



Faecal dry matter, inflammatory cells and antibodies in parasite-resistant sheep challenged with either *Trichostrongylus colubriformis* or *Teladorsagia circumcincta*

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ABSTRACT

Sheep that are highly resistant to parasitic nematodes can suffer bad diarrhoea due to the inflammation associated with rejection of ingested larvae from pasture. We hypothesised that challenging parasite-resistant sheep indoors with nematode larvae would result in reduced faecal dry matter, and that faecal dry matter would be lowest in sheep challenged with a mixture of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* compared to those challenged with either species alone. We also hypothesised that inflammatory cells and serum antibodies and interleukin-5 would be highest in those sheep that received the mixed larval challenge. We found that faecal dry matter was reduced ($P < 0.05$) in challenged sheep compared to unchallenged sheep, with the fastest reduction being in those sheep challenged with only *Tric. colubriformis*. At 14 and 23 days after challenge began, there were no differences in faecal dry matter between the three challenged groups. Within the abomasum, there were no differences in inflammatory cell numbers between unchallenged sheep and those challenged only with *Tric. colubriformis*. Cell numbers in sheep challenged with *Tela. circumcincta* were higher ($P < 0.05$) than those in unchallenged sheep, but there were no differences between sheep challenged only with *Tela. circumcincta* or as a mixed challenge. In the small intestine, inflammatory cell numbers were higher ($P < 0.05$) in sheep that received the mixed challenge compared to controls. Cell numbers in sheep challenged with either *Tela. circumcincta* or *Tric. colubriformis* were also slightly higher than those in controls. Larval challenge increased ($P < 0.05$) levels of IgA and IgE in serum, but there were no differences between the three challenged groups. Larval challenge also increased ($P < 0.05$) levels of IL-5, with the greatest increase being in those sheep challenged with both species. We concluded that both *Tela. circumcincta* and *Tric. colubriformis* can cause immune-mediated diarrhoea in sheep, and that a mixed challenge will not necessarily lead to worse diarrhoea or higher concentrations of antibodies in serum. We also concluded that challenge with *Tric. colubriformis* leads to no inflammation in the abomasum, but challenge with *Tela. circumcincta* may lead to some inflammation in the small intestine.

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1. Introduction

Breeding sheep to be resistant to nematode parasites is a long-term and sustainable parasite-control method due

to minimal reliance on anthelmintic treatment (Karlsson and Greeff, 2006). However, in Western Australia and in New Zealand it has been noted that there is an increased propensity for parasite-resistant sheep to suffer diarrhoea (scouring) (Bisset et al., 1997; Karlsson et al., 2004). Scouring is a major problem in sheep production due to the build-up of faecal material on the wool around the breech, predisposing sheep to flystrike and contaminating meat

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carcasses (Morley et al., 1976; Jacobson et al., 2009). We have established in our previous work that scouring observed in immune, parasite-resistant Merino sheep is due to a reduction in faecal dry matter caused by challenge with nematode larvae (Williams et al., 2008). Faecal dry matter was reduced despite very few worms being present at post-mortem examination. Numbers of eosinophils in intestinal tissue, a hallmark of inflammation associated with Th2 immune responses were negatively correlated with faecal dry matter (Williams et al., submitted for publication-a). Larsen et al. (1994) also demonstrated that eosinophil infiltration in the gut of outbred Merino sheep was associated with increased scouring. This suggests that in resistant sheep it is primarily the immune response that results in scouring.

In our previous work sheep were challenged with a mixture of nematode species, namely *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*. While this is representative of the larval challenge that sheep face while grazing pasture in the southern half of Australia, it means that the effects each species has on the pathology of immune-mediated scouring are confounded. While both *Tric. colubriformis* and *Tela. circumcincta* have similar life-cycles and effects on the host, the magnitude of the immune response towards each species in a mixed infection can vary markedly (Gruner et al., 2004). Therefore, the relative contribution of each species to immune-mediated scouring needs to be quantified.

