Research Article

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FENBENDAZOLE RESISTANCE IN *HAEMONCHUS* SPP. OF CATTLE

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ABSTRACT

The present study was conducted to detect the status of anthelmintic resistance (AR) and to evaluate the efficacy of commonly used Anthelmintic drugs *viz.* fenbendazole, morantel and ivermectin, against gastrointestinal nematodes in cattle under small holder dairy units. Twenty-four cattle with eggs per gram (EPG) of more than or equal to 150 were divided into 4 groups of 6 animals each. Groups I, II and III were administered fenbendazole (5mg/ kg body weight, orally), morantel (10mg/ kg body weight, orally) and ivermectin (0.2mg/ kg body weight, subcutaneously), respectively while Group IV kept as the untreated control. Faecal samples were collected on day of treatments (0 day) and 14th day after treatment from animals of all groups and individual faecal egg counts were determined by modified McMaster technique. Faecal egg count reduction percentage in groups I, II and III were 87.32, 100 and 100%, respectively. Identification of infective larvae from pre-treatment faecal cultures revealed the predominance of *Haemonchus* spp. while post treatment feacal cultures in fenbendazole treated group showed only *Haemonchus* spp. No larvae were recovered from post treatment feacal culture of morantel and ivermectin treated groups. This indicates presence of low level fenbendazole resistance to *Haemonchus* spp. and no resistance to morantel and ivermectin in cattle. This is the first report of fenbendazole resistance to *Haemonchus* spp. in cattle from India.

Key words: Anthelmintic resistance, fenbendazole, *Haemonchus* spp., ivermectin, morantel.

Amongst various parasitic diseases of cattle, gastro-intestinal (GI) nematode infections comprise a major constraint on the efficient raising of cattle throughout the world (Gasbarre *et al.*, 2001). Use of anthelmintics is the only viable, economic and thereby a practical method to control the GI nematode infections. However, the excessive, frequent and indiscriminate use, suppressive dosing and misuse of these drugs had led to development of anthelmintic resistance (AR) (Gilleard, 2006). AR against *Haemonchus* spp. in cattle has been reported from many countries (Pinheiro and Echevarria (1990) against benzimidazoles; Ramos *et al* (2016) against microcyclic lectone and benzimidazole; Yadav and Verma (1997) against morantel). Further, out of various nematodes affecting cattle, *H. contortus* is the main species and is responsible for high mortality and morbidity, as it is a voracious blood sucker. Thus, a regular monitoring of status of AR is required as an integral part of worm control programme. The present study was planned to know the AR status in GI nematodes with special emphasis on *Haemonchus* spp. of cattle under small holder dairy units in Haryana.

MATERIALS AND METHODS

Pre-treatment faecal egg counts

Before the commencement of actual AR trial, pre-treatment faecal egg counts (FEC) were performed on 60 randomly selected cattle in small holder dairy units of the Ludas village, Hisar, to ensure that sufficient eggs per gram (EPG) of faeces were present in the animals to warrant their inclusion in the trial. Small holder dairy units are traditional farming systems and consist of a few milking animals along with calves and heifers. Based on the guidelines published by the World Association for the Advancement of Veterinary Parasitology (WAAVP), a value of more than or equal to 150 EPG was used as a cut off for inclusion in the anthelmintic resistance survey (Coles *et al*., 1992, 2006). For this, fresh faecal samples were collected from all animals and faecal egg countwere performed by the modified McMaster technique (Parfitt, 1958).

Anthelmintic resistance test

Twenty-four cattle which were naturally infected with GI nematodes and had EPG ≥ 150 prior to treatment were used for faecal egg count reduction test (FECRT). The selected animals had not been administered any anthelmintic during the previous two months. These animals were divided into four groups of 6 animals each on the basis of the EPG counts. Group I, II and III were dosed with fenbendazole @ 5 mg/kg b.wt. orally, morantel @10 mg/kg b.wt. orally and ivermectin @ 0.2 mg/kg b.wt. subcutaneously, respectively while Group IV served as untreated control. Faecal egg count (FEC) of each animal was ascertained on 0 day and 14th day post treatment (PT), by the modified McMaster technique. Pooled faecal cultures at 27±2°C for 7 days were made to recover infective third stage larvae, L_3, from each group on day 0 and day 14th PT. The infective larvae were identified as per criteria of Keith (1953).

