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Research Article

EFFICACY OF BENZIMIDAZOLES AGAINST GASTROINTESTINAL NEMATODES OF GOATS FROM THREE AGRO-CLIMATIC ZONES OF HARYANA, INDIA

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ABSTRACT

The present study reveals the status of anthelmintic resistance in goat farms from three agro-climatic zones of Haryana, India. The present research work was undertaken to study the status of benzimidazole group of drugs to GINs in goat farms using *in vivo* faecal egg count reduction test (FECRT) and *in vitro* egg hatch assay (EHA). The FECRT resulted in moderate to severe resistance (54.90-70%) in all the selected goat farms against fenbendazole @ 10 mg/kg b.wt. orally. The post treatment (fenbendazole) faecal culture revealed the predominance of *H. contortus* infective larvae only. However, EHA revealed resistance in three farms (0.119-0.161 µg TBZ/ml) and susceptibility (0.097 µg TBZ/ml) of one farm to thiabendazole i.e. there ED_{50} values were below 0.1 µg TBZ/ml. Therefore, important measures need to be implemented in goat farms to control the spread of anthelmintic resistance against benzimidazole group of drugs. For effective control of GINs regular monitoring for the status of anthelmintic is important in a particular agro-climatic zone. The basic knowledge i.e. resistance of benzimidazoles group of drugs will help in forming a strategy to control gastrointestinal nematodes in goats of Haryana.

Keywords: Fenbendazole, EHA, FECRT, goat, Haryana, Resistance, Thiabendazole

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The goat farming sector is an important source of rural economy of Haryana which needs minimum initial investment, cheap maintenance, fast, highly profitable returns and minimum disease exposure. The state of Haryana lies in the northern region (27° 39' to 30° 35' N and 74° 28' to 77° 36' E) of India. In Haryana state goat population is 0.335 million as per DAHD, Haryana (2020). The major concern is the gastrointestinal nematodes (GINs), which causes significant production losses in the goat farming sector. The most important GINs, *Haemonchus contortus* is highly prevalent and pathogenic worldwide, causing high morbidity and mortality (Piedrafita *et al.*, 2012). Gastrointestinal nematodes depends on anthelmintic drugs should be removed for profitability in the goat farming industry. Three classes of broad-spectrum anthelmintics are used to treat and control of nematodes which are benzimidazole, imidothiazole and macrocyclic lactones. However, continuous use of these anthelmintics without proper dosage and indiscriminate use has led to the development of resistance (Falzon *et al.*, 2013). Resistance is usually defined as the ability to survive doses of drug that would normally kill organisms (Kotze and Prichard 2016). There are many reports of anthelmintic resistance from different parts of India (Rialch *et al.*, 2013; Vohra *et al.*, 2013; Dixit *et al.*, 2017) as well as from other countries (Santiago-Figueroa *et al.*, 2019; Dey *et al.*, (2020). For maintaining the efficacy of the available drugs, regular monitoring of the status of anthelmintic resistance is required, at least once in two years (Rialch *et al.*, 2013). Therefore, we used *in vivo* faecal egg count reduction test (FECRT) and *in vitro* egg hatch assay (EHA) recommended by the World Association for the Advancement of the Veterinary Parasitology (WAAVP) to know the current status of benzimidazoles anthelmintic in agro-climatic region of goat farms in Haryana, India.

MATERIALS AND METHOD

The present work was carried out in Haryana, northwest India, in the latitude and longitude range of 27 degrees 39' N to 30 degrees 35' N and 74 degrees 28' E to 77 degrees 36' E, respectively. Samples were collected from three agro-climatic zones of Haryana according to Thornthwaite's (1948). The three agro-climatic zones were arid (Zone-I), semi-arid (Zone-II) and dry sub-humid (Zone-III). Faecal samples were collected from organized Goat, Central Sheep Breeding Farm, Hisar (G, CSBF) and unorganized farm of Paluwas village, Bhiwani (PVB), Zone-I. While in Zone-II and Zone-III samples were collected from Sighwal village, Jind (SVJ) and Mehmoodpur village, Ambala (MVA), respectively from unorganized farms (Fig. 1).