Challenge with *Tela. circumcincta* leads to increased fluid secretion and increased flow rate of digesta at the abomasum. However, most of this can be re-absorbed in the distal parts of the small intestine (Wilson and Field, 1983). Challenge with *Tric. colubriformis* increases the flow of protein and minerals past the terminal ileum (Bown et al., 1991), thereby reducing the osmotic potential and capacity of the colon to absorb water. This suggests that *Tric. colubriformis* may be more important than *Tela. circumcincta* in the pathology of immune-mediated scouring. In addition, studies that have focused on the interactions between *Teladorsagia* and *Trichostrongylus* on sheep growth rate and/or worm establishment have been conducted with young lambs that have not yet acquired immunity (Sykes et al., 1988; Jackson et al., 1992). Therefore, there is little information on whether clinical signs such as immune-mediated scouring are exacerbated during a mixed challenge. There is evidence of some cross-reactivity in immunity towards different nematode species (Stewart, 1955) suggesting that immunopathology may be greater during mixed infections. This is because part of the immune response towards the other species also acts non-specifically towards the first species (Emery et al., 1993). Therefore, it is feasible that immune-mediated scouring will be more severe during a mixed species challenge, due to a heightened immune response leading to greater immunopathology.

The aim of this experiment was to further investigate immune-mediated scouring in parasite-resistant Merino sheep, particularly the relative contribution that *Teladorsagia* and *Trichostrongylus* species have in the pathogenesis of this condition. We investigated the effects of challenging parasite-resistant sheep with either *Tric. colubriformis* or

Tela. circumcincta, or both species, on faecal dry matter, levels of parasite-specific antibodies and interleukin-5 (IL-5) in serum and numbers of inflammatory cells in the abomasum and small intestine. Our hypothesis is that faecal dry matter will be lowest in those sheep that receive the mixed challenge, followed by those that receive only *Tric. colubriformis*, than by those that receive only *Tela. circumcincta*. In addition, we hypothesise that numbers of inflammatory cells and levels of parasite-specific antibodies and IL-5 in serum will be higher in sheep that receive the mixed challenge, compared to those that are challenged with only one species.

2. Materials and methods

2.1. Experimental design

Twenty Merino rams aged 2 years were selected on the basis of estimated breeding values (EBVs) for faecal worm egg count (FEC) and phenotypic measurements of scouring (dag score at hogget age). Two-year-old rams used as male animals from this flock are culled at this age unless used for breeding. In addition, in Western Australia scouring is normally most severe in animals aged 1–2 years (Jacobson, 2006). EBVs are a measure of the genetic merit of an animal for a particular trait (Nicholas, 1987). A negative EBV for FEC indicates a superior animal, i.e. it has a lower FEC than the within-breed average. Dag scores refer to a subjective assessment to the severity of scouring and consequently faecal build-up on the wool around the breech (Larsen et al., 1994). The rams were from a flock of sheep bred for low FEC. Therefore, all sheep in this flock have low EBV for FEC but the animals used in this experiment were selected as being extremely resistant, i.e. their FEC EBV was among the lowest in the flock. However, all the sheep also had above-average dag scores for this flock so they were assumed to be prone to immune-mediated scouring (Table 1). Five rams were then randomly allocated to one of four treatment groups:

- (1) Challenged with *Tric. colubriformis* only.
- (2) Challenged with *Tela. circumcincta* only.
- (3) Challenged with both *Tric. colubriformis* and *Tela. circumcincta*.
- (4) No challenge (control).

2.2. Location and animals

Rams were selected from the Rylington Merino line owned by the Western Australian Department of Agricul-

Table 1
FEC EBV and dag scores in October (hogget age) for Merino rams used in this experiment. A negative FEC EBV indicates genetic superiority for this trait (i.e. parasite-resistance).

	FEC EBV	October dag score ^a
Experimental rams (n=20)	–96	4.3
Flock average (n=192)	–91.5	2.3

^aDag scores are on a subjective 1–5 scale where 1 is no dag and 5 is severe dag.

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