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Original Research

Detection of Anthelmintic Resistance in Sheep in and around Hassan district, Karnataka by Faecal Egg Count Reduction Test

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Abstract

Anthelmintic resistance against commonly used anthelmintics, like fenbendazole and ivermectin was studied in sheep that are naturally infected with gastrointestinal nematodes in Hassan district, Karnataka by using faecal egg count reduction test (FECRT). Overall, 45 sheep farms were selected in the study out of which 26 farms were treated with fenbendazole and 19 farms were treated with ivermectin. The results of the present study revealed high level of resistance in 15 fenbendazole treated farms with mean FECR per cent ranging from 77 to 94 per cent, whereas among the 19 ivermectin treated farms 2 farms revealed resistance with mean FECR per cent ranging from 90 to 92 per cent. Further morphological characterization of the infective larvae derived from pre- and post- treatment faecal cultures was done. The post-treatment faecal cultures of fenbendazole treated farms revealed Haemonchus contortus as the predominant species in 12 farms and Trichostrongylus spp. in 3 farms. In ivermectin treated farms, H. contortus was predominant.

Keywords: Anthelmintic Resistance, FECRT, Fenbendazole, Ivermectin, Sheep

Introduction

Gastrointestinal nematodes (GINs) are a significant constraint to pasture based sheep rearing worldwide. Parasitic gastroenteritis caused by GINs is widely considered as the most important disease of sheep causing anorexia, weight loss, diarrhoea and death. The economic losses caused by internal parasites are actually not due to mortality but due to associated production losses *viz.,* decreased milk production, wool production, poor fleece growth, cost of treatment, cost of prevention and death of infected animals (Gwaze *et al*., 2009). Gastrointestinal nematodes are commonly controlled by anthelmintic drugs. The extensive use of anthelmintics for the control of GINs has resulted in the development of anthelmintic resistance. Anthelmintic resistance is an intensifying problem in many of the sheep rearing countries worldwide (Papadopoulos, 2008). Anthelmintic resistance has been reported from several states in India (Dhanalakshmi *et al*., 2003; Jeyathilakan *et al*., 2003; Deepa and Devada, 2007; Easwaran *et al*., 2009; Kumar *et al*., 2016). Since, there is paucity on the reports of anthelmintic resistance in Hassan district, Karnataka state, the present study was undertaken to detect anthelmintic resistance against commonly used anthelmintic drugs (fenbendazole, ivermectin) in sheep that are naturally infected with GINs.

Materials and Methods

The Study Area

The study was conducted in Hassan district, located in the south western part of Karnataka state at an altitude of 943.05m with latitude of 12 33^0& 13 33^0 N and longitude of 75 33^0& 76 38^0 E, average rainfall of 456 to 3000mm. It covers an area of 6826.15 sq. kms and has a total of 201155 sheep population according to *19^{th} Livestock Census*-2012.

Collection and Processing of Samples

The farms selected for the study were semi organized, small holder sheep farms of different flock size (80-150). The faecal samples were collected from both male and female sheep of 5 to 15 months age. It was verified from the animal owners that the animals had not been treated with the anthelmintics for the past 8-12 weeks. The faecal samples were collected per rectally and also the freshly voided faecal pellets were collected from the naturally infected animals in a ziplock cover on day zero before treatment and then day 10 after treatment from the animals treated with fenbendazole and day 15 from the animals treated with ivermectin. The samples were clearly labelled for identification and brought to the laboratory for further analysis. Qualitative faecal sample examination was done by using sedimentation method and saturated sodium chloride floatation technique (Soulsby, 1982) for the presence of stongyle eggs. The positive samples were further subjected to quantitative faecal sample examination by modified McMaster's technique (Coles *et al.,* 1992). The faecal egg counts were expressed as eggs per gram (EPG). The animals having EPG of faeces more than 150 were selected for the study. The sick animals were isolated from the rest of the herd.

A pooled faecal culture was prepared for each farm from the faecal samples collected pre- and post- treatment. The cultures were incubated for approximately 14 days at 25^0C in moist conditions. The faecal cultures were then harvested and the larvae were identified based on the morphological keys (Van Wyk and Mayhew, 2013). The generic percentage of the gastrointestinal nematodes present in the culture was determined from a random examination of 100 larvae.

