Response to *Teladorsagia circumcincta* infection in Scottish Blackface lambs with divergent phenotypes for nematode resistance

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A B S T R A C T

The objective of this study was to identify Scottish Blackface lambs that were at the extremes of the spectrum of resistance to gastrointestinal nematodes and characterise their response to an experimental nematode challenge. Lambs ($n = 90$) were monitored for faecal egg count (FEC) (2 samples from each of 2 independent natural infections). The most resistant ($n = 10$) and susceptible ($n = 10$) individuals were selected and challenged with 30,000 *Teladorsagia circumcincta* larvae (L3) at 9 months of age. Response to infection was monitored by measuring FEC, plasma pepsinogen, serum antibodies against nematode larval antigens and haematology profile, until necropsy at 71 days post infection. Worm burden, worm fecundity and the level of anti-nematode antibodies in abomasal mucosa were determined at necropsy.

FEC was consistently higher in susceptible animals ($P < 0.05$), validating the selection method. Worm fecundity was significantly reduced in resistant animals ($P = 0.03$). There was also a significant correlation ($r = 0.88$; $P < 0.001$) between the number of adult worms and FEC at slaughter. There was no effect of phenotype (resistance/susceptibility) on plasma pepsinogen or on haematology profile. Phenotype had a significant effect on the level of anti-nematode IgA antibodies in serum ($P < 0.01$), reflecting a higher peak in resistant animals at day 7 post infection.

It is concluded that significant variation in the response to gastrointestinal nematode challenge exists within the Scottish Blackface population with resistant animals displaying significantly lower FEC, lower worm fecundity and higher concentration of anti-nematode IgA antibodies in serum.

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Introduction

Gastrointestinal nematodes (GIN) are the most serious cause of disease in domestic sheep worldwide with symptoms ranging from clinical disease to ill thrift. In temperate climates, such as in Ireland, the most common infective species are *Teladorsagia circumcincta*, *Trichostrongylus* spp.

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and *Nematodirus* spp. (Burgess et al., 2012; Good et al., 2006). There is a sizeable body of evidence for both within- and between-breed variation in the ability of sheep to resist gastrointestinal nematode infection (Amarante et al., 2004; Good et al., 2006; Vanimisetti et al., 2004). This suggests that breeding for host resistance is a viable strategy to minimise the effects of GIN parasitism.

A number of traits can be used to identify animals that exhibit increased resistance to GIN infection, including faecal egg count (FEC), worm burden, anti-nematode antibody level and plasma pepsinogen concentration (as reviewed in Saddiqi et al., 2012). Of these the most practical and widely used indicator is FEC, which is moderately heritable and shows wide variability among individuals (Bishop and Morris, 2007; Safari et al., 2005). Resistance to GIN infection, as defined by a relatively low FEC, can manifest as a lower number of nematodes, reduced size of adult nematodes, reduced fecundity of females, increased proportion of inhibited larvae, or a combination of the foregoing elements (Balic et al., 2000; Lee et al., 2011). A reduction in either the number of fecund adult females or in female fecundity would have the beneficial effect of reducing worm contamination on pasture (Good et al., 2006).

Previous studies have shown that there is substantial genetic variation among Scottish Blackface lambs in both FEC and worm length (Stear et al., 1999). Resistance to GIN is most likely based on the ability to develop a timely and protective immune response. Therefore, the objective of the present study was to develop a robust method to identify resistant and susceptible Scottish Blackface lambs, and characterise their response to an experimental nematode challenge.

Materials and methods

Ethical approval

The animal procedures described in this study were conducted under experimental license from the Irish Department of Health in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendments of the Cruelty to Animals Act 1976) Regulations, 1994.

Animals

All animals were purebred female Scottish Blackface lambs, sourced from the flock at the Teagasc Hill Sheep Farm, Leenane, Co. Mayo. Two separate grazing groups were run on the farm; lambs and their dams were managed from birth to weaning on either improved lowland pasture (Lowland group, $n = 34$) or unimproved hill pasture (Hill group, $n = 56$). At weaning (14 weeks of age) the lambs in the Hill group were moved to lowland pasture to ensure that they were exposed to a natural GIN challenge. Lambs in the Lowland group remained on lowland pasture after weaning. All lambs received an oral anthelmintic (benzimidazole) treatment at 5 weeks of age to control for *Nematodirus* spp. At approximately 24 weeks of age all lambs were removed to the Teagasc Research Centre, Athenry, Co. Galway.

