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Differences between female and castrated male lambs in susceptibility to natural, predominantly *Teladorsagia circumcincta* infection

O. Abuargob[a], M.J. Stear[b,*]

[a] *Department of Animal Medicine, Faculty of Veterinary Medicine, Tripoli University, PO Box 84575, Tripoli, Libya*
[b] *Institute of Biodiversity, Animal Health and Comparative Medicine, Garscube Campus, University of Glasgow, Bearsden Road, Glasgow G61 1QH, United Kingdom*

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ABSTRACT

The difference between castrated male lambs and entire female lambs in susceptibility to gastrointestinal nematode infection was investigated in a flock of Scottish Blackface sheep. Castrated male lambs had higher faecal egg counts, higher body weights, lower parasite-specific plasma IgA activity and more adult nematodes. There were no detectable differences in the length of adult *Teladorsagia circumcincta*. Heavier egg shedding by males was associated with greater worm burdens. The difference in plasma IgA activity may have been a consequence of larger numbers of adult nematodes inhibiting the transfer of mucosal IgA to the plasma. At least part of the sex difference in egg production could be a consequence of males ingesting larger amounts of grass and hence numbers of infective larvae because of their larger size. The differences between castrated males and females contribute to the observed variation in nematode egg production in naturally infected lambs.

1. Introduction

One of the most intriguing features of parasitic infection is the wide variation among individuals in susceptibility to infection. One of the best understood host–parasite relationships is infection of lambs with the gastrointestinal nematode *Teladorsagia circumcincta* (Lee et al., 2011; Stear et al., 2009). Following natural infection, faecal egg counts and the number of both adult worms and fourth-stage larvae are widely dispersed among lambs (Stear et al., 1998, 2006; Strain et al., 2002). Understanding the sources of this variation is important to identify susceptible animals at risk of developing disease as well as resistant animals for selective breeding.

Clearly, variation is driven either by variation in exposure or by variation in the ability of the potential host to resist infection. In cool temperate areas such as central Scotland essentially all animals are exposed to infection but the intensity of infection depends in part on temperature and moisture which are believed to be the main drivers of the development of nematodes on pasture (Stromberg, 1997). Quantitative genetic analysis has shown that part of the variation in the intensity of nematode infection is due to genetic variation among hosts (Stear et al., 1997a,b) and genetic variation increases as lambs age and the immune response develops (Bishop et al., 1996). In addition, two genetic regions have been repeatedly associated with resistance to infection; the MHC class II region on chromosome 20 (Sayers et al., 2005a; Schwaiger et al., 1995;

* Corresponding author. Tel.: +44 0141 330 5762; fax: +44 1419427215.
E-mail addresses: michael.stear@glasgow.ac.uk, m.j.stear@vet.gla.ac.uk (M.J. Stear).

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Stear et al., 2005) and the interferon gamma locus on chromosome 3 (Coltman et al., 2001; Sayers et al., 2005b).

The gender of the host also has an important effect on susceptibility to nematode infections (Barger, 1993; Graham et al., 2010; Waddell et al., 1971). Males are usually more susceptible than females and this may be due to the immunosuppressive effects of testosterone. However, most lambs on commercial farms are castrated at an early age and the differences between castrated males and female lambs for *T. circumcincta* are unclear. The aim of this study was to explore differences between the sexes in susceptibility of grazing lambs to nematode infection and to examine possible explanations for any differences that do exist.

2. Materials and methods

2.1. Animals

Data were collected each year for five years (1992–1996) from consecutive cohorts of 200 lambs. All lambs were Scottish Blackface sheep from a commercial upland farm in southwest Strathclyde. The dominant nematode on this farm is *T. circumcincta* (Stear et al., 1998). Husbandry procedures followed normal, commercial practice and have been described previously (Stear et al., 1998). Briefly, all lambs were born outside and were continuously exposed to mixed nematode infections by grazing. Every 28 days from 4 to 20 weeks of age, all lambs were treated with a broad-spectrum anthelmintic (albendazole sulphoxide), given according to the manufacturer's recommendations. This anthelmintic is effective against the nematodes that were present (McKellar and Scott, 1990). The efficacy of the drug on this farm was assessed by faecal egg count reduction tests and was consistently greater than 95% in each of the five years of this study. Blood samples were collected in August, September and October every year with the exception of October 1992, October 1993 and August 1995.

Six or seven weeks after the final anthelmintic treatment, about one-half of the lambs in 1992–1995 were slaughtered at the local abattoir. Only surplus males were slaughtered in 1992 and 1993 but both male and female lambs were examined in 1994 and 1995.

