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EMERGENCE OF ANTHELMINTIC RESISTANCE IN NATURALLY INFECTED GOATS OF CUDDALORE DISTRICT, TAMIL NADU

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Abstract

Faecal egg count reduction tests (FECRT) were conducted on goat flocks of all the six taluks of Cuddalore district of Tamil Nadu, India to determine the efficiency of anthelminitics (Feubendazole, Levamisole and Ivermectin) used for treatment against nematode parasites. The results of the present study revealed high levels of anthelminitic resistance to Fenbendazole treated goats of all over the flocks of Cuddalore district with the reduction of 74 – 91 per cent, whereas, the goat flocks of Chidambaram, Cuddlore and Vridhachalam taluks found to be low resisitant to Levamisole with the faecal egg reduction of 91, 91 and 90 per cent respectively. Ivermectin was found to be effective in controlling nematodes in all the farms. The post-treatment (fenbendazole and levamisole) larval culture revealed the presence of *Haemonchus contortus* larvae.
(Key Words: Anthelmintic resistance, goats, Cuddalore)

1. Introduction

Anthelmintic resistance (AR) has developed global issue in the small ruminant industry during past few decades. Most probably, AR is of greater concern in goats than in sheep [1]. Sheep and goats differ in many aspects; as goats have a higher metabolic rate and require higher dose rates for drugs [2-5]. The immune system of goats is also different. The modern broad-spectrum anthelmintics are currently used in prophylaxis and treatment of helminth infections in farm animals [6]. The over usage of anthelmintics ended with the problem of resistance development in the targeted organisms. Anthelmintic resistance is due to traditional treatment, low protein diet and inadequate dose level of anti-parasitic agents [7-10].

In small ruminants, gastrointestinal parasitism is one of the most important cause for production losses around the world. The controlling of G.I parasites can be achieved by various anthelmintics in India despite indiscriminate and frequent usage of anthelmintics exhibits decline in their efficiency and hence resulted in anthelmintic resistance [10-12].

A variety of methods are available to measure anthelmintic resistance including *in vivo* tests such as critical anthelmintic test, controlled anthelmintic test, faecal egg count reduction test and various *in vitro* tests such as egg hatch assay, larval development assay etc [13-16]. The faecal egg count reduction test (FECRT) [17] is recommended by World Association for the Advancement of the Veterinary Parasitology (WAAVP) [18] and is the test of choice especially in the survey for resistance. The status of Fenbendazole, Levamisole and Ivermectin resistances in gastrointestinal nematodes in goat flocks of Cuddalore district, Tamil Nadu, India, has been studied.

2. Materials and Methods

2.1 The study area

The study was conducted in six small holder goat flocks of Chidambaram, Kattumannarkoil, Cuddalore, Panruti, Vridhachalam and Tittakudi taluks of Cuddalore district. One goat flock from each taluk was selected based on good management and having more than 60 animals. Cuddalore district is located between $11^0$11' to $12^0$35' North latitude and $78^0$38' to 80^0 East Longitude and is predominately an agricultural district. Average elevation of the district is 1 m (3 ft) above Mean Sea Level.

2.2 Experimental design and Anthelmintic treatment

The selected goats were of mixed sex and of 5 to 15 months of age. The age of individual goats was determined from birth register maintained in the farm and also by dentition. Each goat was identified using a numbered ear tag. The selected goats were grouped in to four each group consisting of 15 animals in all the field flocks. None of the goats received any anthelmintic two months before the start of the experiment. The goats were then naturally infected on pastures. Faecal egg counts expressed as egg per gram was done on day 0 before treatment and then 10 days after treatment with anthelmintics. The drugs used for the test are given in Table 1.

2.3 Assessment of efficacy of anthelmintics

Rectal faecal samples were collected on day zero before treatment and then day 10 after treatment. Using gloved finger, about 10 gm of samples were obtained from each goat by digital rectal extraction and then immediately placed in a plastic bag. The bag was tightened as close to the faces as possible to keep off air. Each sample was labelled and transported to the laboratory for further analysis.

2.4 Detection of nematode eggs and estimation of faecal egg counts (FEC)

The simple test tube floatation method was used in the detection of the nematode eggs. Identification of nematode

eggs was done as described by soulsby, 1982. FEC were determined as number of eggs per gram for each sample using a modified Mcmaster technique. The detection level of the McMaster method used was 100 epg.

2.5 Faecal egg count reduction test (FECRT)

The EPG of strongyle- type nematodes were subjected to the faecal egg count test (FECRT), to estimate anthelmintic efficiency faecal egg counts were used to calculate the percentage efficacy of each anthelmintic using the following formula:

$$\text{FECR} = \left\{1 - \left[\left(\frac{T_2}{T_1}\right) X \left(\frac{C_1}{C_2}\right)\right]\right\} \text{ X } 100$$

Where $T_1$and T_2 are pre-and post treatment arithmetic means of the egg per gram in treated groups, and C_1 and C_2 are pre-and post-treatment arithmetic means of the egg per gram in the control group.

Efficacy of each anthelmintic was tested and interpreted according to the World association for the advancement of veterinary parasitology (WAAVP) recommendations for efficacy evaluations of anthelmintics [4]. Reduction in efficiency and presence of anthelmintic resistance is considered to exist if the FECRT percentage of an anthelmintic is < 95 %.

2.6 Coproculture and larval identification

Coproculture was done on pooled pre-treatment samples and post treatment samples for identifying the species of infecting nematodes. Mature third stage larvae were identified based on morphological characters (VanWyk *et al*.2004).

2.7 Interpretation of results

The data wereanalysed statistically for finding out the per cent reduction in egg counts using a programme, RESO. Reduction in egg counts of less than 95 per cent with lower 95 per cent confidence limit less than 90 was considered as indicative of resistance against the drug (Coles *et al*. 1992).

