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Research Article

DETECTION OF MULTIPLE ANTHELMINTIC RESISTANCE AGAINST GASTROINTESTINAL NEMATODES IN SHEEP ON CENTRAL SHEEP BREEDING FARM, HISAR

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ABSTRACT

The present study was conducted to evaluate the resistance status against gastrointestinal nematodes in sheep of Central Sheep Breeding Farm, Hisar by using faecal egg count reduction test. Fifty sheep with at least 150 eggs per gram (EPG) of faeces were identified and grouped into 5 groups (A, B, C, D and E) of ten animals each on the basis of EPG. Groups A, B, C and D were treated with fenbendazole (@5 mg/kg of body wt. orally), levamisole (@7.5 mg/ kg b. wt. subcutaneously), morantel (@10 mg/kg b. wt. orally) and ivermectin (@0.2 mg/kg b. wt. subcutaneously), respectively. Group E served as the untreated control. Faecal samples were collected on 13th day after treatment from animals of all groups and individual faecal egg counts were done by modified Mc Master technique. Faecal egg count reduction percentage in groups A, B, C and D was 44.49%, 54.78%, 47.63% and 68.02 %, respectively and the lower confidence limits in these groups were 15.63%, 25.58%, 22.72% and 48.68%, respectively. This indicated severe resistance of fenbendazole, levamisole and morantel. There was moderate resistance against ivermectin. Coprocultures from pre- and post-treatment faecal cultures revealed the predominance of *Haemonchus contortus*. This is the first report of moderate to severe multiple anthelmintic resistance against all the commonly used anthelmintic classes on a single sheep farm from India.

Key words: Anthelmintic resistance, fenbendazole, *Haemonchus contortus,* ivermectin, levamisole, morantel, sheep

Sheep are mainly reared by small farmers and landless labourers in rural India. They contribute greatly to the farmers' economy by providing edible proteins, wool, leather, manure etc. However, sheep production is hindered by many factors including animal health constraints, inadequate nutrition and poor husbandry system. Parasitic gastroenteritis caused by many gastro-intestinal (GI) nematodes like *Haemonchus contortus*, *Trichostrongylus axei*, *Nematodirus* spp. and *Strongyloides papillosus* is the major constraint. Among these GI nematodes, *H. contortus,* is most pathogenic, widely prevalent and important worm in sheep in India which is responsible for high mortality and morbidity (Yadav, 1997).

The GI nematodes are controlled by the use of anthelmintics. There are three broad spectrum anthelmintic classes commonly used for treatment and control of nematodes viz. benzimidazole, imidothiazole and macro-cyclic lactones. The frequent and indiscriminate use and under dosing of these drugs has resulted in widespread occurrence of anthelmintic resistance. There are many reports of anthelmintic resistance from different parts of India (Buttar *et al*., 2012; Meenakshisundaram *et al*., 2014; Singh *et al*., 2015) as well as from other countries (Verissimo *et al*., 2012; Balmer *et al*., 2015) Thus, 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, the present study was planned to find out the current status of anthelmintic resistance in sheep farm and to select the drug of choice to control gastro-intestinal nematodes.

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MATERIALS AND METHODS

The study was conducted to assess the status of anthelmintic resistance of fenbendazole, levamisole, morantel and ivermectin against gastrointestinal nematodes in sheep on Central Sheep Breeding Farm, Hisar by using faecal egg counts reduction test (FECRT). Apparently healthy fifty female sheep (Rambouillet x Nali cross), above 3 months of age, which were being maintained under permanent grazing system and naturally infected with gastro-intestinal nematodes with egg per gram (EPG) of faeces ≥150 counts prior to treatment were used for FECR test. The selected animals had not been administered any anthelmintic during the previous two months. These animals were weighed, identified, their EPG estimated and divided into five groups (A, B, C, D and E) of 10 animals each on the basis of EPG. Groups A, B, C and D were administered fenbendazole (Panacur®, Intervet India Pvt. Limited Wagholi, Pune) @ 5 mg/kg