Faecal egg count reduction test: Fenbendazole status was investigated by in vivo FECRT method as described by (Coles *et al.*, 2006). A total of 30 animals were selected from each goat herd with eggs per gram (EPG) 150 and no anthelmintic were given from last 2-3 months. All the selected animals were divided into two groups, each with 15 animals in the treatment and control group. Goats were

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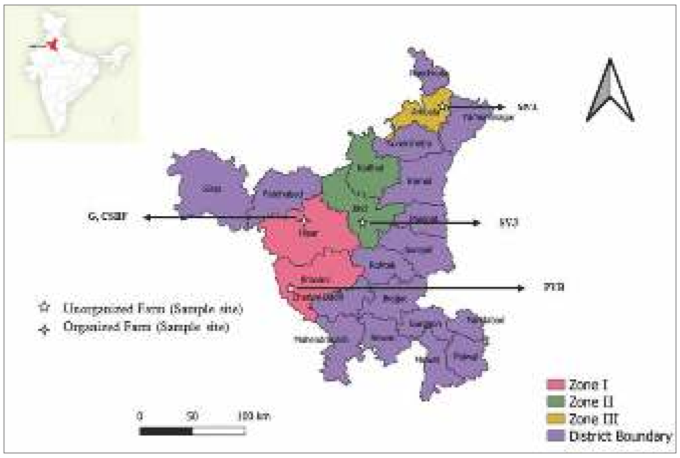

Fig. 1. Locations of the farms for sample collection from three agro-climatic zones of Haryana, India.

selected individually weighed and treated with fenbendazole (Panacur® vet suspension 10% by MSD, Mumbai) @ 10 mg/kg b.wt. orally. Animals in the control group were not treated with anthelmintics. Faecal egg counts (FECs) of each animal was determined on zero and 14th day 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 determined based on the criteria of Van Wyk and Mayhew (2013). The percentage of reduction in the number of eggs passed and confidence intervals (95%) was determined by the WAAVP method using arithmetic mean of the number of eggs. A drug is considered fully effective when it reduces the number of eggs by more than 95% and lower confidence exceeds 90%. While reduction in the number of eggs between 60% to 95% is considered moderately resistant and severely resistant below 60% with confidence level of less than 90%. Chi-square test was used at 5% or 1% significance level.

Egg hatch assay: Thiabendazole (TBZ) status was investigated using the in vitro EHA method as described (Coles *et al.*, 2006). The pooled faecal samples were collected from each herd of goat and under anaerobic conditions (Hunt and Taylor, 1989). Collection of nematode eggs was done using sieve (0.15 mm aperture, 20 cm diameter) and the eggs were isolated by a saturated salt solution. Finally, the eggs are resuspended in distilled water at 100-150 eggs/100 l of water. TBZ (Sigma-Aldrich-T8904) solution was prepared by dissolving 50 mg of TBZ in 5 ml of dimethyl sulphoxide (DMSO) to obtain solution A (10 mg TBZ/ml). From solution A, 1 ml was taken and mixed with 9 ml of DMSO to obtain solution B (1 mg TBZ/ml). 10 l of TBZ working solution i.e. 0.01, 0.025, 0.05, 0.1, 0.2, 0.3 and 0.5 g/ ml in triplicate, were added to seven different wells, followed by 1890 µl of distilled water. 100 µl of egg suspension was added to each test wells. 10 µl of DMSO was added instead of the drug solution for control wells. The plate were then incubated in a BOD incubator at 25 ± 1°C for 48 hrs, after which two drops of 1% iodine were added to each well. The the number of eggs (dead and embryonated) and hatched larvae were then counted in each well using 10X microscope objective lens. At least 100 eggs and larvae were counted from each well. Logistic regression was worked out for EHA.

RESULT AND DISCUSSION

Benzimidazoles such as thibendazole and current benzimidazoles *viz*. albendazole, fenbendazole, oxfendazole and mebendazole were released in the early 1960s and late 1970s, respectively (Gordon, 1961; McKellar and Jackson, 2004). Benzimidazoles act at the cellular level in nematodes, mainly by inhibiting their polymerization and causing cell death (Lacey, 1988; Martin, 1997). Therefore, the present study was planned to determine the current status of anthelmintic benzimidazoles group by FECRT and EHA in goat farms from three agro-climatic zones of Haryana, India.

According to FECRT, all goat farms in three agro-climatic zones of Haryana were moderately to severe resistant to fenbendazole. The complete results of FECRT are summarized in tables 1 and 2, respectively. The highest rate was recorded in MVA (70%) unorganized farms followed by SVJ (63.01%), PVB (54.90%) and G, CSBF (57.83%) in organized farms. Our findings agree with Vohra *et al.* (2013) having 79.04% efficacy in Hisar, Haryana, Pena-Espinoza *et al.* (2014) with 56% efficacy in Denmark and Bihaqi *et al.* (2020) with 62.5% efficacy in Kashmir Valley, India. Percentage of larva composition of faecal culture on day zero had predominantly *H. contortus* (74-84%), followed by *Strongyloides* spp. (5-8%), *Trichostrongylus* spp. (3-7%) and *Oesophagostomum* spp. (2-5%). However, on the 14^{th} day, larval composition produced 100% (91-98%) *H. contortus*. Similar, faecal culture findings were also reported by Vohra *et al.* (2013) in Hisar and Islam *et al.* (2018) in Bangladesh. The EHA was performed using thiabendazole in concentration ranging from 0.01-0.5 g/ml and the result are summarized in Table 3. Resistant farms were G, CSBF (zone I) SVJ (zone II) and MVA (zone III) with ED50 values 0.119, 0.128 and 0.161 µg TBZ/ml, respectively. However, GINs of PVB (zone I) farms were susceptible to TBZ with ED50 values of 0.097 µg TBZ/ml. Three farms (G, CSBF, SVJ and MVA) were in agreement with FECRT i.e. showing resistance to TBZ. The present findings are in agreement with Rialch *et al.* (2013) in sub-Himalayan region of