3. Results and Discussion

All the investigated goats were found positive for GI nematode infection at day 0 of screening. Examination at day 10, the post treatment revealed the variation in the degree of egg reduction in all the faecal samples (Table 2).

The results indicate the development of resistance against fenbendazole in all the field flocks with lower reduction percentage of 74, 88, 90, 90, 91 and 91 in Chidambaram, Kattumannarkoil, Cuddalore, Panruti, Vridhachalam and Tittakudi taluks respectively. Low resistant to Levamisole found in the fieldflocks of Chidambaram, cuddalore, Vridhachalam and Tittakudi flocks. Resistance to fenbendazole could be attributed to the prolonged and intensive use of the drug over the years. The drug is being widely used by the farmers for deworming their livestock even without proper veterinary advice, often leading to under dosing. In the present study, ivermectin was found effective with a percent egg count reduction of 96 – 99 per cent in all the goat farms of Cuddalore district. This can be attributed to the fact that the use of oral ivermectin for deworming has been introduced only recently and its use is not widespread.

From the above results, it is noted that Fenbendazole at the recommended dosage was not effective against gastrointestinal nematodes at the field goat flocks of Cuddalore district and levamizole were also not effective against GI nematodes of field goat farms in certain taluks of Cuddalore district.

The post-treatment (fenbendazole and levamizole) larval culture revealed the presence of *Haemonchus contortus* larvae.

Reports of anthelmintic resistance are mainly from organized farms with intensive anthelmintic treatment schedules. Existence of drug resistant GI nematodes in breeding animals in farms increases the risk of dissemination of resistant strains to small holder farmers' flocks as farm bred animals are distributed to farmers [6]. Reports of anthelmintic resistance from small holder farmers' flocks are rare or uncommon, but if the present use of anthelmintics is continued, the situation can become unmanageable [9]. Thus the detection of anthelmintic resistance in small holder farmers' flocks is significant and warrants implementation of proper anthelmintic treatment strategies to check further development of resistance [16]. It clearly demonstrated that the goats in the coastal areas of Tamil Nadu have retained resistance to both Fenbendazole and Levamisole as a result of frequent and routine usage. Withdrawing that type of anthelmintic from use and replacing it with an alternate drug along with suitable grazing methods could be the need of this hour.

Conclusion

Faecal egg count reduction tests (FECRT) were conducted in goat flocks of all the 6 taluks of Cuddalore district, Tamil Nadu to determine the efficiency of anthelminitics (Fenbendazole, Levamisole and Ivermectin) used for treatment against nematode parasites. The results of the present study revealed high levels of anthelminitic resistance to Feubendazole treated goats of all the field flocks of the district and some of the flocks also revealed the resistance against Levamizole. Ivermectin was found to be effective in reducing the EPG in all the field flocks. It clearly demonstrated that the goats in the Cuddalore district have retained resistance to both Fenbendazole and Levamisole as a result of frequent and routine usage. Withdrawing that type of anthelmintic from use and replacing it with an alternate drug along with suitable grazing methods could be the need of this hour.

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Annexure

Table 1. Anthelmintics used in the field flocks

Animals	Drugs	Company	Dose (mg / kg BW)	Route of Administration
Group I	Fenbendazole	Intervet India pvt Ltd	7.5	per os
Group II	Levamisole Hydrochloride	Virbac Animal Health India pvt Ltd	22.5	per os
Group III	Ivermectin Oral solution	Virbac Animal Health India pvt Ltd	2.5 ml / 10 kg BW	Per os
Group IV	Control	-	-	-

Table 2. Mean faecal egg counts and faecal egg count reduction values on pre and post anthelmintic treatments in goat

Sl No	Smallholder flocks	Anthelmintic	Mean faecal egg count (EPG)		Mean faecal egg count in control group (EPG)		FECR (%)	95% confidence limit	
			before treatment	after treatment	before treatment	after treatment		Upper	Lower
1.	Chidambaram Taluk	FBZ	1540±50.30	406.66±34.20	1786.66 ± 48.30	2133.33± 53.13	74	78	68
		LEV	1786.66±96.29	160±22.13			91	93	88
		IVM	1920±84.03	73.33±15.13			96	98	94
2.	Kattumannarkoil Taluk	FBZ	1613.33±58.78	186.66±26.47	1680 ± 73.67	2093.33 ± 62.65	88	91	84
		LEV	1666.66±56.84	153.33±30.07			91	94	86
		IVM	1793.33±80.47	66.66±16.49			96	98	94
3.	Cuddalore Taluk	FBZ	1733.33±55.93	166.66±39.98	1680 ± 64.04	2253.33 ± 93.60	90	94	84
		LEV	1660±84.39	166.66±29.73			90	93	85
		IVM	1953.33±70.61	40±16.90			98	99	95
4.	Panruti Taluk	FBZ	1440±84.39	140±31.62	1106.66 ± 46.80	1553.33 ± 58.78	90	94	84
		LEV	1680±72.98	46.66±17.10			97	99	94
		IVM	1333.33±69.74	40±13.55			97	99	94
5.	Vridhachalam Taluk	FBZ	1220±51.70	106.66±18.80	1113.33 ± 52.35	1593.33 ± 61.83	91	94	87
		LEV	1320±54.58	66.66±13.04			95	97	92
		IVM	1146.66±49.34	73.33±15.86			94	96	90
6.	Tittakudi Taluk	FBZ	1253.33±63.78	106.66±21.34	973.33 ± 52.94	1513 ± 50.37	91	94	87
		LEV	1160±54.21	60±13.55			95	97	92
		IVM	1273.33±79.19	53.33±13.80			96	98	93