Veterinary Parasitology 171 (2010) 263–272

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Relationships between faecal dry matter, worm burdens and inflammatory mediators and cells in parasite-resistant Merino rams

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ARTICLE INFO

Article history:
Received 6 October 2009
Received in revised form 16 March 2010
Accepted 24 March 2010

Keywords:
Sheep
Nematodes
Diarrhoea
Trichostrongylus colubriformis
Teladorsagia circumcincta

ABSTRACT

Immune-mediated scouring due to ingested parasite larvae is a major concern for sheep producers in Mediterranean climates. We investigated immune-mediated scouring in parasite-resistant Merino sheep in Australia. Forty-adult, parasite-resistant Merino rams were judged to be either susceptible or non-susceptible to immune-mediated scouring on the basis of dag scores taken under field conditions. We hypothesised that the susceptible rams would have lower faecal dry matter during larval challenge than non-susceptible rams and that, at post-mortem examination, inflammatory mediators and granulocytes would be negatively correlated with both faecal dry matter and worm numbers. In pens, the rams received a dose of 500 *Teladorsagia circumcincta* L_3 and 500 *Trichostrongylus colubriformis* L_3 each day for 6 weeks before euthanasia. Ten rams acted as unchallenged controls. Challenging sheep with larvae reduced faecal dry matter at 2, 3 and 4 weeks after challenge began and the greatest reductions were with the sheep susceptible to scouring. The sheep showed good resistance to the parasite challenge as evidenced by low faecal worm egg counts and low total worm counts at post-mortem, with the numbers of *T. colubriformis* particularly low. Sheep with low faecal dry matter had significantly higher numbers of eosinophils in small intestine tissue. Sheep with low total worm counts had significantly higher levels of bradykinin in abomasum mucus. Sheep with more granulocytes in tissue and inflammatory mediators in mucus tended to have fewer numbers of *T. circumcincta* but there was little relationship with numbers of *T. colubriformis*. Our results show that dag scores are correlated to a reduction in faecal dry matter, which can be attributed to the challenge with infective parasite larvae. Inflammation during worm infection is associated with rejection of the worm challenge and may result in more fluid faeces and consequently diarrhoea. Therefore, sheep breeders should focus on breeding for both low worm egg counts and also low dag scores.

1. Introduction

Breeding sheep to be naturally resistant to nematode parasites is a potential solution to rising levels of anthelmintic resistance and consumer demand for organic animal products (Karlsson and Greeff, 2006). Researchers in Western Australia, which is characterised by a Mediterranean, winter-rainfall climate, have demonstrated the

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doi:10.1016/j.vetpar.2010.03.031

feasibility of breeding sheep for low faecal egg counts (FEC) without unfavourable correlated responses in production traits such as bodyweight (Pollott et al., 2004). The major parasitic nematodes of sheep in this climatic zone are the abomasal nematode *Teladorsagia circumcincta* and the intestinal nematode *Trichostrongylus colubriformis*. Infection with these parasites leads to scouring (diarrhoea), which is a major management and animal welfare problem due to the build up of faecal material on wool which leads to flystrike (Morley et al., 1976). It has long been considered that diarrhoea was a symptom of a heavy adult worm burden. However, it is now apparent that most parasite-related diarrhoea in sheep of post-weaning age may be due to a hypersensitive immune response to ingested larvae-'immune-mediated scouring' (Larsen et al., 1999). As a result, breeding sheep on the basis of low FEC may bring about a moderate increase in scouring (Bisset et al., 1997; Karlsson et al., 2004).

The immune response of sheep to nematode parasites depends on Th2 cytokines such as IL-4, IL-5 and IL-13 which recruit mast cells and eosinophils into the abomasal and intestinal mucosa (Miller and Horohov, 2006). These recruited cells release potent inflammatory mediators, such as histamine, and also arachidonic acid metabolites such as leukotrienes and prostaglandins (Prussin and Metcalfe, 2006). These mediators, as well as potent vasodilators such as bradykinin, probably act to remove worm larvae by causing leakage of plasma protein into the abomasum and intestinal lumen, and contracting non-vascular smooth muscle. A side-effect of this immune response may be diarrhoea.

In this experiment we investigated low worm egg count or 'immune-mediated' scouring in a flock of parasite-resistant sheep. In our previous work we were able to reproduce faecal softening in sheep housed indoors and given a trickle infection of nematode larvae. We found that concentrations of leukotrienes and prostaglandin E_2 (PGE_2) were higher in parasite-challenged Merino rams than unchallenged controls, and was accompanied by a concurrent decrease in faecal dry matter and egg counts (Williams et al., 2008). In the current paper, we investigated the pattern of faecal dry matter in penned, parasite-resistant Merino rams judged as being susceptible or otherwise to immune-mediated scouring, when challenged with both *T. circumcincta* and *T. colubriformis* L_3. We also quantified numbers of granulocytes and the production of inflammatory mediators (cysteinyl leukotrienes, prostaglandin E_2 and bradykinin) in abomasal and intestinal mucus. It was postulated that rams susceptible to immune-mediated scouring would have reduced faecal dry matter compared to rams that are not susceptible. In addition, we expected that in all challenged sheep there would be negative correlations between granulocytes, inflammatory mediators and both faecal dry matter and worm burdens at post-mortem examination.

2. Materials and methods

2.1. Experimental design

Fifty Merino rams, aged 20 months and from a flock genetically selected for low FEC, were selected on the basis of Estimated Breeding Values (EBVs) for FEC and phenotypic measurements of scouring (dag score at hogget age). Dag score refers to a subjective measurement of the severity of faecal accumulation on the wool around the breech ('dags') and is scored on a scale where 0 or 1 refers to no faecal material and 5 refers to a heavily soiled breech (Larsen et al., 1994). While this Merino flock is highly resistant to parasites, there is still considerable variation in EBV for FEC, i.e. some sheep are more resistant than others. Likewise, there is also variation in dag scores. Therefore, for this experiment we used FEC EBV and dag scores to select forty sheep such that there were ten sheep in each of the following four categories:

(1) high FEC, high dag score
(2) high FEC, low dag score
(3) low FEC, low dag score
(4) low FEC, high dag score

The EBV and dag scores are shown in Table 1. A further group of 10 sheep was selected to act as a control group—its EBV values and dag scores were average for the flock. A negative EBV for FEC is desirable, i.e. indicates that the animal has genetic superiority for that trait.

Rams were treated with an effective anthelmintic. Then, after a 2-week break, rams in groups 1–4 were challenged daily with L_3. After 6 weeks of challenge, all rams were necropsied for total worm counts. Tissue and mucus samples were taken from the abomasum and duodenum and analysed for numbers of inflammatory cells and concentrations of inflammatory mediators.

2.2. Location and animals

Rams were sourced from the Rylington Merino line selected by the Western Australian Department of Agriculture and Food. Sheep from this line have been bred for worm resistance since 1987. Worm resistance was deter-

Table 1
Mean EBV for faecal egg count (FEC) and mean dag scores in winter and spring 2006 for rams used in this experiment and flock averages.

	High FEC EBV		Low FEC EBV		Control ($n=10$)	Flock average ($n=233$)
	High dag ($n=10$)	Low dag ($n=10$)	High dag ($n=10$)	Low dag ($n=10$)		
FEC EBV	−78.3	−80	−96.5	−98.5	−91.7	−89.4
Winter dag	2.7	1.5	2.3	1.2	1.7	2.1
Spring dag	3.3	1.2	3.6	1.3	2.1	2.4

Dag scores are on a subjective 1–5 scale where 1 is no dag and 5 is severe dag (Larsen et al., 1994).