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Study on anthelmintic resistance in sheep flocks of Cuddalore district, Tamil nadu

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ABSTRACT

Anthelmintic resistance against commonly used anthelmintics (ivermectin, levamisole, and fenbendazole) was studied in naturally occurring gastrointestinal (GI) nematodes in adult sheep flocks of Cuddalore district, Tamil Nadu by using Faecal egg count reduction tests (FECRT) and also 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 Fenbendazole treated sheep flocks in Cuddalore district with the reduction of 50 - 89 per cent, whereas, a low resistant to Levamisole with the faecal egg reduction of 93 - 95 per cent. Ivermectin was found to be effective in controlling nematodes in all the sheep farms. The post-treatment (fenbendazole and levamisole) larval culture revealed the presence of *Haemonchus contortus* larvae.

(***Index terms:*** Anthelmintic resistance, Sheep, Cuddalore)

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INTRODUCTION

The infections of gastrointestinal parasites among small ruminants were most prevalent throughout the year in organized and small holding flocks in varying intensity (Varadharajan and Vijayalakshmi, 2015a). Parasite control, especially the control of helminth parasites, predominantly relied upon the use of anthelmintic drugs and it will continue to remain as the cornerstone of helminth control in the foreseeable future (Sanyal, 2004). Therefore, periodic evaluation of efficacy of commonly used anthelmintics against gastrointestinal nematodosis in sheep to detect the emergence of resistance is an important area of helminth control. This would help in strategic and judicious use of anthelmintics in a particular area and further limit the development of resistance. Anthelmintic resistance has become an increasingly widespread problem in recent years in many parts of India (Dhanalakshmi *et al.*, 2003; Das and Singh, 2005; Chaudhary *et al*., 2007; Singh *et al*., 2010, Varadharajan and Vijayalakshmi, 2015b). As, the informations available on anthelmintic resistance is sparse in Cuddalore district, Tamil Nadu, so the present study was undertaken to detect anthelmintic resistance against commonly used anthelmintics (ivermectin, levamisole, and fenbendazole) in naturally occurring gastrointestinal (GI) nematodes in sheep flocks.

MATERIALS AND METHODS

The study area

The study was conducted in six small holder sheep flocks of Chidambaram, Kattumannarkoil, Cuddalore, Panruti, Vridhachalam and Tittakudi taluks of Cuddalore district. One sheep 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.

Experimental design

The selected sheep were of both male and female and of 5 to 15 months of age. The age of individual sheep was determined from birth register maintained in the farm and also by dentition. Each sheep was identified using a numbered ear tag. None of the sheep received any anthelmintic two months before the start of the experiment. The sheep 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.

Anthelmintic treatment

Each sheep was treated with an anthelmintic with a dose recommended according to the manufacturers. The anthelmintics used were: Fenbendazole - 5 mg/kg body weight [PANACUR –

Intervet India pvt Ltd]; Levamisole Hydrochloride- 15 mg/kg body weight [NILVERM – Virbac Animal Health India pvt Ltd] and Ivermectin - 0.1 mg/kg body weight [HITEK – Virbac Animal Health India pvt Ltd]. Fenbendazole and levamisole were administrated orally using calibrated syringes whereas Ivermectin was administrated via subcutaneous injection route with calibrated syringes and needles. Pretreatment fasting of 8 hrs was instituted to facilitate effectiveness of the anthelmintics administered.

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 sheep by digital rectal extraction and then immediately placed in a plastic bag. The bag was tightened as close to the faeces as possible to keep off air. Each sample was carefully labeled with the details of the individual sheep for identification, and put in a cold box containing ice packs. The samples were transported to the laboratory for further analysis.

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.

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 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 epg in treated groups, and C_1 and C_2 are pre-and post-treatment arithmetic means of the epg 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 %.

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 and Mayhew, 2013).

Interpretation of results

The data were analysed 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).

RESULTS AND DISCUSSION

All the investigated sheep were found positive for GI nematode infection on day 0 of screening and on day 10, the post treatment revealed the variation in the degree of egg reduction in all the faecal samples (Table 1).