2.2. Parasitology

Faecal egg counts were estimated by the McMaster technique (Gordon and Whitlock, 1939). Each egg counted represented 50 eggs per gram of faeces. At slaughter the lambs were 6 to 7 months of age. The abomasum and small intestine were removed, opened along the greater curvature and washed with tap water under moderate pressure. The contents and washings were made up to 2 l, from which ten 4 ml aliquots were examined to estimate the size of the adult nematode population. The mucosa from one half of the washed abomasum was digested with pepsin–HCL for 6 h at 42 °C; the digest was then made up to 2 l and 4 ml aliquots taken to estimate the number of larvae (Armour et al., 1966).

Standard procedures were used to estimate the mean length of adult female nematodes (Stear et al., 1997b). The mean length (mm) was estimated by image analysis of at least 25 female adult worms from each sheep. The procedures and the worm species present in this study have been described elsewhere in more detail (Stear et al., 1998). In 1992, the proportions of adult nematodes recovered at necropsy were 95.8% *T. circumcincta*; 0.6% *Cooperia* species, 2% *Trichostrongylus vitrinus*, 1.7% *Nematodirus* species and 0.01% *Bunostomum trigonocephalum*. In 1993 the proportions were 76.8% *T. circumcincta*; 1.5% *Trichostrongylus axei*, 8.8% *Cooperia* species, 2.6% *T. vitrinus*, and 10.3% *Nematodirus* species. In 1994 the proportions were 71.0% *T. circumcincta*; 0.04% *T. axei*, 17.3% *Cooperia* species, 8.0% *T. vitrinus*, and 3.7% *Nematodirus* species. In 1995 the proportions were 50.8% *T. circumcincta*; 2.9% *T. axei*, 0.14% *Haemonchus contortus*, 35.4% *Cooperia* species, 8.3% *T. vitrinus*, and 2.4% *Nematodirus* species. Low numbers of *Nematodirus battus* eggs were also present earlier in the grazing season (Denwood et al., 2008).

2.3. IgA activity

The activity of plasma IgA against a somatic extract of fourth-stage larvae from *T. circumcincta* was measured by indirect ELISA. Fourth-stage larvae were harvested 4 days after infecting helminth-naïve lambs with 150,000 infective larvae. The abomasum was washed with tap water and cut into strips. These strips were suspended in Baermann funnels containing PBS (pH 7.4) at 37 °C. The larvae were then placed onto surgical swabs and the migrating larvae were recovered in PBS. These larvae were washed 5× in PBS, once in PBS containing 100 iu penicillin ml^{-1}, 0.1 mg streptomycin ml^{-1}, 2.5 μg amphotericin B ml^{-1}, and 0.05 mg gentamicin ml^{-1} and once in Tris-inhibitor solution (pH 8.3; 10 mM Tris containing 1 mM EDTA (disodium ethylene diamine tetraacetic acid), 1 mM EGTA (ethylene glycol bis (2-amino ethyl ether)–*N*,*N*,*N*′*N*′-tetraacetic acid), 1 mM NEM (*N*-ethylmaleimide), 0.1 μM pepstatin, 1 mM PMSF (phenyl methyl sulphonyl fluoride) and 0.1 mM TPCK (*N*-tosylamide-L-phenylalanine chloromethyl ketone). After centrifugation, the pellet was resuspended in 1% sodium deoxycholate v/v in Tris-inhibitor solution and stored at −20 °C. After thawing, the sample was homogenised on ice with a hand-held electric homogeniser (Janke & Kunkel IKA Labortechnik). The supernatant was filtered through a 0.2 μm filter and aliquots stored at −80 °C. The protein concentration was estimated with Bicinchoninic acid (Pierce) and adjusted to 5 μg ml^{-1} in 0.06 M bicarbonate buffer (pH 9.6) before use.

The wells on a flat-bottomed microtitre plates (Nunc) were coated with 100 μl parasite solution and left overnight at 4 °C. The plate was washed 5 times in PBS-Tween (0.1% v/v Tween 20 in PBS), incubated for 2 h with 200 μl blocking buffer (4% skimmed milk powder in PBS-Tween), then again washed 5 times in PBS-Tween. 100 μl of plasma sample diluted 1:10 in blocking buffer was added to each of three wells and incubated at 37 °C for 30 min. After another five washes in PBS-Tween, 100 μl of a rat monoclonal anti-sheep IgA at a dilution of 1:50 in blocking buffer was added and incubated for 30 min at 37 °C.