For each group, faecal egg count reduction, percentage and confidence intervals (95%) were determined following the method of the WAAVP using arithmetic mean egg counts (Coles *et al*., 1992). Resistance was considered to be present in the worm population when the egg count reduction following treatment was less than 95% and the confidence limits

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Table 1

Response to various anthelmintics in cattle naturally infected with GI nematodes

Group	Anthelmintic	Dose (mg/ Kg)	No. of cattle treated	Route of administration	FEC on days (Mean ±SE)		FECR on 14th day PT		Confidence limits at 95%	
					0	14	%	Variance (y^2)	Upper	Lower
I	Fenbendazole	5	6	Oral	300 ±25.82	33.33 ±21.08	87.32	0.4062	96.611	53.88
II	Morantel	10	6	Oral	300 ±25.82	0.00 ±0.00	100	0.0000	0.00	0.00
III	Ivermectin	0.2	6	S/C	316 ±30.73	0.00 ±0.00	100	0.0000	0.00	0.00
IV	Control	----	6	----	250 ±34.15	263 ±34.15	0	-----	------	------

FECR= Faecal egg counts reduction

were less than 90% (Coles *et al*., 1992). Worm populations were considered as severely resistant when percent reduction in EPG was less than 60% and moderately/slightly resistant when EPG was reduced between 60 to 95% (Sharma, 2015).

RESULTS AND DISCUSSION

The FECRT is the major and most widely used *in vivo* test for resistance detection and is suitable for all anthelmintic classes (Coles *et al*., 2006). The WAAVP has established guidelines that give precise details and recommendation for the use of this detection method (Coles *et al*., 1992, 2006). The FECRT provides a good estimation of anthelmintic resistance with comparatively low costs and labour input (Cabaret and Berrag, 2004). Furthermore, this test allows identifying problems with the application of anthelmintic under field conditions. The simplicity and accuracy of FECRT in AR survey in cattle have been reported by various workers in India (Yadav and Verma, 1997; Lalchhandama, 2010).The faecal culture revealed predominance of *Haemonchus* spp. infective larvae (Table 2). Larvae were slender, tail sheath of medium length, tapering to point and kinked (Fig 1 and 2).

Table 2
Anthelmintic effect on different genera of GI nematodes of cattle

Group	Species	Percent larval composition on day	
		0	14
I-Fenbendazole	*Haemonchus spp.*	95	100
	Trichostrongylus spp.	1	0
	Strongyloides spp.	4	0
II-Morantel	*Haemonchus spp.*	95	0
	Trichostrongylus spp.	1	0
	Strongyloides spp.	4	0
III-Ivermectin	*Haemonchus spp.*	95	0
	Trichostrongylus spp.	1	0
	Strongyloides spp.	4	0
IV-Control	*Haemonchus spp.*	95	94
	Trichostrongylus spp.	1	3
	Strongyloides spp.	4	3

The other genera of nematodes identified (*Trichostrongylus* spp. and *Strongyloides* sp.) were present only in smaller proportions. This finding is consistent with findings of earlier workers (Marskole *et al*., 2016; Vanisri *et al*., 2016). Previously, Yadav (1997) had also reported *Haemonchus* spp. to be most prevalent and pathogenic species among various GI nematodes which is responsible for high mortality and morbidity in India.

Fenbendazole, morantel and ivermectin are widely used anthelmintics for the control of GI nematodes, worldwide. The report of AR to morantel on an organized farm of cattle by strains of *Haemonchus* spp. in Haryana made the situation worse (Yadav and Verma, 1997). In the present study, percent reduction in FEC by fenbendazole was less than 95% (87.32%) and 95% upper and lower confidence limits were 96.61% and 53.88%, respectively (Table 1). The result based on larval culture showed that the worms surviving fenbendazole treatment were only *Haemonchus* spp. (100%) (Table 2). Thus, the results revealed presence of low level fenbendazole resistant in *Haemonchus* spp. population in cattle under small holder dairy production system. So far current information available hitherto, this is the first report of fenbendazole resistance against *Haemonchus* spp. in cattle in India. Fenbendazole belongs to benzimidazole class and benzimidazole resistance to GI nematodes in cattle had been reported by many workers (Suarez and Cristel, 2007; Cotter *et al*., 2015). Among various GI nematodes, *Haemonchus* spp. has been reported to be the main species involved in benzimidazole resistance in most of the cases. History of deworming under small holder dairy production system revealed that fenbendazole was used frequently in calves below one year of age as well as in sheep and goats of this village. The anthelmintic in calves, sheep and goats was being administered on the basis of approximate weight of the animals which might have resulted in under dosing. It has been reported that resistance often arises faster in *Haemonchus* spp. than in other nematode genera (van Wyk, 1990). Under dosing is generally considered an

