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Genetic and phenotypic relationships between carbohydrate larval antigen (CarLA) IgA, parasite resistance and productivity in serial samples taken from lambs after weaning

R.J. Shaw [a,*], C.A. Morris [b], M. Wheeler [b]

[a] AgResearch Limited, The Hopkirk Research Institute, Private Bag 11008, Palmerston North 4442, New Zealand
[b] AgResearch Limited, Ruakura Research Centre, Private Bag 3123, Hamilton 3240, New Zealand

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ABSTRACT

Genetic selection for enhanced levels of protective antibody to specific nematode antigens may be a more user-friendly means of selecting animals for resistance to gastrointestinal nematodes than obtaining faecal samples and selecting on the basis of faecal egg counts. Saliva IgA antibody levels to the L3-specific surface glycan known as carbohydrate larval antigen were measured on six occasions over a 5 month period in approximately 350 lambs. The carbohydrate larval antigen IgA response increased markedly with time as the lambs grazed on pasture naturally contaminated with nematode parasite larvae. The monthly $\log_e$ transformed carbohydrate larval antigen IgA levels were moderately heritable at all samplings, with a combined value of 0.28 ± 0.10 and a repeatability of 0.35 ± 0.03. The genetic correlations between all samplings were high (0.86), suggesting that testing for a carbohydrate larval antigen IgA response could be carried out at any time in the 5 months post-weaning. The transformed carbohydrate larval antigen IgA levels were genetically and phenotypically correlated negatively with $\log_e$ transformed (faecal egg count + 50), averaging −0.57 ± 0.20 and −0.12 ± 0.03 ($P < 0.05$), respectively. The correlations between carbohydrate larval antigen IgA and breech-soiling (dag score) never reached significance. However, genetic correlations between carbohydrate larval antigen IgA and live weight were always positive and significantly so, especially at the beginning and end of the trial, indicating that carbohydrate larval antigen IgA production may be an important genetic determinant of growth rate for lambs experiencing a larval challenge. The data suggest that the ideal time to sample for a carbohydrate larval antigen IgA response and maximise selection for lowered faecal egg count and increased live-weight would be in the first 2 months after weaning.

1. Introduction

Gastrointestinal nematodes (GINs) have a large influence on the productivity of livestock farming in pastoral grazing situations. Without the routine use of anthelmintic drugs, current levels of animal production would be severely limited by GINs. However the heavy reliance on just three main families of broad-spectrum anthelmintics over the last 10–30 years has resulted in parasite populations that have developed resistance to these drugs. Without prudent use, the recent addition of two new anthelmintic classes may also result in parasite resistance developing to these. Selective breeding of animals better able to resist nematode infection and remain productive could be a sustainable long-term option for farmers. Sheep selected for enhanced parasite resistance would lead to lower pasture larval contamination and these sheep would require fewer anthelmintic treatments to keep parasite burdens below an acceptable level (Bishop and Stear, 2003).

Considerable research effort has gone towards vaccine development and the identification of phenotypic and genetic markers of protective immunity (Hein and Harrison, 2005; Saddiqi et al., 2012). Other than the use of faecal egg counts (FECs) as a means of selecting breeding stock expressing protective immunity, there has been limited success in developing new parasite control technologies. In part the failure to develop vaccines or new markers of resistance to GINs is due to the fact that there is a diverse range of effector mechanisms which operate against individual GIN species and their developmental stages (Kemper et al., 2010; Li et al., 2012).

Genetic variation in protective antibody responses offers the potential for identifying animals that are better able to develop protective immunity to infection by GINs (Douch et al., 1996; Davies et al., 2005; Shaw et al., 2012). Secretory IgA is the main immunoglobulin of mucosal surfaces and its best-known function

* Corresponding author. Tel.: +64 6 3518644; fax: +64 6 3537853.
E-mail address: richard.shaw@agresearch.co.nz (R.J. Shaw).

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is in containing commensal bacteria within the intestinal lumen, neutralising toxins and viruses, and providing a barrier to the entry of pathogens (Brandtzaeg, 2007). There is growing evidence that IgA is also involved with protection against nematode parasite infections in humans e.g. *Trichuris trichura* (Needham and Lilly-white, 1994), *Strongyloides stercoralis* (Atkins et al., 1999), bancroftian filariasis (Sahu et al., 2008) and hookworms (Bungiro et al., 2008). A number of studies have shown that IgA responses are similarly associated with protective immunity to nematode infections in sheep (Strain et al., 2002; Harrison et al., 2003a; Davies et al., 2005; Beraldi et al., 2008; Shaw et al., 2012). Interestingly, IgA is not thought to be involved in protective immune responses in many mouse model infections (Patel et al., 2009).

Sheep made highly immune by multiple truncated infections with *Trichostrongylus colubriformis* develop a mucosal antibody response to an L3-specific epicuticle glycan known as carbohydrate larval antigen (CarLA) (Harrison et al., 2003a,b). Likewise, challenge infection of sheep made immune to *Teladorsagia circumcincta* induces a local antibody response detected in supernatants of cultured abomasal lymph node cells to a molecule with very similar properties to CarLA (Balic et al., 2003). Previously, it was shown that mucosal IgG1 and IgA antibody responses specific to CarLA can be measured in the saliva of sheep exposed to natural GIN larval challenge (Shaw et al., 2012). The genetic relationship between CarLA IgA and FEC has been determined, but over just a 2-week period (Shaw et al., 2012).

