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Status of anthelmintic resistance in gastrointestinal nematodes of sheep in Rajasthan*

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ABSTRACT

A study was conducted to assess the status of anthelmintic resistance in gastrointestinal nematodes (predominantly *Haemonchus contortus*) of sheep maintained at organized farms and farmer's field in Rajasthan through *in-vivo* faecal egg count reduction test and *in-vitro* egg hatch assay. The magnitude of reduction in the faecal egg counts by fenbendazole (@ 5.0 mg kg^{-1} body weight) revealed emergence of benzimidazole resistance in *H. contortus* of sheep from all the farm and field flocks except in field flocks from north-eastern Rajasthan where 66.7% flocks possessed benzimidazole-resistant *H. contortus*. With tetramisole (@ 15 mg kg^{-1} body weight) resistance in *H. contortus* was observed in farm flock of north-eastern region whereas among field flocks it ranged from 33.33% (north region) to 83.33% (eastern region). On egg hatch assay, strains of worms were found susceptible to benzimidazole in farm flocks of northern region while in field flocks prevalence of benzimidazole resistance strongyle worms was 100% in eastern and northern region and 83.33% in north-eastern region. A high agreement (86.4%) with poor linear correlation (r = 0.087) was observed between results of both faecal egg count reduction test and egg hatch assay for benzimidazole resistance.

Key words: Anthelmintic resistance, Benzimidazole, Egg hatch assay, Faecal egg count reduction test, *Haemonchus contortus*, Tetramisole

Gastrointestinal (GI) parasites are considered as major cause responsible for morbidity and mortality losses in sheep flocks throughout the world including India (Gupta *et al.* 1987, Singh *et al.* 1997). Various chemical (anthelmintics) and non-chemical methods (pasture rotation, nutritional interventions and high degree of sanitation) have been developed to manage gastrointestinal nematodes (Singh and Swarnkar 2007). Use of anthelmintics is the dominant form of worm control worldwide including India (Sanyal 1998) and benzimidazole and levamisole class of anthelmintics have been regularly and widely used to control the GI nematodes in India in sheep since four decades (Gill 1996, Anon 2004). The extensive use and sole reliance on anthelmintics with their indiscriminate use resulted in emergence of anthelmintic resistant parasite strains (Singh *et al.* 2002, Yadav and Garg 2007). Knowledge about status of anthelmintic resistance and its monitoring becomes a vital component of worm management strategy in order to maintain efficacy of available anthelmintics with simultaneous reducing the selection pressure in worm population (Várady *et al.* 2007). The present communiqué entails results of survey on the prevalence of anthelmintic resistance against GI nematodes from sheep maintained at organized farms and farmer's field in different agroclimatic conditions of Rajasthan.

MATERIALS AND METHODS

The study was conducted in 3 different agro-climatic (eastern, north - eastern and northern) regions of Rajasthan. From each region 1 flock maintained by farm and 6 flocks maintained by farmers around farms were undertaken. The farms selected were Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Tonk (eastern region), Sheep Breeding Farm (SBF), Fatehpur, Sikar (north-eastern region) and Arid Research Centre (CSWRI), Bikaner (northern region). The flocks selected were having history of not using any anthelmintic for the last 3 months. All the flocks were managed under semi-intensive system by grazing during day hours and rest in corrals at night. The field flocks were exclusively managed on common property resources with top feeds while farm flocks were maintained on fenced pastures, top feeds and supplemented with concentrate ration.

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For worm management, farmers used 2–4 drench per annum while in farm flocks from northern and eastern regions, single drench per annum and from north-eastern region two drenches per annum were being practiced since last one decade. The benzimidazole and imidazothiazole were the drugs used in both the systems of management

Faecal egg count reduction test: The test was performed as per guidelines of World Association for Advancement of Veterinary Parasitology (Coles *et al.* 1992). Animals within a flock were randomly divided into 3 groups (12–16 animals / group). Animals of group 1 and 2 were drenched with fenbendazole orally @ 5.0 mg kg^{-1} BW and tetramisole orally @ 15.0 mg kg^{-1}, respectively. Group 3, kept as untreated control for observing the natural changes in faecal egg counts during the test period. All the animals within flock were kept together and allowed to graze on similar pasture. Faecal samples were collected *per rectum* from each animal on the treatment day (0 day) as well as on 10–14 days post treatment and faecal counts were estimated by modified McMaster technique (MAFF 1986). The left over faecal samples were pooled on both pre-treatment (flock-wise) and post-treatment (group-wise) occasions and subjected to coproculture. The larval identification was made according to key provided by Solusby (1965). The data were analyzed for % faecal egg count reduction (%FECR) by calculator (Martin and Wursthorn 1991) for combined species of strongyle infection as well as for *H. contortus* only. Resistance to anthelmintic was considered present if (i) the % FECR was less than 95 and (ii) the lower 95% confidence limit was < 90. If only one of these 2 criteria was met, the resistance was suspected.

