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Salivary IgA: A suitable measure of immunity to gastrointestinal nematodes in sheep

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ABSTRACT

Infection with gastrointestinal nematodes (GIN) is a major constraint on the productivity of grazing livestock. The development of selection methods to quickly and accurately identify animals capable of developing an effective natural immunity to infection would contribute to the development of sustainable worm control programs. A carbohydrate larval surface antigen (CarLA), present on the infective-stage larvae (L3) of all trichostrongylid nematodes, is a target antigen for host antibody (Ab). The levels of various Ab isotypes in serum and/or saliva of field-grazed lambs were assessed by ELISA, and Ab titres compared with parasite faecal egg counts (FECs) and a range of animal productivity parameters. Levels of anti-CarLA IgA in saliva proved to be the most heritable ($h^2 = 0.3$), and had the closest genetic correlation with FEC ($r = -0.5$). Those animals identified as having 'high levels' of anti-CarLA IgA typically have 20–30% lower FEC than animals with low or undetectable titres. Furthermore, animals with 'high levels' of anti-CarLA IgA tend to have improved growth rates post-weaning, and have no tendency for increased breech-soiling. The assay performed well regardless of parasite genera present on pasture. The saliva assay has a number of key practical advantages over the use of FEC for selection purposes: animals can be identified without a requirement to withhold anthelmintic treatment; sampling is rapid and easy and there is a significantly reduced barrier to adoption within the farming community. Measurement of anti-CarLA IgA in saliva by ELISA offers a practical, rapid and easy method of selecting for natural immunity to GIN in sheep.

1. Introduction

Parasitism with gastro-intestinal nematodes (GIN) is a significant limitation on animal productivity in all major sheep-producing regions (McLeod, 1995; Perry et al., 2002), and can result in reduced feed intake and feed utilization, leading to reductions in live-weight gains of up to 60–100% (Abbott et al., 1988; Coop et al., 1977; Sykes et al., 1997). While infection with GIN may result in severe clinical disease, particularly in warmer regions where the haematophagous *Haemonchus contortus* can be prevalent, subclinical effects from a range of mucosal browsing species is more common in temperate climates (Stear et al., 2003). Effective control of GIN parasitism in sheep is heavily reliant on the administration of effective broad-spectrum anthelmintic drugs (Vlassoff et al., 2001). The introduction of these compounds has enabled the intensification of sheep farming in many regions. Not only have farmers been able to increase stocking density, but productivity of the individual animals appears to have increased markedly.

Unfortunately, the regular and sustained widespread use of a limited number of drench families has led to the development of anthelmintic resistance. While resistance has not yet been detected to recently introduced anthelmintics, history suggests that it will, and that careful use will be required to minimise the selective process.

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Of further concern is the impact that production-focused selection, made possible by the use of anthelmintics, may have had on the development of host immunity. A recent New Zealand study compared a range of selection parameters in modern day dual-purpose sires, with those measured in animals generated using 30-year old semen. The results suggested that while breeding values for a range of productivity parameters, such as live-weight, have improved markedly, those for parasite resistance have declined significantly (Meat and Wool New Zealand Central Progeny Test Results 2008–2009). It is proposed, therefore, that breeding for more productive animals with limited or no selection pressure for 'parasite resistance' has resulted in sheep becoming ever more dependent on regular anthelmintic use to maintain acceptable levels of productivity (McEwan et al., 1992; Williamson et al., 1995; Morris et al., 1996).

Attempts to develop sustainable control strategies less reliant on anthelmintics have largely concentrated on either (a) breeding for natural resistance or (b) manipulating anti-parasite immunity through the development of vaccines. Of these two strategies, most progress to date has been made using the former approach.

