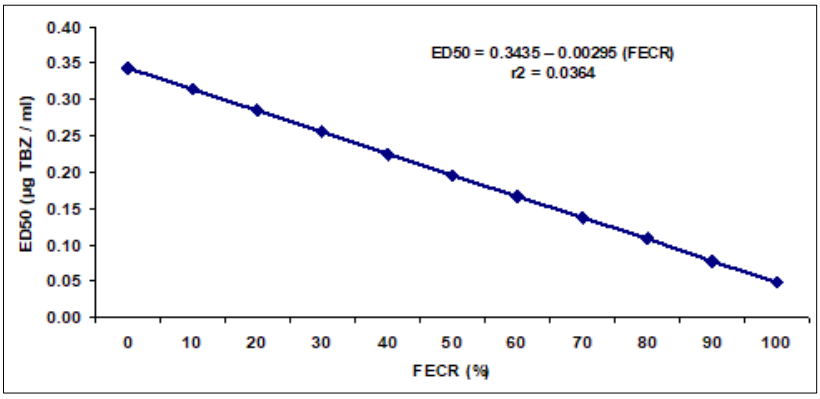

Fig 1: Correlation between % FECR (on *in-vivo* FECRT) and ED_{50} values (on *in – vitro* EHA) for benzimidazole against *Strongyle* worms

Present study exhibited high level of benzimidazole and levamisole resistance in strongyle worms in goat of Jaipur region of Rajasthan. Similar trends of high benzimidazole and levamisole closantel resistance were recorded in earlier studies also from field flocks (Swarnkar *et al*., 2004; Garg *et al.,* 2007; Cezar *et al.*, 2010) [4, 16, 6]. Similarly, Dhanalakshmi *et al.,* (2003) [13], Das and Singh (2005) [12], Jaiswal *et al.,* (2013) [21], Sharma *et al.,* (2015) [32], Varadharajan *et al.,* (2019) [40], Bihaqi *et al.,* (2020) [4] and Hafiz *et al.,* (2020) [19] reported % FECR with benzimidazole anthelmintic varying from 60 to 73%. The efficacies of LEV obtained in the present study are in agreement with the findings of Easwaran *et al.,* (2009) [14], Godara *et al.,* (2011) [18], Gelot *et al.,* (2016) [17], and Vohra *et al.,* (2019) [41] who reported 53-82% efficacy against GI nematodes in Tamil Nadu, Rajasthan, Gujarat and, Haryana respectively. The ED_{50} values obtained in the present study are in agreement with the findings of Arunachalam *et al.,* (2005) [2], Easwaran *et al.,* (2009) [14], Maharshi *et al.,* (2011) [24], Amulya *et al.,* (2016) [1], Rajagopal *et al.,* (2017) [27], Sankaralingam *et al.,* (2018) [30] who reported 0.586, 0.388-0.678, 0.103-0.353, 0.24-1.56, 0.25 and 0.247 µg of Thiabendazole/ml against GI nematodes in Tamil Nadu, Karnataka, Rajasthan, Karnataka, Kerala and Tamil Nadu, respectively. In the present study, the average ED_{50} value for benzimidazole susceptible strongyle worms was 0.095±0.014 µg TBZ ml^{-1} which is in conformity with the findings of Rialch *et al.,* (2013) [29] who found ED_{50} value to the tune of 0.037-0.096 µg TBZ ml^{-1}, respectively.

The comparison of results for BZ resistance in strongyle worms based on both FECRT and EHA revealed high agreement (96.43%). Similar findings were reported by Chartier *et al.* (1998) [8] and Maharshi *et al*. (2011) [24]. The minor discrepancy between *in-vivo* FECRT and *in-vitro* EHA results is probably due to the ability of some immature nematodes to survive a standard drench and then to develop into egg laying adults within 14 days or the effect of diet on the pharmacokinetics of anthelmintic resistances (Singh *et al.* 1999) [34]. On linear regression analysis, Boersema and Pandey (1997) [5], Chartier *et al.* (1998) [8] and Maharshi *et al.* (2011) [24] also reported poor correlation (r=0.087 to 0.11) between the LC_{50} values on EHA and % FECR after treatment with FBZ. The poor correlation between FECR and ED_{50} values as well as variation in ED_{50} value during patent period might be due to fact that the anthelmintic activity of benzimidazole in the host is not necessarily in a linear relationship with the ovicidal effect of benzimidazole anthelmintics as measured by *in vitro* EHA.