The mean FECR after treatment with fenbendazole ranged from 50 to 91 per cent in sheep flocks of various taluks indicating resistance for fenbendazole to GINs in Cuddalore district. The mean FECR values after treatment with levamisole ranged from 93 to 96 per cent for sheep flocks of Cuddalore district indicating the susceptibility of nematodes to levamisole. The mean FECR values after treatment with ivermectin ranged from 94 to 97 per cent in sheep is suggestive of susceptibility to ivermectin in nematodes of sheep flocks of Cuddalore district.

The results of the present study indicated multiple resistance for *H. contortus* against fenbendazole and susceptible to levamizole nd ivermectin in all the sheep flocks of Cuddalore district. The prevalence of fenbendazole and levamisole resistant nematodes in sheep using FECRT was earlier reported by various authors in Tamil Nadu; Arunachalam *et al.* (2005); Lourderaj, (2005); Easwaran *et al.* (2009) and Meenakshisundaram *et al.* (2014).

In this study, *H. contortus* was the predominant nematode involved in resistance. This is in accordance with earlier surveys on anthelmintic resistance in Tamil Nadu; Meenakshisundaram, (1999); Jeyathilakan *et al*. (2003) and Lourderaj, (2005).

Anthelmintic resistance in *H. contortus* to fenbendazole was also detected in small holder sheep flocks of Cuddalore district, albeit not multiple anthelmintic resistance. A varied response either resistance or susceptibility was exhibited by each flock depending upon the type of anthelmintic drug. This may be due to frequent and indiscriminate use of anthelmintic drugs by the farmers that led to anthelmintic resistance in animals as reported by Jaiswal *et al.* (2013). Thus, the present study indicated that the anthelmintic resistance against fenbendazole in small holder sheep flocks was significant and warrants implementation of proper anthelmintic treatment strategies to check further development of resistance against levamizole and ivermectin.

CONCLUSION

Faecal egg count reduction tests (FECRT) were conducted in sheep 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 Fenbendazole treated sheep of all the field flocks in the district. It was found that Levamizole and Ivermectin were effective in reducing the EPG in all the field flocks. It was clearly demonstrated that the sheep in the Cuddalore district developed resistance to Fenbendazole as a result of frequent and routine usage. The resistant anthelmintic to be withdrawn from use and replace it with an alternate drug along with suitable grazing methods could be the need of the hour.

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Table 1. Mean faecal egg counts and faecal egg count reduction values on pre and post anthelmintic treatments in Sheep

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	1386.66±74.13	700±41.64	1806.66 ± 46.80	2326.66± 104.71	50[R]	57	41
		LEV	1793.33±127.56	86.66±19.86			95	97	92
		IVM	2226.66±46.80	100±24.74			96	97	92
2.	Kattumannarkoil Taluk	FBZ	1673.33±96.07	426.66±42.21	1626.66 ± 111.33	2293.33 ± 107.12	75[R]	80	68
		LEV	1840±107.76	80±20.70			96	97	93
		IVM	1766.66±54.08	93.33±15.86			95	96	92
3.	Cuddalore Taluk	FBZ	1273.33±76.57	160±34.69	1186.66 ± 63.78	1753.33 ± 57.91	87[R]	92	80
		LEV	1226.66±63.46	53.33±24.46			96	98	89
		IVM	1233.33±63.78	66.66±19.34			95	97	90
4.	Panruti Taluk	FBZ	1580±64.83	206.66±39.72	1633.33 ± 57.73	1993.33 ± 71.04	87[R]	91	81
		LEV	1640±60.44	113.33±26.47			93	96	89
		IVM	1793.33±66.59	106.66±25.68			94	96	90
5.	Vridhachalam Taluk	FBZ	1780±87.01	166.66±32.99	1480 ± 72.28	1800 ± 76.93	91[R]	94	86
		LEV	1986.66±76.17	93.33±21.34			95	97	92
		IVM	1766.66±67.51	60±16.90			97	98	94
6.	Tittakudi Taluk	FBZ	1826.66±60.15	206.66±34.20	1513.33 ± 94.14	1873.33 ± 74.55	89[R]	92	84
		LEV	1953.33±57.02	93.33±25.68			95	97	92
		IVM	1513.33±94.14	66.66±19.34			96	98	93

R – Resistance by calculation method