Table 1. Pre- and post-anthelmintic treatment faecal egg counts treated with fenbendazole in goat farms of three agro-climatic zones of Haryana, India

Zone	Farms	Faecal egg counts on days (Mean ± S.E.)		Faecal egg counts reduction on 14th day (Post treatment)		Confidence limits at 95%		Resistance status
		0	14	%	Variance (y^2)	Upper	Lower	
Zone I	G, SCBF	473.33^a±101.16	233.33^b±37.37	57.83	0.04	73.10	53.17	Resistant
	PVB	793.33^a±103.03	306.66^b±73.33	54.90	0.07	74.65	19.70	Resistant
Zone II	SVJ	600^a±112.54	180^b±39.27	63.01	0.06	78.15	37.38	Resistant
Zone III	MVA	653.33^a±96.04	200^b±62.48	70	0.13	85.98	35.77	Resistant

Means with the same superscripts are not significantly different (P<0.01)

Table 2. Anthelmintic effect on different genera of gastro-intestinal nematodes of goat farms of three agro-climatic zones of Haryana, India

Drug	Species	Per cent larval composition on day							
		Zone I				Zone II		Zone III	
		G, SCBF		PVB		SVJ		MVA	
		0	14	0	14	0	14	0	14
Fenbendazole	*Haemonchus* spp.	84	100	77	100	74	100	83	100
	Trichostrongylus spp.	6	0	3	0	6	0	7	0
	Oesophagostomum spp.	2	0	4	0	5	0	5	0
	Strongyloides sp.	8	0	6	0	5	0	5	0

Table 3. Egg hatch assay (ED50) values using thiabendazole in goat farms of three agro-climatic zones of Haryana, India

Zone	Farms	*ED_{50} (µg TBZ/ml)	Confidence	R^2 limits at 95%	Thiabendazole/ Resistance status
Zone I	G, SCBF	0.119	0.10-0.34	0.95	Resistant
	PVB	0.097	0.09-0.12	0.88	Susceptible
Zone II	SVJ	0.128	0.12-0.18	0.81	Resistant
Zone III	MVA	0.161	0.10-0.15	0.93	Resistant

*ED_{50} (dose required to prevent 50% of the viable eggs from hatching) value above 0.1 µg TBZ/ml is indicative of resistance.

northern India (0.111 µg TBZ/ml), Arunchalam *et al.* (2015) in Tamil Nadu (0.659 µg TBZ/ml), Santiago-Figueroa *et al.* (2019) in Mexico (0.01 µg TBZ/ml).

The benzimidazole resistance to GINs in organized and unorganized goat farms has been reported by many workers from India and other countries (Vohra *et al.*, 2013; Dixit *et al.*, 2017; Vadlejch *et al.*, 2021). The infective third stage larvae, *H. contortus* is the main GIN associated with benzimidazole resistance. Development of resistance is faster for *H. contortus* as compared to other nematode species (Van Wyk, 1990). Resistance to benzimidazole in goats farming may be due to its frequent use, double dose is given recommended for goat as compared to sheep, easy availability, low cost and more government supply in veterinary hospital leading to indiscriminate usage. The prevalence of benzimidazole resistance in three zones of Haryana and other parts of the world shows the continuous monitoring for anthelmintic status for better control of resistance. The G, CSBF, SVJ and MVA goat farms which were resistant by in vivo FECRT were also resistant by in vitro EHA. The PVB goat farm is only susceptible to EHA but resistant to FECRT. The present findings are in agreement with Maingi *et al.* (1998). However, our findings differ from Rialch *et al.* (2013) in sub-Himalyan region of northern India who found higher difference in the result of FECRT when compared to EHA. The difference in results by FECRT and EHA in goat flocks may be due to the fact that adult worms are examined by FECRT and eggs in EHA. According to von Samson-Himmelstjerna *et al.* (2009), the pharmacokinetics of drug in different hosts and immunological factors also influence the difference in results.

CONCLUSION

In conclusion, goat farms from three agro-climatic zones of Haryana, India have high anthelmintic resistance to the benzimidazole group of drugs. Predominance of infective larvae of *H. contortus*, the most pathogenic gastrointestinal nematode was observed. Therefore, a strategic eradication program should be established to prevent further development of resistance. This is the first report of moderate to severe anthelmintic resistance against benzimidazole drug group in goat farms from three agro-climatic zones of Haryana, India.

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