Journal of Helminthology (1999) **73,** 137–142

Variation in resistance to haemonchosis: selection of female sheep resistant to *Haemonchus contortus*

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Abstract

Seventy female lambs (6–7 months old) which were exposed to natural infections of *Haemonchus contortus* were designated as responders or non-responders on the basis of 10 weekly cumulative faecal egg counts. Selected responder and non-responder lambs were treated with ivermectin, housed separately and 6 weeks post-housing, seven lambs from each group were given a trickle infection of *Haemonchus contortus* at 1000 L_3 daily for 5 days per week up to 2 weeks and examined weekly for 10 weeks after first infection. Analysis of data revealed significantly lower mean faecal egg counts and non-significantly less weight loss in responder than non-responder lambs. Mean values of haemoglobin, packed cell volume, total serum protein and peripheral eosinophil counts were significantly higher in responders than non-responders. In contrast, serum pepsinogen concentration was significantly less in responders than in non-responders. At 10 weeks post-infection, there were fewer pathological lesions and significantly lower worm burdens in responders than in non-responders. These results demonstrate a distinct resistance in responders to *Haemonchus contortus* infection.

Introduction

Gastrointestinal nematodosis, especially haemonchosis, is a major cause of a staggering economic loss to the sheep industry worldwide, particularly in tropical and sub-tropical regions. Immunoprophylaxis is one of the most effective means of preventing the occurrence of bacterial and viral diseases. However, the development of effective vaccines against parasitic diseases has not met with similar success and is further compounded by a variety of host–parasite related factors (Smith, 1992). Helminth diseases have been controlled to some extent by the use of anthelmintics but the development of resistance to drugs limits their use (Yadav *et al*., 1993). Therefore, breeding animals with a higher genetic resistance against important parasites appears to complement and prolong the usefulness of the existing drug families (Gray, 1991).

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Genetic variation in resistance to *Haemonchus contortus* between and within breeds and its exploitation for selective breeding for resistant sheep has been reported by Gray *et al*. (1995) and Yadav *et al*. (1997) and is widely used in breeding programmes in Australia (Albers *et al*., 1987; Woolaston *et al*., 1991) and New Zealand (Baker *et al*., 1991).

The aim of the present study is to identify female sheep resistant to *H. contortus* on a sheep farm for use in breeding programmes.

Materials and methods

Field study

A flock of 70 Sonali × Corriedale female cross-bred lambs (6–7 months old), born in January–February 1996, weaned at the age of 3 months and grazed with a flock of 500 sheep on 9–15 ha permanent pasture seeded with *Cyanodon dactylon* grass were maintained at the Central

Sheep Breeding Farm, Hisar. All the lambs had become naturally infected with *H. contortus*.

Selection of responders and non-responders

On 24 August 1996, the infected lambs were faecal sampled and treated with ivermectin (Ivomec, Dynamic Phamacols, Bombay) at 0.4 mg kg^{-1} body weight sc. After overnight housing, they were turned out to graze on the same pasture which was contaminated with *H. contortus*. Faecal samples were collected weekly for estimation of eggs per gram (EPG) by the modified McMaster technique (Anon., 1986) until early November, i.e. 10 weeks after turn out. At the end of the field study, responder and non-responder lambs were selected on the basis of their cumulative EPG obtained from 10 weekly samples. The lamb with lowest EPG was ranked 1 and that with the highest EPG was ranked 70. The group of eight lambs with the lowest cumulative EPG and the group of eight with the highest EPG were designated responders and non-responders, respectively.

Experimental infections

On November 7, selected responders and non-responders were treated with ivermectin at 0.4 mg kg^{-1} body weight and housed on a raised wooden floor to avoid the risk of any further parasitic infection. The lambs were fed berseem (*Trifolium alexandricum*), oats (*Avena sativa*) and provided water *ad libitum*. One of the three non-responders showing bottle jaw died 2 days post-housing as the result of a natural infection. To equalize the number of lambs in each group, one responder lamb with ranking 8 was withdrawn leaving seven responders and seven non-responders. Six weeks post-housing all lambs were experimentally infected with 1000 *H. contortus* L_3 daily for 5 days per week for 2 weeks.