Anthelmintic Treatment

Overall, 45 farms were randomly selected from different taluks of Hassan district, Karanataka state and were treated with anthelmintics (Fenbendazole, Ivermectin). Twenty-six farms were treated with fenbendazole and 19 farms were treated with ivermectin.

Faecal Egg Count Reduction Test

Efficacy of the anthelmintics were tested and interpreted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations for efficacy evaluations (Das and Singh, 2005). The efficacy of oral formulations of both, a benzimidazole (BZ) group - fenbendazole (PANACUR, dose- 5mg/kg body

weight) and a macrocyclic lactone (ML) group – ivermectin (IVOMEC, dose- 0.2mg/kg body weight) was investigated by faecal egg count reduction test (FECRT). The faecal egg count reduction (FECR) per cent estimated as per the formula given by McKenna (2006).

$$FECR\ \% = 100 \times (1 - T_2/T_1)$$

T_1 - pre-treatment faecal egg count; T_2 - post-treatment faecal egg count

Interpretation of Results

The data were statistically analysed to determine the per cent reduction in egg counts using a programme, RESO. Resistance to a particular class of anthelmintic was considered if the percentage reduction in egg count was less than 95 per cent and also the lower 95 per cent confidence level was less than 90 per cent. If only one of the two criteria was met, the resistance was classified as suspected (Coles *et al*., 1992).

Results and Discussion

In this study the anthelmintic resistance levels in field population of nematode parasites from sheep farms was investigated in Hassan district. The resistance status against the benzimidazole and macrocyclic lactone group drugs *viz*., fenbendazole and ivermectin was determined by FECRT. Faecal samples of 45 farms were screened for GIN infections. Arithmetic mean and the standard deviation (SD) of the pre- and post- treatment faecal egg counts expressed as EPG of faeces in the farms are presented in Table 1. It was observed that the post treatment arithmetic mean EPGs were low in general.

Table 1: Arithmetic mean ±standard deviation (SD) of the pre- and post- treatment faecal egg counts in the farms

Farm No.	N	Benzimidazole group – Fenbendazole				Farm No.	N	Macrocyclic lactone group- Ivermectin			
		Pre-treatment		Post-treatment				Pre-treatment		Post-treatment	
		Mean	±SD	Mean	±SD			Mean	±SD	Mean	±SD
1	15	1033	302	127	121	23	16	1534	2491	0	0
2	10	555	323	70	110	24	16	76	93	0	0
3	15	27	57	0	0	29	10	570	560	0	0
4	12	308	636	0	0	30	15	335	105	0	0
5	12	800	1288	92	164	31	14	650	273	57	112
6	10	210	230	0	0	32	10	395	300	0	0
7	12	117	172	0	0	33	12	550	218	17	55
8	10	8	28	0	0	34	14	200	209	0	0
9	8	581	234	38	70	35	15	530	516	0	0
10	11	70	155	0	0	36	15	329	181	0	0
11	8	550	229	44	58	37	11	564	161	55	99
12	10	1000	819	160	174	38	12	675	299	17	55
13	14	1537	1213	277	263	39	11	886	1305	0	0
14	12	1375	1447	217	182	40	10	1397	1779	0	0
15	8	14	20	0	0	41	10	3144	2680	0	0
16	10	857	667	86	83	42	10	595	891	0	0
17	8	100	120	0	0	43	9	55	133	0	0
18	15	1257	2505	104	221	44	15	211	98	0	0
19	12	1496	1594	158	166	45	14	443	209	0	0
20	15	390	170	47	96						
21	14	326	271	0	0						
22	11	117	181	36	64						
25	15	703	320	53	109						
26	9	220	134	0	0						
27	10	770	246	50	81						
28	10	240	154	0	0						

Out of 45 farms, 26 farms were treated with fenbendazole and 19 farms were treated with ivermectin. Overall, resistance to fenbendazole was found on 15 out of 26 (57.69 %) sheep farms. The anthelmintic resistance to fenbendazole have also been reported from various parts of India (Arunachalam *et al*., 2005; Das and Singh, 2005;

Singh *et al.*, 2017; Varadarajan *et al*., 2019). Out of 19 farms screened, two farms (10.53 %) revealed resistance to ivermectin. Detection of ivermectin resistance in GIN was found to be alarming. These findings were in concurence with those of Makawana & Singh (2009). There are comparatively fewer reports of resistance to ivermectin from India (Vieira *et al*., 1992; Ranjan *et al*., 2002). The per cent reduction in faecal egg counts and upper and lower 95 per cent confidence limit of all the farms treated with fenbendazole and ivermectin are presented in Table 2.