Development of anthelmintic resistance among the GI nematode populations in goats of Jaipur region of Rajasthan could be attributed to indiscriminate mass deworming by paravets and quacks, practice of mass deworming during unsuitable period of year when climatic factors naturally eliminate the free-living infective stages of nematodes from the pasture and use of low quality drugs of less bioavailability (Terril *et al.,* 2001) [38]. The role of management practices and the frequent use of anthelmintics are very important factors for the development of resistance (Martin *et al.,* 1982) [26]. The selection pressure exerted by regular use of anthelmintic is responsible for the development of anthelmintic resistance. Another factor which may have contributed to the high worldwide prevalence of anthelmintic resistance in small ruminants is the common use of the sheep dosage of these products in both sheep and goats (Conder and Campbell, 1995) [11]. In this situation, resistance nematodes may have been transmitted from goats to sheep, if they were grazed together or sequentially on the same pasture during the same year or in the following years. Coles (1997) [9] recommended that goats require higher dosage of anthelmintics than sheep to achieve similar efficacy against GI nematodes. In the present study, goats were grazed together or sequentially on the same pasture and received similar dosages of anthelmintics. Hence, constant monitoring for anthelmintic resistance is essential in both sheep and goat farms to determine the effectiveness of anthelmintics before their use, where resistance has not already emerged. This in turn is expected to help in taking timely measures to be taken to prevent or to delay the occurrence of anthelmintic resistance based on minimum anthelmintic usage. Further, it is apparent that small ruminant producers and veterinarians can no longer rely solely on anthelmintics for parasite control. To preserve the few drugs that are still effective, veterinarians and producers must change their attitudes and approaches to parasite control. The worm control programme relying on the sole use of anthelmintics need to be replace with other sustainable ones, combining chemical, environmental, managemental and other measures. Anthelmintics should be thought of as extremely valuable, limited resources that should be used less frequently and only in conjunction with non-anthelmintic parasite-control measures (Howell *et al*., 2013) [20].

Acknowledgements

The research is part of M.V.Sc. work of the first author. The authors are thankful to the authorities of the PGIVER, Jaipur and ICAR-CSWRI, Avikanagar for the facilities provided. The cooperation by goat farmers and the staff of the Department of Veterinary Parasitology, PGIVER, Jaipur is also acknowledged.

