ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODES OF SHEEP

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ABSTRACT

Anthelmintics are used traditionally as an integral part of helminthic control strategies for grazing livestock to prevent production losses from parasitic infections. The continuous and indiscriminate use of the same anthelmintics over years together as the sole means of control are now failing due to the emergence of resistance strains of helminths. Resistance to the commonly used anthelmintics in gastrointestinal nematodes of sheep has become an increasingly wide spread problem throughout the world. The present study was aimed to determine the resistance against albendazole, fenbendazole, levamisole and closantel in gastrointestinal nematodes of sheep. Fifty five naturally infected Madras Red lambs of 6 - 12 months of age were selected and distributed randomly into five treatment groups of eleven animals each. Four groups were treated orally with albendazole (5mg/kg), fenbendazole (7 mg/kg), levamisole (7.5m/kg) and closantel (10 mg/kg) respectively, while the fifth group served as untreated control. Faecal samples were collected per rectum of each lamb just prior to treatment (pre treatment) and then on days 7, 14, 21 and 28 after treatment. The anthelmintic resistance was evaluated by in vivo faecal egg count reduction test (FECRT), post treatment larval culture and in vitro egg hatch assay. In the faecal egg count reduction test, albendazole reduced the faecal egg count by 86.50 per cent, 84.81 per cent, 85.28 per cent and 84.47 per cent respectively for four weeks after treatment. Faecal egg count reduction using fenbendazole was 92.64, 93.04, 90.80 and 90.06 per cent respectively for four weeks after treatment. The per cent efficacy for levamisole and closantel was more than 95 per cent. The post treatment larval culture contained only Haemonchus contortus. In the in vitro egg hatch assay, the ED_{50} value for benzimidazole was 0.299 µg thiabenazole/ml and levamisole showed an ED_{50} value of 0.283 µg /ml.

Key words: Benzimidazole resistance, Sheep, GI Nematodes. FECRT, Egg hatch assay

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INTRODUCTION

The extensive use of anthelmintics for control of gastrointestinal nematodes has resulted in development of resistance to one or more of the widely used anthelmintics in many countries. (Maingi et al., 1998). Resistance to anthelmintics by gastrointestinal nematodes of sheep and goat is widespread problem. A recent study performed on anthelmintic worm control practices in Norwegian sheep and goat flocks has indicated the occurrence of under dosing, a lack of anthelmintic class rotation and, in some breeding areas, a high drench frequency, which alone or in combination, are likely to increase the risk for anthelmintic resistance (Domke., et al., 2011). Further, mixed grazing of sheep and goats has been evoked as a possible risk factor for the spread and emergence of anthelmintic resistance. A number of reports on anthelmintic resistance were documented in many countries (Waller, 1987; Taylor and Hunt, 1989). In addition, multiple resistance to most of the anthelmintics against gastrointestinal nematodes have also been detected in many countries (Paraud et al., 2009). In India, even though many reports have been undertaken to highlight the resistant status among the gastrointestinal nematodes in Northern states (Uppal et al., 1992; Singh et al., 1995), the reports about the same in South India are very limited. Hence, the present study was designed to detect the resistance to the most commonly used anthelmintics viz. albendazole, fenbendazole, levamisole and closantel against gastrointestinal nematodes of sheep in an organized farm by the widely used in vivo faecal egg count reduction test and in vitro egg hatch assay.

MATERIALS AND METHODS

In vivo assay - Faecal Egg Count Reduction Test (FECRT)

The present study was carried out at Livestock Research Station, Kattupakkam, a research unit of Tamilnadu Veterinary and Animal Sciences University. Madras Red breed of sheep formed the experimental animals for this study, which were managed in a semi-intensive system of rearing. Regular deworming was carried out under a scheduled programme viz. once in three months. Fifty five naturally infected Madras Red lambs of 6 - 12 months of age were randomly distributed into four treatment groups of eleven animals each and the fifth one was maintained as control group. Four anthelmintics viz. albendazole (Azole, Vet India, 5.0 mg/kg b.wt), fenbendazole (Fzole Vet India, 7.0 mg/kg b.wt), levamisole hydrochloride (Alved, 7.5 mg/kg b.wt) and Zycloz (Closantel, Zydus Agrovet, 10.0 mg/kg b.wt) were used in the study. All the drugs were administered orally.

About five grams of faecal sample was collected per rectum from each lamb just prior to treatment (Pre-treatment sampling) and then on days 7, 14, 21, 28 after treatment (Post-treatment sampling) for assessing the gastrointestinal nematode infection in terms of eggs per gram (epg) using modified McMaster method (Coles et al., 1992). The data were calculated according to the method laid down by the World Association for the Advancement of Veterinary Parasitologists (WAAVP) (Coles et al., 1992). Reduction in the egg count of less than 95 per cent and lower confidence level of less than 95 per cent compared with the untreated control animals are considered as resistant.
Coproculture and larval identification

After examination of the faecal samples for nematode egg counts, the pooled faecal samples were cultured to determine the species spectrum of mixed gastrointestinal nematode species. The coproculture was done and the larvae were identified as described by MAFF (1971).

In vitro assay - Egg Hatch Assay

Pooled faecal samples were obtained by mixing several samples collected per rectum from a number of sheep. Eggs were isolated from the faeces as per the method described by Coles et al. (1992) and used in the assay.

Preparation of albendazole stock solution and Test Protocol

A stock solution of albendazole was prepared by dissolving 50 mg of the pure chemical albendazole (Vet India Pharmaceutical Limited) in 250 ml of methanol. Working solutions of the same containing albendazole 200 µg/ml to 2 µg /ml were prepared by further dilutions with methanol.