Egg hatch assay: It was performed on eggs separated from faeces collected during pre-treatment period using pure thiabendazole in a dilution series of 0.0625 to 1.00 µg TBZ ml^{-1} as per Coles *et al.* (1992). The eggs along with varying concentrations of TBZ were incubated for 24 h at 28°C in BOD, after which a drop of Lugol's iodine was added and the number of unhatched eggs and larvae (L_1) were counted to determine the proportion of egg hatched. The data were analysed by probit analysis to obtain ED_{50} value for egg hatch. ED_{50} value in excess of 0.1 µg TBZ ml^{-1} was suggestive of benzimidazole resistance.

RESULTS AND DISCUSSION

With FBZ, on FECRT in farm flocks the % FECR for *H. contortus* in sheep was 26, 72 and 94% in eastern, north-eastern and northern region, respectively, revealing emergence of benzimidazole resistance in all the farms. Among field flocks from eastern region, the flock-wise % FECR ranged from 26 to 84 with overall average to the tune of 56.33±9.11%, indicating that benzimidazole resistance in *H. contortus* is prevalent in all the flocks. In field flocks of north-eastern region, the overall efficacy of FBZ was 91.00±2.67% and the magnitude of FECR and its 95% lower confidence limit exhibited that out of 6, 4 flocks (66.7%) have possessing benzimidazole resistant *H. contortus.* In northern region, the% FECR varied from 39.0 to 79.0% with an overall efficacy of FBZ to the tune of 59.17±5.73%. Similar to eastern region, all the flocks were found to have strain of *H. contortus* that had developed resistance to benzimidazole (Table 1).

The present study exhibited high level of benzimidazole resistance and moderate level of levamisole resistance in *H. contortus* from sheep flocks of Rajasthan. Further region based variation in prevalence of both benzimidazole and levamisole resistance with low prevalence in north - eastern region would be attributed to low drench frequency (2 drench per annum) in this region compared to other regions (3 or more drench per annum). Lower efficacy (< 60%) of benzimidazole in field flocks of eastern and northern regions compared to 91% efficacy in field flocks of north-eastern region could be due to the fact that in eastern and northern regions, drenching of flocks during extreme summer (June) is common practice (Anon 2002) when there are no *refugia*. Under these circumstances, it is expected that the eggs only from resistant worms reside in the body of host and contribute to genetic pool in next monsoon season which were available to sheep with likely deceased efficacy of anthelmintics during monsoon (Swarnkar *et al.* 2004). Regional differences in the susceptibility of parasites to chemotherapy suggested that gene frequency for resistant worms can differ from area to area before a drug is used (Coles *et al.* 1987, Coles 2005). These findings of the benzimidazole and levamisole resistance status at the farms are in conformity with those reported earlier on eastern region (Singh *et al*. 1995, Swarnkar *et al*. 2001), north-eastern region (Swarnkar *et al*. 1999b) and on northern region (Swarnkar *et al*. 1999a).

Relatively higher efficacy in field flocks of north-eastern region during present investigation was due to the fact that in these flocks test was carried out in late monsoon period when there were sufficient *refugia* and greater availability of a mixture of dry and green fodder increases bioavailability of anthelmintic metabolites (Singh *et al.* 1999). A moderate rise in proportion of susceptible genotype in *refugia* during late monsoon was reported to increase the efficacy of benzimidazole compounds against gastrointestinal nematodes (Swarnkar *et al.* 2006). The fact that resistance to LEV could not be shown, even after its prolonged use, indicates delayed onset of resistance to this anthelmintic group (Prichard 1990). LEV resistance in *H. contortus* is inherited as an autosomal recessive trait that is not sex-linked (Dobson *et al.* 1996) and leads to low prevalence (Sangster 1999).

The comparison of results for BZ resistance in strongyle worms based on both FECRT and EHA revealed high agreement (86.4%) with minor discrepancy. Similar findings were reported by Chartier *et al.* (1998). The discrepancy between *in-vivo* FECRT and *in-vitro* EHA results is probably due to the ability of some immature nematodes to survive a

Table 1. Prevalence of anthelmintic resistance in strongyle worms (predominantly *Haemonchus contortus*) in sheep of Rajasthan