In this study the CarLA IgA responses were examined over a 5-month period post-weaning to determine the optimal time to saliva-sample sheep. Genetic and phenotypic correlations between CarLA IgA, FEC, live weight and breech soiling (dags) were examined to determine the best options for improving protective immunity to GINs and animal production.

2. Materials and methods

2.1. Animals and experimental design

Commercially sourced 2007-born crossbred ((Finnish Landrace × Texel)♂ × Romney♀) ewe lambs (n = 353), with known parentage representing progeny from 14 sires, were grazed from just after weaning in December, 2007 (~90 days of age) on AgResearch's Aorangi farm in the Manawatu region of New Zealand (NZ). Immediately prior to transportation to the Aorangi farm the animals were treated with a quarantine drench consisting of a triple combination of Abamectin, Levamisole and Oxfendazole (Matrix, Ancare NZ Ltd., New Zealand).

The animals were run as a single flock from December until late April, (~90 to 210 days of age), at which time they were split into two balanced groups which grazed separately during mating from May to early June, when they were returned to one mob. Throughout the entire trial period, from December 2007 until early July 2008, the animals were rotated around a set of eight paddocks of approximately 2.0 hectares in size with an average stay of 4 days. During April leading up to the start of mating in May, the flock grazed on a faster rotation so as to maximise weight gain before mating.

The whole flock was sampled for saliva in late January 2008 (sampling 1) and then approximately every month until the end of June (samplings 2–6). The saliva samples were assayed for CarLA-specific IgA antibodies by an immuno-assay described in Section 2.4.

Sampling for FECs was carried out on three occasions, when monitor samples reached a mean count of over 800 eggs per gram of faeces (epg), or if the lambs' condition was of concern, or at the end of the trial. At each 'sampling occasion' for FEC animals were faecal sampled twice over 2–3 days. On each occasion, immediately after the second sampling, the animals were weighed, dag scored using a severity score of 0–4 (Sheep Improvement Ltd., New Zealand, Dag Score Technical Note, where score 0 = 'no dags'), drenched (as mentioned earlier) and crutched to remove dags. The trial was carried out with the approval of, and in accordance with, the requirements of the AgResearch Grasslands Research Centre Animal Ethics Committee, NZ.

2.2. Faecal sampling

Faecal samples (~2 g each) were taken manually from the rectum of each lamb and worm eggs counted using a modified McMaster method in which one egg represented 50 epg of faeces. Faecal cultures were set up on pooled surplus material at each sampling, infective larvae recovered and 100 were identified to genus and counted, as described by Shaw et al. (2012).

2.3. Collection of saliva in field-grazed sheep

Sheep saliva samples were collected and processed as described by Shaw et al. (2012), except that saliva samples were diluted 1/20 for the assay of CarLA-specific IgA.

2.4. CarLA-specific IgA analysis

Saliva samples were diluted with sample dilution buffer and assayed in a procedure similar to that described by Shaw et al. (2012), with the exception that a reference standard method was used to obtain concentration values for CarLA-specific IgA (Peterman and Butler, 1989). To prepare the reference standard a pool of sera with high CarLA-specific IgA levels was diluted 1/1,000 with sample dilution buffer and this was given a nominal value of 1 unit/mL. A fivefold dilution series of the standard (1/1,000 to 1/32,000) was then prepared and loaded onto each plate in duplicate (100 μL/well). The standard curve generated was transformed by taking the natural logarithm of unit values and a logit (Peterman and Butler, 1989) of the reference standard absorbance. A linear regression was fitted to the linear region of the logit-log standard curve and the equation for this straight line remodeled to calculate sample concentration. To determine the minimal detectable value for the assay, the mean absorbance value plus three S.D.s of wells consisting of sample dilution buffer multiplied by 20 (sample dilution) were calculated. This was always <0.3 units/mL. The effective range of this standard curve was 0.3–20 units/mL. Samples above the standard curve (>20 units/mL) were diluted further and re-assayed. Internal controls were made by spiking saliva collected from parasite-free sheep with two different amounts of standard serum. The mean coefficient of variation (CV = (S.D./mean) × 100) of the replicates of the standard was 4.2% within assays and 10.2% between assays; the mean CV of internal controls was 21.2% within assays and 23.5% between assays. Plates with standard curves that varied by >10% were repeated.

2.5. Statistical analysis

DNA analysis was used to determine the dam and sire pedigree of lambs. Then birth-rank of lambs (i.e. single, twin or triplet) was predicted from the dam's ultrasound scan results, and their date of birth was projected from the dam's estimated stage of pregnancy at scanning time.

FEC data were transformed to natural logarithms, after first adding a constant of 50 (the smallest non-zero count) due to the presence of zero values. CarLA IgA data were also transformed to natural logarithms. As the smallest reliably measurable response