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Multiple anthelmintic resistance in *Haemonchus contortus* of sheep

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ABSTRACT

Faecal egg count reduction test (FECRT) and *in vitro* egg hatch assay (EHA) detected anthelmintic resistance in *Haemonchus contortus* in sheep. On FECRT, fenbendazole and tetramisole were, respectively, found 0 and 25% effective against *H. contortus*, while closantel showed 100% efficacy. Though rafoxanide was 96% effective against *H. contortus*, but lower 95% confidence limit of less than 90 suggested suspected resistance against the rafoxanide. The EHA showed LC_{50} value of 0.265 ± 0.098 µg thiabendazole / ml against *H. contortus*. It was concluded that *H. contortus* developed resistance against benzimidazole, tetramisole and low level resistance against rafoxanide.

Key words: Anthelmintic resistance, Egg hatch assay, Faecal egg count reduction, *Haemonchus contortus*, Sheep

Anthelmintic resistance of gastrointestinal nematodes (GIN) has become a widespread problem throughout the world (Jackson 1993, Waller 1994) in sheep and goats in organized farms where anthelmintics are extensively used. In India, the emergence of anthelmintic resistant *H. contortus* strains to various anthelmintics were recorded from sheep and goats reared in different agro-climatic zones (Singh *et al.* 1992, Gill 1993, Yadav *et al.* 1993, Singh *et al.* 1995, 1996). There are also a few reports of multiple anthelmintic resistance (Uppal *et al.* 1992, Gill 1996). This paper reports an occurrence of anthelmintic resistance in field strain of *H. contortus* of sheep reared in an organized farm.

MATERIALS AND METHODS

Farm history

The study was conducted at large scale sheep breeding farm, Fatehpur (Sikar) located in the arid region of Rajasthan. Around 5 500 sheep of exotic (Rambouillet) and crossbred (Rambouillet × Nali) were kept at 6 different sites situated in an area of around 7 000 ha. The animals were fed gram hay, *beri pala* (*Zizyphus nummularia*) and *khejari* (*Prosopis cineraria*) and supplemented with 100-250 g concentrate /head/ day. There was no rational policy for use of anthelmintics and worm control. In 1997-1998 the animals were dosed 10 times with various anthelmintics, viz. mixture of copper sulphate and nicotine, rafoxanide, levamisole, mebendazole and albendazole.

During April-May 1998, heavy mortality in sheep was reported at this farm. Though the deaths were due to varied reasons (pneumonia, GIN, hepatitis, debility etc.), but most of the sheep on necropsy showed heavy load of *H. contortus*. The animals were treated several times with different anthelmintics, but the problem of haemonchosis remained. The present trial was conducted in crossbred ewes and hoggets, which were previously drenched 1 month ago with levamisole.

Faecal egg count reduction test (FECRT)

Faecal samples from 65 naturally infected hogget sheep were evaluated for worm egg count by modified McMaster technique (Coles *et al.* 1992). Based on pre-treatment EPG, the animals were randomly divided into 5 groups of 13 animals each. Sheep of groups 1, 2, 3 and 4 were orally drenched with fenbendazole, tetramisole, closantel and rafoxanide @ 5, 15, 7.5 and 7.5 mg kg^{-1} body weight, respectively. The animals of group 5 were kept as untreated infected control. Faecal samples were collected from the rectum of each sheep on day 12 post-treatment and EPG were determined.

Pre-treatment pooled faecal samples from all the animals were cultured and third stage larvae (L_3) were identified (Soulsby 1965, Anonymous 1971). Similarly on day 12 post-treatment faecal sample from each group were pooled, cultured separately according to group and L_3 were identified.

The per cent faecal egg count reduction (FECR) was calculated by using RESO computer programme (Martin and Wursthorn 1991). The FECR was analyzed on the basis of post-treatment arithmetic group mean with lower and upper 95% confidence limit (Coles *et al.* 1992).

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In-vitro *egg hatch assay (EHA)*

Pooled faecal samples from adult flock of the same site were collected and eggs recovered using flotation technique (Coles *et al.* 1992). EHA was performed using pure thiabendazole in a dilution series of 0.0625 to 1.00 µg TBZ ml^{-1} as per Le Jambre (1976). Probit analysis was performed on EHA data to obtain TBZ concentration which on average would prevent 50% of eggs from hatching (LC_{50}) at 95% confidence limit (Finney 1965).

RESULTS AND DISCUSSION

The pre-treatment mean faecal egg counts varied from 3 208 (group 2) to 4 054 (group 1) eggs / g of faeces. On day 12 post-treatment, mean faecal egg counts were 4 092, 2 677, 8 131 and 3 569 in fenbendazole, tetramisole, closantel, rafoxanide and control group respectively. The fenbendazole and tetramisole reduced faecal egg count by 0 and 25%, respectively, with less than 90 lower 95% confidence limit. The per cent reduction in faecal egg count in group treated with rafoxanide was 96 but its lower 95% confidence limit was less than 90. However, closantel was 100% effective in eliminating the infection. The results of EHA showed that LC_{50} value of eggs was 0.625 ± 0.098 µg TBZ ml^{-1}.

At the study area benzimidazoles (BZD) and tetramisole were being used since a long time and rafoxanide was introduced in the last couple of years. The choice of anthelmintic was based on the availability of the drug instead of any rational scheme.

Coles *et al.* (1992) suggested that any anthelmintic having efficacy less than 95% with lower 95% confidence limit less than 90 should be classified as confirmed resistant in the target parasite against the drug based on FECRT. In this study, fenbendazole and tetramisole had only 0 and 25% efficacy with less than 90 lower 95% confidence limit which suggested that the parasite has developed resistance against these anthelmintics. The high LC_{50} value of EHA further confirmed the existence of BZD resistance at the farm. Le Jambre (1976) and Coles *et al.* (1992) reported that BZD susceptible parasites did not hatch in concentration higher than 0.1 µg TBZ ml^{-1} and had LC_{50} value of about 0.06 µg TBZ ml^{-1}. The lower efficacy and resistance to both these anthelmintic groups at the farm observed might be attributed to their continuous and prolonged use to control GIN at the farm. Although the efficacy of rafoxanide was 96% but its lower 95% confidence limit was less than 90 which indicated suspected resistance as per Coles *et al.* (1992). On FECRT the parasite was fully susceptible to closantel as there was 100% reduction in egg count following treatment with closantel.

Strains of *H. contortus* resistant to benzimidazole (Yadav 1990, Singh *et al.* 1992,Singh *et al.* 1995), levamisole/tetramisole (Yadav and Uppal 1992), rafoxanide (Singh *et al.* 1996) and multiple anthelmintics (Uppal *et al.* 1992, Gill 1996) were reported from various parts of India.

The very high frequency of anthelmintic treatment at the farm might have selected the *H. contortus* strain resistant to benzimidazole and tetramisole. However, closantel, not been used previously, showed 100% efficacy and this could be used for satisfactory control of *H. contortus*.

The pre- and post-treatment faecal cultures revealed predominance of *H. contortus* in fenbendazole and tetramisole treated groups. Therefore, it could be concluded that *H. contortus* had developed resistance against fenbendazole and tetramisole. Anthelmintic resistance in nematodes has great epistemologies significance and the resistant parasites were not only more fecund and pathogenic, but also had increased establishment rate in the host and increased survivability of free living stages (Kelly *et al.* 1978). Thus, regular monitoring of the status of anthelmintic resistance is required as an integral part of worm control programme, particularly in organized sheep farms.

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