body weight (b. wt.) orally, morantel (Banminth®, Pfizer Limited Mumbai) @ 10 mg/kg b. wt. orally, levamisole (Lemasole®-75, Zoetis India Limited, Haridwar, Uttarakhand) @ 7.5 mg/kg b. wt. subcutaneously and ivermectin (Trumectin®, Zydus Animal Health Limited, Ahmadabad) @ 0.2 mg/kg b. wt. subcutaneously, respectively. Group E served as untreated control. Faecal egg count of each animal was ascertained on 0 day and 13th day post treatment (PT) by the modified Mc Master technique to an accuracy of one egg counted representing 50 EPG. Pooled faecal cultures at 27±2^{0}C for 7 days were made to recover infective third stage larvae i.e. L_3 from each group on day 0 and 13 PT. The infective larvae were identified as per criteria of Keith (1953). Faecal egg count reduction percentage and confidence intervals (95%) were determined following the method of the World Association for the Advancement of Veterinary Parasitology (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 were less than 90% (Coles *et al*., 1992).

RESULTS AND DISCUSSION

Faecal egg counts (Mean±S.E.) on 0 and 13th day post- treatment (PT), percent reduction in faecal egg counts (FECR %), variance, upper and lower confidence limits (95%) of sheep naturally infected with gastrointestinal nematodes and treated with different anthelmintics are given in Table 1. The results revealed that fenbendazole (group A) reduced the faecal egg counts by 44.49% on 13th day post-treatment with 95% upper and lower confidence levels as 63.46 and 15.63, respectively. Fenbendazole belongs to benzimidazole class and its resistance to gastrointestinal nematodes in sheep had been reported by many workers from our country (Yadav *et al*., 1995; Melo *et al*., 2009; Maharshi *et al*., 2011; Sarika 2012; Sharma *et al*., 2015). Earlier, Singh and Yadav (1997) detected 74% efficacy of fenbendazole at this farm. The anthelmintic resistance status of fenbendazole against *H. contortus* changed from moderate to severe on this farm as the use of albendazole, another drug of this class, continued though rarely, as per availability and convenience of management (Table 2).

Levamisole (group B) caused 54.78% reduction in faecal egg counts with 95% upper and lower confidence levels as 72.52% and 25.58%, respectively indicating severe resistance. Previously, Singh and Yadav (1997) reported 72% efficacy of levamisole in this farm. Thus, there is change of resistance status from moderate in 1997 to severe in 2016. This may be due to continuous use of levamisole upto the year 2010 and permanent grazing system at the farm.

Further, morantel (group C) caused 47.63% reduction in faecal egg counts with 95% upper and lower confidence levels as 64.22% and 22.72%, respectively. Previously, Singh and Yadav (1997) reported 66% efficacy of morantel at this farm. So the status of resistance for morantel changed from moderate in 1997 to severe in 2016 as a result of frequent use of morantel during the year 2008 to 2010 on this farm (Table 3). Singh and Rayulu (2007) reported partial reversion of susceptibility to morantel resistant strain of *H. contortus* in an organized sheep farm after with drawl of drug for 10 years. Singh and Gupta (2009) reported partial reversion of susceptibility of fenbendazole and levamisole resistant strain of *H. contortus* in an organized sheep farm after switching over to ivermectin and closantel for 12 years. Therefore, it is inferred that reversion to susceptibility takes much longer time under permanent grazing system.