Faecal egg count (eggs per gram (epg); FEC) was monitored weekly, on a flock basis, from when lambs were approximately 8 (Lowland group) or 14 (Hill group) weeks of age, using the FECPAK system (www.fecpak.co.nz). Once flock FEC reached approximately 600 epg the lambs were individually sampled twice (FEC1A and FEC1B), 1 week apart, and FEC was determined for each sample using the modified McMaster method (Ministry of Agriculture and Food, 1986). Samples with a FEC of 0 epg were subjected to an additional floatation step using the method of Kelly et al. (2009). Faecal egg counts were distinguished as *Nematodirus* and 'other trichostrongyles'. Following the second sample (FEC1B) the lambs were treated with a macrocyclic lactone (ML; Oramec, Merial Animal Health Ltd) in accordance with manufacturer's recommendations. Flock FEC was again monitored weekly (FECPAK), and once it reached approximately 600 epg two more FEC counts (1 week apart) per individual were completed (FEC2A and FEC2B).

Selection of HighFEC and LowFEC animals

Individual animal values for $\ln(\text{FEC} + 25)$ were used to identify the most resistant (subsequently known as "LowFEC") and susceptible (subsequently known as "HighFEC") lambs; average values for individual lambs were estimated using mixed model procedures of SAS® (v9.1). As the lambs were raised in two separate grazing groups, selection involved combining the animal effects from two sets of analyses. Due to differences in variance estimates (among animals and residuals) between the grazing groups, samples from each natural infection (FEC1 and FEC2) were analysed separately by group (Hill or Lowland) using a model that included rearing type (single or twin) and sample date (A or B sample of round) as fixed effects. To get the selection differential for each animal, the estimated animal effect for each round was scaled by the standard error of prediction and averaged; these average values were used to identify the 10 individuals with the highest egg counts (HighFEC) and the 10 lambs with the lowest egg counts (LowFEC).

Experimental infection

The selected lambs were cleared of helminth infection by administration of an amino-acetonitrile derivative (Zolvix, Novartis) and housed on straw bedding until slaughter, with free access to water and 600 g commercial lamb ration per day. Lambs were additionally treated with an ML (Oramec, Merial Animal Health Ltd) to eliminate *Strongyloides papillosus*, and a flukicide (Duotech, Norbrook Laboratories Ltd; closantel and oxfendazole combination) to clear infection with *Fasciola hepatica*. Fluke infection was determined using the fluke sedimentation test as described in Mooney et al. (2009). All lambs were free of both helminth and fluke infection at the start of the experimental infection, based on FEC measurements on 3 consecutive days. All chemicals were administered in accordance with manufacturer's recommendations.

On day 0, the selected lambs ($n = 20$) received an oral challenge of approximately 30,000 *T. circumcincta* larvae (L3). Response to infection was monitored over the course

of the infection by measuring FEC (3 times per week), along with weekly assessments of plasma pepsinogen concentration, anti-nematode antibody level in serum, and haematology parameters. Lambs were moved to the abattoir facility at Teagasc, Ashtown, Co. Dublin to acclimatise for 2 days prior to slaughter. Animals were slaughtered at 71 days post infection by electrical stunning followed immediately by exsanguination. The concentration of anti-nematode antibodies in abomasal mucosa, abomasal worm burden, and worm fecundity were determined at slaughter.

Pepsinogen and haematology

Blood samples, in lithium heparin-containing vacutainers, were spun on the day of collection, in a bench top centrifuge at $1000 \times g$ for 15 min at 8 °C, and plasma was harvested and stored at −20 °C until assayed for pepsinogen concentration using the Ross et al. (1967) modification of the method of Hirschowitz (1955). Blood samples for haematology were assayed within 6 h of collection using an ADVIA® 2120 haematology system (Siemens Healthcare Diagnostics Inc.) as per manufacturer's recommendations.

Worm burden

The abomasum was removed at slaughter, and its contents recovered; the abomasum was then opened along the greater curvature, and digested in 1 L physiological saline for 4 h at 37 °C to recover nematodes from the tissue wall (Eysker and Kooyman, 1993). The contents and the digest were washed through a 75 μm sieve followed by a 38 μm sieve, before being preserved in 5% formalin. Adult and larval nematodes were counted from both the abomasal contents and the abomasal digest as previously described (McKenna, 2008); sex was determined where possible. Total nematode burden was calculated, for each stage by sex category, by extrapolating from 2% (75 μm sieve samples) or 5% (38 μm sieve samples) aliquot counts.