Haematological and faecal examination

Venous blood in tubes with anticoagulant (ethylene diamine tetra-acetic acid, EDTA) for determination of haemoglobin (Hbg%), packed cell volume (PCV%), peripheral eosinophil counts (cmm) and without anti-coagulant for analysis of total serum protein (TSPg%) and pepsinogen levels were collected weekly for 10 weeks post-infection (PI). Haemoglobin and PCV were estimated by the cyanmethaemoglobin and Wintrobe tube methods (Coles, 1974), respectively. Blood eosinophils were counted by the direct method using as improved Newbauer counting chamber (Zarrow *et al.*, 1964). Total serum protein and pepsinogen were determined according to the methods of Lowry *et al.* (1951) and Mylrea & Hotson (1969), respectively. Faecal samples were taken weekly and egg counts determined by the modified McMaster technique (Anon, 1986).

Worm recovery and histopathology

Five lambs selected randomly from each group were separated, necropsied and abomasa were processed for the recovery, counting and identification of adult and larval stages on day 70 PI as per procedure of Anon. (1986).

The abomasa from responder and non-responder lambs were fixed in 10% neutral formalin, processed and stained with haematoxylin and eosin for histopathological alterations as previously described by Cullings (1963).

Statistical analysis

The data was analysed using the Student's t-test (Snedecor & Cochran, 1968).

Results

Mean faecal egg counts (fig. 1) resulting from pasture infection between days 42 and 70 post-grazing were significantly ($P < 0.05$) lower in responders (262.5 ± 67.2 to 1400 ± 65.5) than in non-responders (4775 ± 1035.6 to $10,900 \pm 918$). Three non-responders exhibited varying degrees of submandibular oedema between days 63 and 70 post-grazing. One of the three lambs died within 2 days of housing despite its treatment on day 70 post-grazing. The other two lambs recovered completely by day 7 post-housing.

Selected non-responders (9100 ± 1050.6 to $10,542.9 \pm 1651.8$) had significantly ($P < 0.05$) higher egg counts than the responders (5085.7 ± 1065.3 to 5414.3 ± 923.4) between days 56 and 70 (fig. 2).

Lambs from both groups lost weight over the 10 week experimental infection period and the weight loss in responders and non-responders was 2.0 and 3.93 kg, respectively. Despite these weight losses lambs did not exhibit any adverse clinical signs.

Mean values of Hb and PCV fell throughout the post-infection period in both groups of lambs (figs 3 and 4). Responders had significantly ($P < 0.05$) higher Hb and PCV from day 35 to 70 and from day 42 to 70, respectively in comparison with non-responders. The pattern of increase in mean eosinophil counts of both groups was similar (fig. 5) but it was highly significant ($P < 0.01$) from day 28 to 56 PI in responders compared with non-responders.

Mean values of TSP were significantly ($P < 0.05$) higher

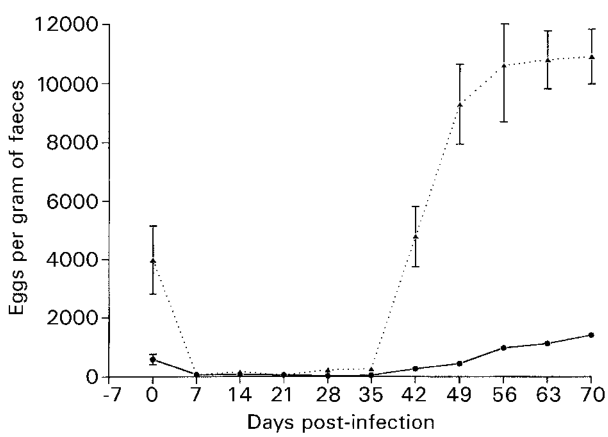

Fig. 1. Mean (± SE) faecal egg counts for responder (—●—) and non-responder (···▲···) lambs following natural pasture infection.