References

1. Amulya G, Sudharani R, Shareef MI, Gopinath SM. *In vivo* and *in vitro* detection of anthelmintic resistance against gastrointestinal nematodes in sheep. Indian Vet. J. 2016;93:81-83.
2. Arunachalam K, Raman M, Jeyathilakan N, Karunanidhi K. Multiple anthelmintic resistances in gastrointestinal nematodes from Mecheri sheep in an organized farm in Tamil Nadu. Indian J Anim. Sci. 2005;7:203-205.
3. BAHS. Basic Animal Husbandry Statistics. Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India, 2019.
4. Bihaqi SJ, Allaie IM, Banday MAA, Sankar M, Wani ZA, Prasad A. Multiple anthelmintic resistance in gastrointestinal nematodes of caprines on Mountain Research Centre for Sheep and Goat at Kashmir Valley, India. Parasitol.Epid. Cont. 2020;11:163.
5. Boersema JH, Pandey VS. Anthelmintic resistance of trichostrongylids in sheep in the highveld of Zimbabwe. Vet.Parasitol. 1997;68:383-388.
6. Cezar A, Toscan G, Camillo G, Sangioni L, Ribas H, Vogel F. Multiple resistance of gastrointestinal nematodes to nine different drugs in a sheep flock in southern Brazil. Vet. Parasitol. 2010;173:157-60.
7. Chandra S, Prasad A, Yadav N, Latchumikanthan A, Rakesh RL, Praveen K, *et al*. Status of benzimidazole resistance in *Haemonchus contortus* of goats from different geographic regions of Uttar Pradesh, India. Vet. Parasitol. 2015;208:263-267.
8. Chartier C, Pors I, Hubert J, Rocheteau D, Benoit C, Bernard N. Prevalence of anthelmintic resistance in sheep and goats in Western France. Small Rumin. Res. 1998;29:33-41.
9. Coles GC. The control of parasites in goats. Goat Vet. Soc. J. 1997;17:28-32.
10. Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, *et al*. World Association for Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 1992;44:35-44.
11. Conder GA, Campbell WC. Chemotherapy of nematode infection of Veterinary importance, with special reference in drug resistance. Adv. Parasitol. 1995;35:1-184.
12. Das M, Singh S. Anthelmintic resistance to nematodes in sheep and goat farms in Hisar. J Vet. Parasitol. 2005;19:103-106.
13. Dhanalakshmi H, Jagannath MS, D'souza, P.E. 2003. Multiple anthelmintic resistances in gastrointestinal nematodes of sheep. J Vet. Parasitol. 2005;17:89-91.
14. Easwaran C, Harikrishanan TJ, Raman M. Multiple anthelmintic resistance in gastrointestinal nematodes of sheep in Southern India. Vet. Arhiv. 2009;9:611-620.
15. Finney DJ. Probit Analysis. A Statistical Method of the Sigmoid Curve. 2nd edn.,Cambridge University Press, London, 1965.
16. Garg R, Kumar RR, Yadav CL, Banerjee PS. Duration of anthelmintic effect of three formulations of ivermectin (oral, injectable and pour-on) against multiple anthelmintic-resistant Haemonchuscontortus in sheep. Vet. Res. Commun. 2007;31:749-755.
17. Gelot IS, Singh V, Shyma KP, Parsani HR. Emergence of multiple resistances against gastrointestinal nematodes of Mehsana-cross goats in a semi-organized farm of semi-arid region of India. J Appl. Anim. Res. 2016;44:146-149.
18. Godara R, Sharma RL, Sodhi SS. Efficacy of fenbendazole, levamisole and ivermectin against gastrointestinal nematodes in Jamunapari goats. J Parasit. Dis. 2011;35:219-221.
19. Hafiz A, Kalita DN, Dutta TC. Emergence of anthelmintic resistance in an organized goat farm in Assam. Rumin. Sci. 2020;9:263-266.
20. Howell SB, Burke JM, Miller JE, Terrill TH, Valencia E, Williams MJ, *et al*. Prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States. J Amer. Vet. Med. Assoc. 2008;233:1913-1919.
21. Jaiswal AK, Sudan V, Shanker D, Kumar P. Emergence of ivermectin resistance in gastrointestinal nematodes of goats in a semi-organized farm of Mathura district - India. Vet. Arhiv. 2013;83:275-280.
22. Kalkal H, Vohra S. Multiple anthelmintic resistance in goat farms from semi-arid zone of Haryana. J Anim. Res. 2021;11:933-937.
23. MAFF. Manual of veterinary parasitological techniques. Ministry of Agriculture, Fisheries and Food, Technical Bulletin No. 18, Her Majesty's Stationary Office, London, 1986, 129.
24. Maharshi AK, Swarnkar CP, Singh D, Manohar GS, Ayub M. Status of anthelmintic resistance in gastrointestinal nematodes of sheep in Rajasthan. Indian J Anim. Sci. 2011;81:105-109.
25. Martin PJ, Wursthorn L. RESO faecal egg count reduction test calculator. Melbourne, Australia, Council of Scientific and Industrial Research Organization, Division of Animal Health, 1991.
26. Martin PJ, Anderson NJ, Jarrett RG, Brown TH, Lond GE. Effects of preventive suppressive control scheme on the development of thiabendazole resistance in Ostertagia sp. Aus. Vet. J. 1982;58:185-190.
27. Rajagopal A, Sabu L, Devada K, Radhika R, Gleeja VL. Assessment of benzimidazole resistance status in an organized goat farm by egg hatch assay in Kerala. Int. J Sci, Envr. Tech. 2017;6:2112-2117.
28. Rathod Y, Kasaralikar VR, Ravindra BG, Sunilchandra U, Halmandge SC, Patil NA. Assessment of anthelmintic resistance against strongylosis in goats. Intern. J Livestock Res. 2019;9:111-115.
29. Rialch A, Vatsya S, Kumar RR. Detection of benzimidazole resistance in gastrointestinal nematodes of sheep and goats of sub-Himalayan region of northern using different tests. Vet. Parasitol. 2013;198:312-318.
30. Sankaralingam G, Raman M, Harikrishnan T, Pandiyan V, Sahoo S, Prakash O. *Haemonchuscontortus* load and anthelmintics resistance in sheep and goats in Chennai, Tamil Nadu, India. Intern J Chem. Stud. 2018;6:2712-2716.
31. Shah HL, Chaudhry RK. Parasitism in dairy animals in India: present status and its impact. In: Proceedings of the workshop on control strategy against gastrointestinal parasites in dairy animals in India using medicated urea molasses blocks. National Dairy Development Board, Anand, India, 1995, 1-5.
32. Sharma S, Kumar S, Farooq J, Katoch R. Comparative study of efficacy of fenbendazole and ivermectin against *Haemonchus contortus* in goats of Jammu region. J Anim. Res. 2015;5:293-295.
33. Singh AK, Das G, Roy B, Nath S, Naresh R, Kumar S.