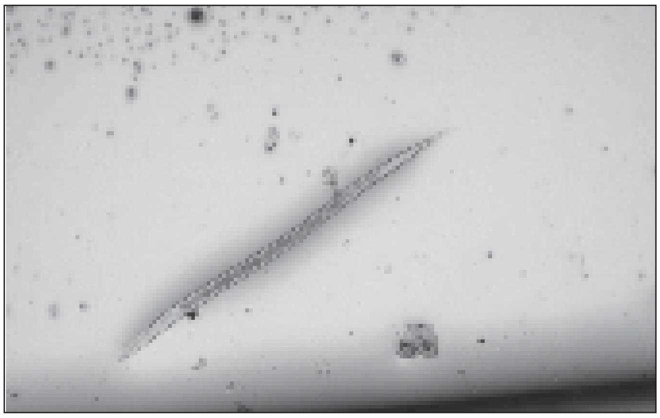

Fig. 1: Larva of *Haemonchus* spp

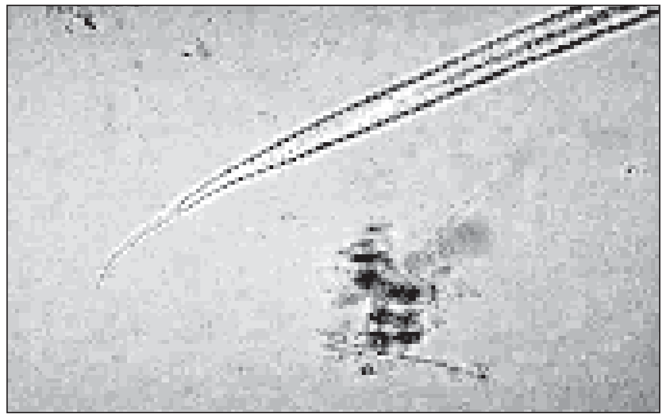

Fig. 2: Tail of *Haemonchus* spp. (kinked)

important factor in the development of anthelmintic resistance (Edwards *et al*., 1986) because sub-therapeutic doses might allow the survival of heterozygous resistance worms (Smith, 1990). Under dosing occurs when a host is administered dose that is less than the therapeutic dose recommended by the manufacturer (Smith *et al*., 1999).

Further, the percent reduction in FEC by morantel was more than 95% *i.e.* 100% and the 95% upper and lower confidence levels were 0% and 0%, respectively (Table 1). The result based on larval culture showed that no GI nematode species survived after the treatment with morantel (Table 2). Thus, the morantel is fully effective against GI nematodes of this area. Yadav and Verma, (1997) reported morantel resistance on an organized cattle farm where morantel was used frequently. But in present study under traditional system, mortantel was not being practised, therefore strongyles are susceptible.

The percent reduction in FEC by ivermectin was more than 95% *i.e.* 100% and the 95% upper and lower confidence levels were 0% and 0%, respectively. The result based on larval culture showed that no GI nematode species survived after the treatment with ivermectin (Table 2). Thus, the present study revealed that ivermectin is fully effective against GI nematodes. These results are in contrary with Geurden *et al*. (2015) and Ramos *et al*. (2016). History of deworming also revealed that ivermectin has not been used in the traditional system under study.

Based on the results of this study, it may be concluded that the choice of anthelmintic should be based on the previous history of use of drug and status of anthelmintic resistance. It should always be considered primarily to use an anthelmintic judicially and AR may be estimated yearly or at least once in two years. The drug which shows partial resistance should be changed immediately and discontinued for some years so that the larval population resistant to the drug is diluted. This is the first report of fenbendazole resistance against *Haemonchus* spp. in cattle in India.

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