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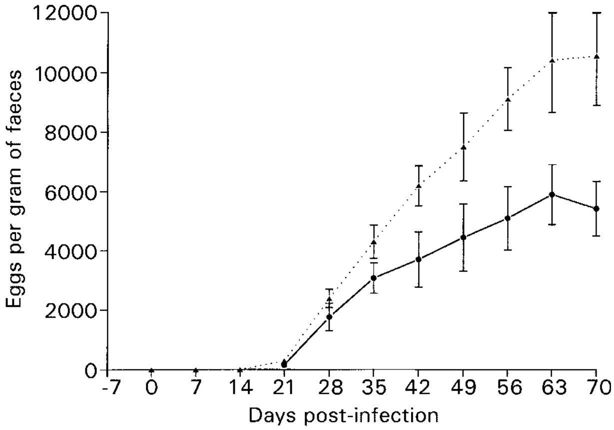

Fig. 2. Mean (± SE) faecal egg counts for responder (—●—) and non-responder (···▲···) lambs following experimental infection.

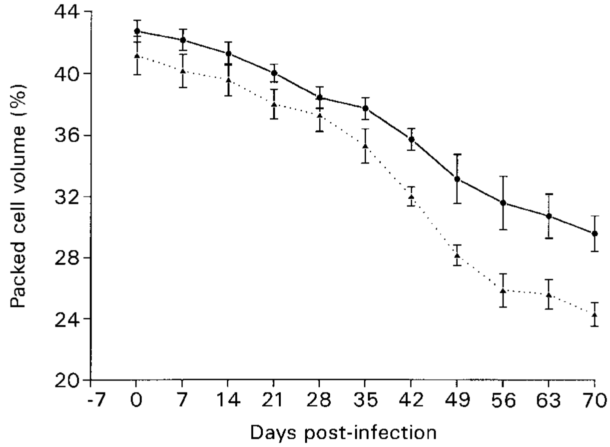

Fig. 4. Mean (± SE) packed cell volume for responder (—●—) and non-responder (···▲···) lambs following experimental infection.

in responders (6.95 ± 0.12 g% to 8.21 ± 0.11 g%) than in non-responders (5.70 ± 0.31 g% to 7.87 ± 0.15 g%) from day 7 to 70 PI.

The *H. contortus* infection had a significant effect on serum pepsinogen between groups as early as day 14 PI (fig. 6). The increased values of this enzyme were significantly higher in non-responders than in responders ($P < 0.01$). Between days 14 and 70 PI responders showed less than a 2-fold increase in comparison with a 3-fold increase in non-responders.

Mean worm counts were significantly ($P < 0.01$) lower in responders than in non-responder lambs (table 1). The abomasal mucosa of all non-responder lambs revealed congestion, oedema and a heavy infiltration of mononuclear cells with a large number of eosinophils in the lamina propria of plicae in comparison to a mild infiltration of mononuclear cells in the lamina propria of responders.

Discussion

Faecal egg counts have previously been used as a routine parasitological indicator for the selection of responsiveness in nematode infections of sheep (Gray, 1991; Sreter *et al.*, 1994) and goats (Patterson *et al.*, 1996a,b). Faecal egg counts correlate well with total trichostrongyle numbers (Bisset *et al.*, 1991) and in particular with *H. contortus* (Roberts & Swan, 1981). Therefore, there is justification for using faecal egg counts, which correlate well with current worm burdens as indicators of parasite resistance.