Prevalence of gastro-intestinal parasitic infections in goat of Madhya Pradesh, India. J Parasit. Dis. 2015;39:716-719.
34. Singh D, Sanyal PK, Swarnkar CP, Khan FA, Bhagwan PSK. Influence of diet type and pretreatment fasting on kinetics of albendazole in sheep. Vet. Res. commun. 1999;23:229-240.
35. Singh D, Swarnkar CP, Khan FA. Anthelmintic resistance in gastrointestinal nematodes of livestock in India. J Vet. Parasitol. 2002;16:115-130.
36. Soulsby EJL. Helminths, Arthropods and Protozoa of Domesticated Animals. 7thedn., Bailliere, Tindall and Cassell Ltd. London, 1982, 824.
37. Swarnkar CP, Singh D, Khan FA, Tiwari R, Dubey SC. Prevalence of anthelmintic resistance and seasonal variation in efficacy of anthelmintics in sheep. In: XV National Congress of Indian Association for the Advancement of Veterinary Parasitology, GBPUAT, Pantnagar, 2004 October 25-27, 72.
38. Terrill TH, Kaplan RM, Larsen M, Samples OM, Miller JE, Gelaye S. Anthelmintic resistance on goat farms in Georgia: efficacy of anthelmintics against gastrointestinal nematodes in two selected goat herds. Vet. Parasitol. 2001;97:261-268.
39. Torres-Acosta JFJ, Sandoval-Castro CA, Hoste H, Aguilar-Caballero AJ, Camara-Sarmiento R, Alonso-Diaz MA. Nutritional manipulation of sheep and goats for the control of gastrointestinal nematodes under hot humid and subhumid tropical conditions. Small Rumin. Res. 2012;103:28-40.
40. Varadharajan A, Gnanasekar R, Vijayalakshmi R. Anthelmintic resistance in naturally infected sheep flocks of Cuddalore district, Tamil nadu. Intern. J Scient. Res. Pub. 2019;9:127-130.
41. Vohra S, Singh S, Kumar V. Status of anthelmintic resistance of fenbendazole, closantel and levamisole against gastrointestinal nematodes in sheep of Haryana. Intern. J Livestock Res. 2019;9:91-96.
42. Wyk JAV, Mayhew E. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. J Vet. Res. 2013;80:1-14.
43. Yadav CL, Garg R. Anthelmintic resistance in India: Current status and future directions. In: Proceedings of XVIII National Congress of Veterinary Parasitology, SKUAT, Jammu, 2007, 121-128.