Ten microlitre of the working solution of albendazole was pipetted into each well of the tissue culture plate. Two ml distilled water containing a minimum of 200 eggs was added to each well so as to get the final concentration of 1 µg to 0.01 µg of albendazole/ml. Assays were conducted in triplicate with a minimum of ten serial concentrations for each. Eggs in distilled water alone and in distilled water containing 10 µg methanol were used as controls. After incubation at 27° C for 48 hrs, two drops of aqueous iodine was added to each well to prevent further hatching and hatched larvae and the number of eggs in each well were counted. The percentage of eggs, which had failed to hatch at each drug concentration was calculated. The data were subjected to arcsin transformation to find out the ED₅₀ value as per Rahman (1993).

Preparation of levamisole stock solution and Test protocol

A stock solution of levamisole was prepared by dissolving 50 mg of the pure chemical levamisole (Vet India Pharmaceutical limited) in 250 ml of deionised distilled water. Test solutions were then made by standard dilution techniques as per Cawthorne and Whitehead (1983).

Suspensions of 0.1 ml containing 70 - 199 eggs were dispensed into each well of a flat-bottomed microtitration plate. The plate was covered and incubated at 26° C in a saturated atmosphere to prevent evaporation. When the first stage larvae became transparent, 0.1 ml of prepared range of levamisole hydrochloride concentrations (0.05 - 0.7 µg) was added to each well and incubated for 6 hrs at 26° C to allow hatching in the control well. The plate was then snap cooled for 5 minutes and then 0.1 ml of chilled 40 per cent formaldehyde was added to each well. The plate was then held at 4° C for overnight cooling. The percentage of eggs, which had failed at each drug concentration, was calculated as described by Dobson et al. (1986). The data were subjected to arcsin transformation to find out the ED₅₀ value as per Rahman (1993).
RESUL TS AND DISCUSSION

The pre-treatment, post treatment egg count and the per cent reduction in the faecal egg counts are presented in Table 1. The faecal egg count reduction of above 95 per cent was obtained with the drug levamisole and closantel for all the four weeks after treatment.

Albendazole had efficacy of 86.50, 84.81, 85.28 and 84.47 per cent, respectively at weekly intervals for four weeks after treatment with lower than 95 per cent confidence limits amounting to 82.86, 80.55, 81.14 and 79.57 per cent respectively for four weeks. Fenbendazole reduced the egg count by 92.64, 93.04, 90.80 and 90.06 per cent respectively at weekly intervals for four weeks after treatment with lower than 95 per cent confidence limits amounting to 89.05, 89.71, 86.01 and 85.22 per cent respectively for four weeks. The mean faecal egg count reduction in levamisole treatment group was 98.77, 97.47, 97.55 and 97.52 percent respectively, whereas in closantel treated group, the faecal egg count reduction was 100 per cent in all the four weeks.

The post treatment (albendazole and fenbendazole) larval culture revealed the presence of Haemonchus contortus larvae. However, in the other two groups no larvae were found.

The ED$_{50}$ value obtained after arcsin transformation of the data using albendazole as a reference drug in the egg hatch assay was 0.299 µg/ml (Fig. 1).

An ED$_{50}$ value was 0.283 µg levamisole /ml obtained after arcsin transformation of the data in the study (Fig. 2).

The results of faecal egg count reduction test in this study confirmed the presence of benzimidazole (albendazole and fenbendazole) resistance confirming the findings of Coles et al. (1992) who reported that there was resistance, when an anthelmintic showed efficacy less than 95 per cent and when the lower confidence limit was less than 95 per cent. Similar results were also recorded by Yadav et al. (1993); Srivastava et al. (1995) and Kochapakdee et al. (1995).

The observation on the fenbendazole resistance in the farm under study was expected since cross resistance between benzimidazole would equally be reflected in the use of another anthelmintic having similar mode of action (Maingi, 1991). Similar observations were also documented by many workers (Prichard et al., 1980; Pandey and Sivaraj, 1994).

The survival of Haemonchus contortus in albendazole and fenbendazole treated groups are in agreement with the findings of Höglund et al. (2009) and Atle et al. (2012).

The ED$_{50}$ value of 0.299 µg albendazole/ml obtained in the egg hatch assay for bezimidazole group confirmed resistance as it was well above the limit (0.1 µg/ml) prescribed by Coles et al., (1992) and the same was higher than the limit of 0.24 µg albendazole/ml as suggested by Smith_Bujs and Borgsteede (1986) who also used albendazole as a reference drug in the egg hatch assay. Similar observations on benzimidazole resistance with ED$_{50}$ value greater than 0.10 µg/ml of thiabendazole were previously recorded by Cawthore and Whitehead (1983) in United Kingdom, Singh et al. (1995) in India and Borgsteede et al. (1996) in the Netherlands.
The ED$_{50}$ value of 0.283 µg levamisole/ml recorded in this study indicated susceptibility which may be attributed to the fact that levamisole was not used as frequently as that of benzimidazole group of anthelmintics. In earlier studies, Maingi (1993) recorded an ED$_{50}$ value of 3.12 µg levamisole/ml indicating resistance. Since, benzimidazole are the most widely used anthelmintics, the development of resistance to albendazole was understandable. Frequent using of albendazole in the farm might have contributed to the resistance, since the drug had been used for years together as per the available records which agreed with the findings of Round et al. (1974), Barton (1983) and Martin et al. (1984).

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Anthelmintic Resistance in Gastrointestinal nematodes of sheep

Figure 1
*In vitro* egg hatch assay - Albendazole

Figure 2
*In vitro* egg hatch assay – Levamisole
CONCLUSION

Based upon the results, it is confirmed that *Haemonchus contortus* resistant to benzimidazole in sheep was detected in this farm.

REFERENCES


Maingi, N. (1991). Resistance to thiabendazole, fenbendazole and levamisole in *Haemonchus contortus* and
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