The results of this study provide direct evidence of differences in resistance to natural infections of *H. contortus* and the ability to tolerate the effects of parasitism between responders and non-responders. Further experimental infection of naturally infected and treated lambs also confirmed a similar degree of

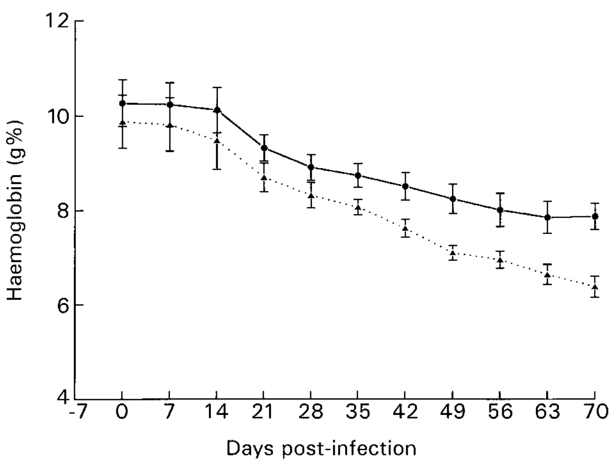

Fig. 3. Mean (± SE) haemoglobin for responder (—●—) and non-responder (···▲···) lambs following experimental infection.

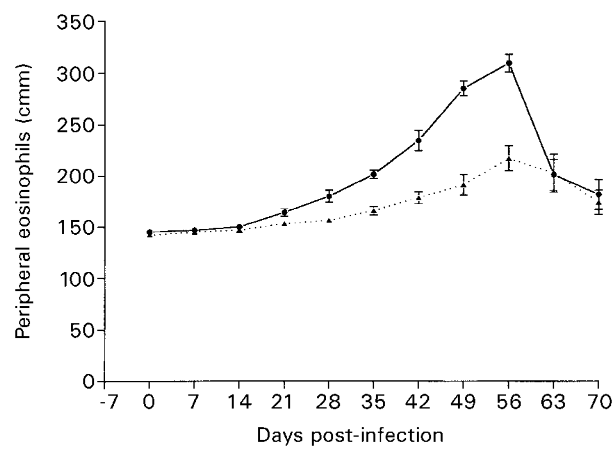

Fig. 5. Mean (± SE) peripheral eosinophils for responder (—●—) and non-responder (···▲···) lambs following experimental infection.

https://doi.org/10.1017/S0022149X00700320 Published online by Cambridge University Press

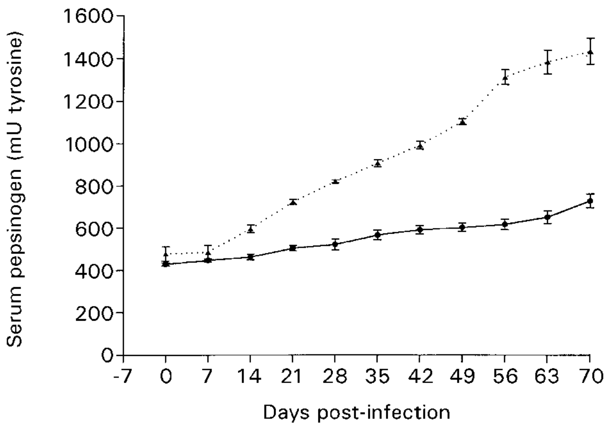

Fig. 6. Mean (± SE) serum pepsinogen for responder (—●—) and non-responder (···▲···) lambs following experimental infection.

difference in resistance between responders and non-responders. On pasture, the genetic variation in resistance to nematode infection between and within breed genotypes is well documented (Cabaret & Gruner, 1988; Gray *et al*., 1995; Yadav *et al*., 1997) and is influenced by genetic and environmental factors (Barger & Dash, 1987). Host age, sex, climate, nutrition, grazing management, physiological conditions and immunity affect the susceptibility of animals to nematode parasites (Gruner, 1991; Wakelin, 1992). All sheep used in this study were bred and maintained on the farm under similar conditions of management practices and were of similar age and sex and therefore, these factors are unlikely to play a significant role in causing differences in resistance between selected responders and non-responders. Woolaston *et al*. (1991) showed that faecal egg count is under genetic control and in the present study, individual hosts were identified as highly resistant or susceptible based on high and low egg counts, respectively. Genetic variation in resistance to *H. contortus* within the sheep flocks has been demonstrated and used in breeding programmes in Australia (Albers *et al*., 1987; Woolaston *et al*., 1991) and New Zealand (Baker *et al*., 1991).