Ivermectin (group D) caused 68.02% reduction in faecal egg counts with 95% upper and lower confidence levels as 80.06% and 48.68%, respectively. Thus, there was moderate ivermectin resistant *H. contortus* population in sheep at this farm. Earlier Singh and Yadav (1997)

Table 1

Pre- and post-anthelmintic treatment faecal egg counts in sheep naturally infected with gastro-intestinal nematodes at Central Sheep Breeding Farm, Hisar

Group (Anthelmintic)	Dose (mg/kg) and route of administration	Faecal egg counts on days (Mean±S.E.)		Faecal egg counts reduction on 13th day PT		Confidence limits at 95%	
		0	13	%	Variance (y^2)	Upper	Lower
A (Fenbendazole)	5; Oral	1690^a±450.91	755^a±114.85	44.49	0.0417	63.46	15.63
B (Levamisole)	7.5; S/C	1535^a±383.55	615^a±123.84	54.78	0.0591	72.52	25.58
C (Morantel)	10; Oral	1235^a±134.58	715^a±92.51	47.63	0.0353	64.22	22.72
D (Ivermectin)	0.2; S/C	1460^a±232.47	435^a±80.98	68.02	0.0532	80.06	48.68
E (Control)	----	1435^a±199.31	1360^b±185.56	0	----	----	----

Means with same superscripts are not significantly different (P<0.01) column wise. No. of sheep in each group was 10.

Table 2
Management routines (2006-2015) on the Central Sheep Breeding Farm, Hisar

Duration	Grazing system	Anthelmintics used	Frequency of deworming
2006-2010	Permanent	Albendazole, moxidectin, closantel, levamisole, morantel	5-6 times/year
2011-2015	Permanent	Closantel, moxidectin, doramectin, ivermectin, levamisole	6-7 times/year

found that ivermectin was 100% effective against *H. contortus* in this farm. Since then macrocyclic lactone class was proposed to be used in this farm and doramectin, ivermectin and moxidectin had been used continuously during the last 10 years (>2 times per year) which also belong to same anthelmintic class i.e. macro-cyclic lactones. Thus, continuous use of these anthelmintics for the last 10 years at this farm could be the reason for development of resistance.

The coproculture of pooled faecal cultures of infective third stage larvae (Fig.1) in different groups and untreated control on day 0 and 13 were performed. A total of 500 infective larvae in each group (A, B, C, D and E) were counted. The infective larvae were identified as per criteria of Keith (1953). The result showed different genera of GI nematodes of sheep with the predominance of *H. contortus* (95%) followed by *Trichostrongylus* sp. (4%) and only 1% *S. papillosus* larvae in all the treatment and untreated control groups on day 0. After 13 days of treatment, *H. contortus* was the only species found to survive in all treatment groups. The strain of *H. contortus* resistant to various anthelmintics in small ruminants/ sheep have already been reported by Swarnkar *et al.* (1999), Fleming *et al.* (2006) and Singh *et al.* (2013).

Based on the results of the this study it may be concluded that the choice of anthelmintic in a flock should be based on the previous history of use of drug, frequency of use of drug and status of anthelmintic resistance. It should always be considered primarily to use an anthelmintic judiciously and the anthelmintic resistance may be estimated at least once in two years. The drugs which show partial resistance should be changed immediately and discontinued for some years so that the larval population resistant to the drug is diluted and the portion of susceptible larval population is increased in the sheep flocks. Due to frequent use of all classes of anthelmintics on this farm, resistance against all the classes of anthelmintics has been developed in this farm. This is the first report of moderate to severe multiple anthelmintic resistance against all commonly used anthelmintic classes on a single sheep farm from India.

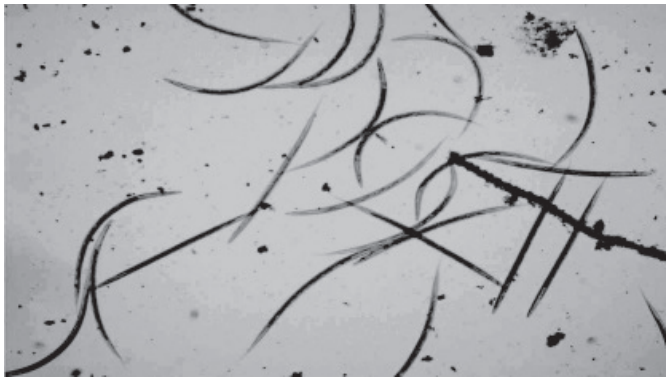

Fig 1. Infective third stage larvae of strongyles (10X)

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