Table 2: The per cent reduction in faecal egg counts and upper and lower 95 per cent confidence limit of farms with fenbendazole and ivermectin resistance

S. No.	Farm No.	Drug	FECR per cent	95 per cent Confidence limits	
				Upper	Lower
1	1	Fenbendazole	77	72	52
2	2		87	89	74
3	5		87	93	82
4	9		93	97	86
5	11		90	95	83
6	12		75	81	68
7	13		82	91	65
8	14		84	87	80
9	16		90	94	84
10	18		94	99	66
11	19		89	92	86
12	20		88	96	61
13	22		93	97	88
14	25		92	95	88
15	27		94	96	89
16	31	Ivermectin	92	95	88
17	37		90	94	85

The mean FECR per cent after treatment with fenbendazole ranged from 77 to 94 per cent in sheep farms of various taluks indicating resistance to fenbendazole in GINs. The mean FECR per cent values after treatment with ivermectin ranged from 90 to 92 per cent which is suggestive of resistance to ivermectin. Overall, the resistance status to fenbendazole was higher when compared to ivermectin. This could be attributed to the prolonged and intensive use of the drug. As per the history collected from the animal owners, fenbendazole was the most commonly used anthelmintic for many years and the drug is being widely used by the animal owners for deworming without consulting the veterinarians, which might often lead to under dosing or over dosing of the animal. In addition to this the number of the farms involved in the study of anthelmintic resistance against fenbendazole was more as compared to ivermectin. These might be the probable reasons for detection of more resistance to fenbendazole. The grazing behaviour is attributable to the increased load of GI parasites in sheep. Their close grazing behaviour allow them to feed upon even small amount of parasites/eggs that reside on the ground, hence the GI parasites are more prevalent in sheep than goats (Waruiru *et al*., 2005). Hence anthelmintic treatments are frequently administered in sheep herds (Singh *et al*., 2017).

In the present study pre-treatment faecal culture of all the farms collectively revealed *Haemonchus contortus* as the predominant parasite, followed by *Trichostrongylus* spp., *Oesophagost omum* sp., *Bunostomum* sp. and *Cooperia* sp. by morphological identification. The findings are consistent with the findings of other workers from different parts of the world (Tsotetsi *et al*., 2013; Aruna *et al*., 2016; Vanisri *et al*., 2016). Out of the 15 farms found resistant to fenbendazole, post-treatment faecal cultures showed the predominance of *H. contortus* in 12 farms and *Trichostrongylus* spp. in 3 farms. This propound the emergence of resistance in *Trichostrongylus* spp. The results were in agreement with Jeyathilakan *et al*. (2003) and Vardharajan *et al*. (2019).

In the ivermectin resistant farms the post treatment faecal cultures revealed *H. contortus* as the predominant species. The results were in accordance with Scheuerle *et al.* (2009) where the author reported *H. contortus* as the predominant species in sheep and Jaiswal *et al.* (2013) who reported resistance to mainly *Haemonchus* spp. and to a lesser degree against *Trichostrongylus* spp. against ivermectin in goats.

Conclusion

Overall, these results have practical implications for sheep owners in terms of anthelmintic resistance. In the light of this animal owners should be advised for rotational use of different group of anthelmintics and the advantage of grazing management. It is the need of the hour to educate the farmers about the problems of development of anthelmintic resistance. Control strategies for gastrointestinal nematode infections should be directed towards the management rather than the use of anthelmintics until and unless it is essential. Appropriate use of anthelmintics and good management will lead to delay in onset of anthelmintic resistance in GINs affecting small ruminants.

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Conflict of Interests

There is no conflict of interest.

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