The benefit of selecting responders for use in breeding programmes may be exploited for raising the overall level of flock resistance, resulting in fewer anthelmintic treatments being required. It would thus reduce the risk of development of anthelmintic resistance, a serious problem observed on some sheep breeding farms in Western Haryana, India (Yadav, 1990; Yadav *et al*., 1993, 1995; Singh & Yadav, 1997).

Elevation in serum/plasma pepsinogen is a common response of ruminants to gastrointestinal nematodes (Thomas & Walter, 1975). Increase in pepsinogen is indicative of abomasal damage which is associated with the emergence of developing larval stages from gastric glands (Holmes & Mclean, 1971). In the present study, lower levels of pepsinogen in responders than in non-responders suggests less abomasal damage by the nematode and a higher ability of the former to exploit a more protective role to nematode infection than the latter.

The association of peripheral eosinophil response with parasite infection, though complex, is well documented (Jones, 1993). Eosinophil counts are considered to be dependent on host age and immunological responses and the degree of response appears to be genetic in origin (Vadas, 1982; Sewell & Vadas, 1983). The higher peripheral eosinophilia in responders compared with non-responders in the present study suggests a high degree of host resistance in the responders to *H. contortus* (Dawkins *et al*., 1989; Buddle *et al*., 1992).

Changes in the blood parameters during the course of infection show highly significant differences between the selected responders and non-responders. The period of significant change in blood constituents reflects the stage of larval development and at the same time anaemia and hypo-proteinaemia are considered to be common features of *H. contortus* infections (Rahman & Collins, 1990). In view of the high genetic correlation between EPG and PCV (Albers *et al*., 1987; Romjali *et al*., 1996) and between EPG and the degree of anaemia (Luffau *et al*., 1990) seen in infected sheep, the degree of anaemia has been suggested as an alternative for supporting selection criteria along with EPG. In this study, the results show that decreases in blood parameters are related to an increase in EPG suggeting that these criteria can be used as pathological indicators to determine resistance or susceptibility.

The effects of nematode infection and genotype on host physiology and pathology have previously been reported by Yadav *et al*. (1993) and Romjali *et al*. (1996). The body weight of responders and non-responders decreased during the course of the experimental period indicating that *H. contortus* infections were sufficient to interfere with the body weight gains, although the average decrease in body weight was lower in responder than non-responder lambs. Necropsy findings also revealed four times higher worm burdens and more pathological changes in non-responders compared with responders. Albers *et al*. (1987) found that low weight gains in lambs infected with *H. contortus* were negatively

Table 1. Individual and mean (± SE) worm counts for each of 5 responder and non-responder lambs killed on day 70 post-infection with *Haemonchus contortus*.

Lambs	Worm counts	
Status	Individual	Mean (± SE)
Responders	221, 569, 590, 19, 51	290.00 ± 21.11
Non-responders	1278, 614, 1243, 1254, 1322	1142.00 ± 132.54

https://doi.org/10.1017/S0022149X00700320 Published online by Cambridge University Press

correlated with EPG. Therefore, it can be deduced from the present observations that higher worm burdens causing greater pathology might be responsible for more reduction in the body weight of non-responders.

The present results demonstrate the use of routine parasitological techniques and blood parameters for the identification of female lambs resistant to *H. contortus*. Such resistant individuals can be selectively used in breeding programmes for increased resistance to *H. contortus*, which is regarded as one of the most pathogenic gastrointestinal parasites of small ruminants.

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(Accepted 12 November 1998)