Region	Flock	FECRT						EHA	
		FBZ			TEM				
		% FECR	95% lower limit	Result	% FECR	95% lower limit	Result	Mean ED_{50} (μg TBZ / ml)	Result
Eastern	Farm								
	1	26	0	R	97	94	S	0.196±0.011	R
	Field								
	1	77	58	R	91	84	R	0.205±0.051	R
	2	84	71	R	100	100	S	0.154±0.005	R
	3	33	0	R	75	44	R	0.207±0.009	R
	4	64	15	R	78	53	R	0.122±0.010	R
	5	49	11	R	94	86	R	0.265±0.029	R
	6	26	0	R	90	83	R	0.219±0.014	R
North-Eastern	Farm								
	1	72	45	R	91	81	R	0.421±0.017	R
	Field								
	1	97	90	S	96	86	L-R	0.117±0.006	R
	2	96	84	L-R	95	81	L-R	0.030±0.022	S
	3	79	56	R	100	100	S	0.126±0.011	R
	4	89	74	R	100	100	S	0.103±0.016	R
	5	92	80	R	100	100	S	0.253±0.011	R
	6	93	76	R	100	100	S	0.172±0.021	R
Northern	Farm								
	1	94	79	R	100	100	S	0.062±0.039	S
	2	94	79	R	100	100	S	0.060±0.034	S
	Field								
	1	71	39	R	75	46	R	0.347±0.005	R
	2	79	56	R	80	49	R	0.246±0.020	R
	3	39	0	R	100	100	S	0.353±0.134	R
	4	55	19	R	95	80	L-R	0.125±0.002	R
	5	56	22	R	95	76	L-R	0.212±0.043	R
	6	55	0	R	100	100	S	0.184±0.015	R

R, Resistant; L-R, low resistance; S, susceptible.

standard drench and then to develop into egg laying adults within 14 days (Hong *et al.* 1996) or the effect of diet on the pharmacokinetics of anthelmintic resistances (Singh *et al.* 1999). From the observations on variation in resistance factor for BZ against different stages of *Haemonchus contortus*, Hall *et al.* (1978) postulated that following a single dose of a benzimidazole anthelmintic a variable effect would be seen against the eggs, larvae and adults. While comparing the

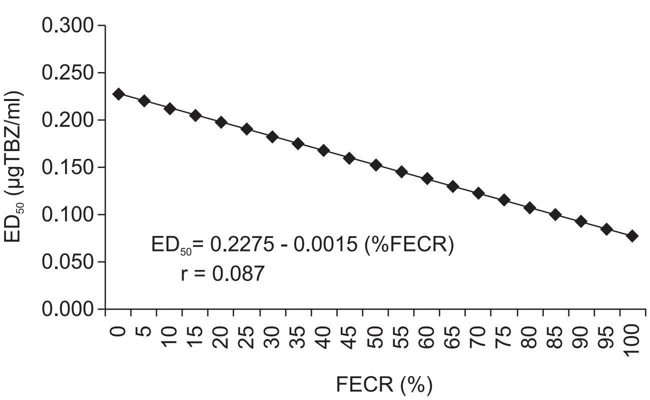

Fig. 1. Correlation between % FECR and ED_{50} values for benzimidazole against strongyle worms.

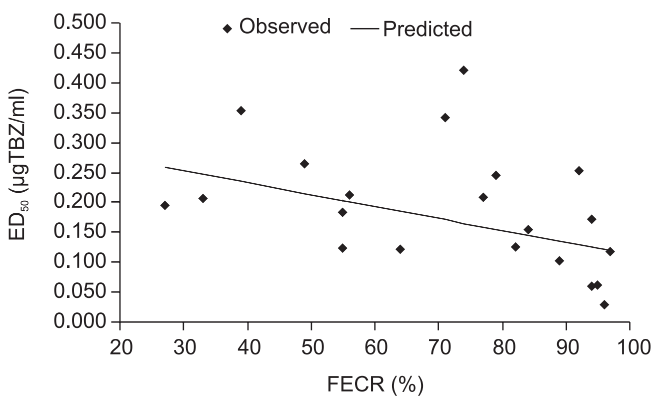

Fig. 2. Comparison between predicted and observed ED_{50} values for benzimidazole against strongyle worms.

results from these tests Martin *et al.* (1989) demonstrated that both tests had similar ability to detect BZ resistance but egg hatch assay provided a better quantitative estimate of the level of resistance.

On linear regression analysis the correlation coefficient (r) between percentage reduction in faecal egg counts and ED_{50} value was low (0.087) indicating poor linear correlation among them (Figs. 1 and 2). However, comparison of results on status of BZ resistance on both the assays, using Kappa statistics, indicated good agreement between both tests (K=0.640). Similarly, Boersema and Pandey (1997) reported no correlation (r=0.11) between the LC_{50} values of the EHA and percentage reduction in FEC after treatment with FBZ in sheep infected with *H. contortus*. Chartier *et al.* (1998) surveyed 23 sheep and 15 dairy goat farms in Western France for anthelmintic resistance and failed to get any relationship between% FECR and LC_{50} values. The poor correlation between faecal egg count reduction 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* egg hatch assay.

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