The most commonly used measure of the ability of an animal to withstand or remove GIN has been to enumerate the numbers of parasite eggs in faeces (faecal egg count, FEC). A number of studies have demonstrated that FEC is influenced by host genetics in grazing livestock (Gasbarre et al., 1990; Bisset et al., 2001; Vagenas et al., 2002; Bishop and Morris, 2007). In some countries this had led to the development of large-scale recording programs in ram-breeding flocks for example, WormFEC in New Zealand (Bisset et al., 2001) and Nemesis (now through Sheep Genetics) in Australia (Eady et al., 1998). However, the use of FEC as a marker in sheep has drawbacks. For example, FEC are subject to variability due to factors such as the level and composition of feed intake, animal parameters such as stress levels and variation in the fecundity of individual GIN species (Stear et al., 1999). Across a flock, it is normal for there to be a heterogeneous distribution of eggs in faeces and a skewed distribution amongst animals, including a proportion of zero counts (Stear et al., 2006). There are also negative connotations amongst farmers regarding the sampling procedure; the process is not amenable to high volume automated processing and generally lacks good quality control procedures between laboratories. Furthermore, FEC requires a significant level of infection for differences in 'resistance' to be expressed. This may result in a necessity for animals to be left untreated for significant periods, which can impact on productivity. Nevertheless, FEC in lambs post-weaning has a moderate heritability, and an acceptable repeatability for ranking like-treated animals (Morris et al., 2000).

Identifying components of the host response to GIN which confers 'resistance' to GIN, and which are measurable, reliable and heritable, would potentially provide an alternative to FEC testing. Various attempts to do this have concentrated on comparing components of the immune response of animals to infection. Natural protection against GIN in sheep develops via an acquired immune response (Stear et al., 1999), although this requires a lengthy acquisition phase before protective immunity is expressed. Furthermore the outcomes of protective immunity are different for various sheep parasite species (Smith et al., 1985; Stear et al., 1995, 1999; Kemper et al., 2010). It is proposed, therefore, that studying the immune response to parasites may identify immune markers that could supplement or replace FEC as a measure of host 'resistance'. It is further proposed that the term 'resistance' is a relative term, and should be replaced by the more accurate 'protective immunity'.

Of all the immune markers studied in response to GIN infection, measuring anti-parasite antibody appears to offer the most potential, due to the ease of collection of relevant bodily fluids and the availability of assay techniques that allow high sample throughput.

Several studies have demonstrated a relationship between anti-parasite antibody responses and protection against GIN (for reviews see Stear et al., 2009; Shaw et al., 2009). Serum IgG1 antibody responses to *Trichostrongylus colubriformis* and *H. contortus* L3 antigens in sheep genetically selected for low FEC were significantly elevated compared with those from unselected control animals (Gill et al., 1993) or from high FEC selection-line sheep (Douch et al., 1995). Furthermore, higher levels of anti-parasite IgG1 was associated with reduced FEC and was demonstrated to be a heritable trait (Douch et al., 1995, 1996). Based on these results a test for host resistance was marketed by a New Zealand vaccine supply company for several years.

Elevated serum antigen-specific IgE responses to *T. colubriformis* L3 antigens (Shaw et al., 1999) and defined nematode allergens (Shaw et al., 2003; Murphy et al., 2010) have also been associated with reduced FEC, with a heritability of 0.36 in field grazed sheep (Shaw et al., 1999). However, the IgE response was unfavourably associated with live-weight gain and was therefore not considered to be a suitable measure of immunity to parasites.

Numerous studies have investigated the IgA response to *Teladorsagia circumcincta* infections in sheep, and have determined that the major genetic influence on *T. circumcincta* nematode egg counts is mediated via the retardation of worm growth and not in a reduction in worm numbers in lambs (Stear et al., 1999). Increases in abomasal IgA also appears to be a major mechanism in controlling worm length and fecundity of adult female *T. circumcincta* (Smith et al., 1985; Stear et al., 1995). Furthermore, sheep with increased mucosal and plasma IgA activity against fourth-stage larvae (L4) tend to have more inhibited L4 (Stear et al., 1995, 2004, 2009).

In naturally infected sheep, plasma IgA titre to L4 antigens was repeatable and highly heritable (0.56) and was significantly associated with lower FEC and shorter adult females (Strain et al., 2002; Davies et al., 2005). The strong association between the IgA response and measures of resistance to *T. circumcincta* has led to suggestions that IgA activity in plasma may be more useful than FEC as a marker of susceptibility to *T. circumcincta* infection (Stear et al., 1999; Strain et al., 2002). For *H. contortus*, elevated serum IgA to L3 antigens is negatively correlated with FEC (Gill et al., 1993) and is associated with a suppression of adult worm growth (Strain and Stear, 2001). The reduction