Fecundity

All mature female worms (vulva and uterine structure present) recovered whilst enumerating worm burden were mounted on slides with lactophenol, and the number of eggs *in utero* recorded. Whenever possible, measurements on at least 30 individual worms were taken per animal. Where 30 worms had not been obtained from the worm burden aliquots, samples were taken at random from the abomasal digest (75 μm sieve) until 30 female worms in total had been obtained. Females were photographed, under the dissecting microscope, with a digital camera and length determined using the public domain software (ImageJ 1.37; http://rsb.info.nih.gov/ij) after calibration with a stage micrometre slide.

Mucosal and serum collection and antibody recovery

The abomasal surface layer together with the mucus epithelial layer was removed at necropsy, by scraping with a glass slide, placed in cryovials and snap frozen in liquid nitrogen. The samples were stored at −80 °C until use. Mucosal samples were prepared for antibody recovery using a modified version of the method of Sinski et al. (1995). A sample of tissue (~50 mg) was homogenised in 1 mL of phosphate buffered saline (PBS) and 40 μL protease inhibitor cocktail (Sigma-Aldrich). After centrifugation of the homogenate at $14,000 \times g$ for 15 min the supernatant was removed, and protein concentration determined using a Qubit® protein assay kit (Invitrogen) before storage at −20 °C. Blood collected for serum antibody measurements was stored in a refrigerator overnight for clotting. Serum was extracted by centrifugation at $2000 \times g$ for 5 min before storage at −20 °C.

CarLA-specific IgA analysis

CarLA is a carbohydrate larval antigen found on the epicuticle of L3 ruminant nematode species and is shed during the moult to L4 (Harrison et al., 2003b). The CarLA-specific immunoglobulin A (IgA) analysis was carried out by AgResearch (New Zealand), using the method of Shaw et al. (2012). The CarLA antigen used in this test was isolated from *Trichostrongylus colubriformis*. A reference standard method (Peterman and Butler, 1989) was used to obtain concentration values for CarLA-specific IgA (Shaw et al., 2013). The coefficient of variation (CV) for replicates of the standards at each dilution point were ≤7% within assays and ≤10% between assays; the CV for internal controls (high, medium and low anti-CarLA IgA) were ≤12% within assays and ≤23% between assays.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was used to determine *T. circumcincta* specific IgA and IgG in serum and mucosal samples. Antigen from *T. circumcincta* L3 was kindly supplied by Tom McNeilly (Moredun Research Institute). The wells of a 96-well polystyrene ELISA plate (Thermo Fisher Scientific, USA) were coated with 100 μL of L3 antigen (2 mg/mL) in carbonate–bicarbonate buffer (Sigma-Aldrich, UK) at pH 9.6 and left overnight at 4 °C. Subsequent steps were undertaken using the DSX® ELISA processing system (Dynex Technologies, USA). All sample and antibody dilutions were in PBS-T (PBS + 1% Tween 20; Sigma-Aldrich, UK) + 3% BSA. The plates were washed four times using PBS-T. An aliquot (100 μL) of either serum (diluted 1:50 or 1:25,000 for IgA and IgG, respectively) or mucosal sample (adjusted to 50 μg/mL or 10 μg/mL for IgA and IgG, respectively) was added to each of 3 wells and incubated at 37 °C for 30 min, followed by another 4 washes in PBS–T. The plates were then incubated for 30 min at 37 °C with 100 μL of mouse anti bovine/ovine IgA (AbD Serotec, UK), diluted 1:1000 or rabbit anti-sheep IgG (AbD Serotec, UK), diluted 1:20,000. Plates were washed four times with PBS-T and samples were then incubated for 30 min at 37 °C with 100 μL of goat anti-mouse Ig:HRP (Dako, UK; diluted 1:2000) or goat anti-rabbit IgG:HRP (AbD Serotec, UK; diluted 1:2000) for IgA and IgG, respectively. After four final washes with PBS-T, chromogen tetramethylbenzidine (TMB) (Novex, UK) was added (100 μL) to each well and the plates were incubated for 15 min at room temperature. The reaction was stopped by adding 100 μL of 10% 1N HCl

to each well and the optical density (OD) read at 450 nm. Each plate included a blank (PBS-T + 3% BSA) as a negative control and a pooled plasma or mucosal sample from a subset of infected animals as a positive control.

Statistical analysis

Log transformation ($\ln(X + 25)$) was applied to adult worm burden, eggs per worm, and faecal egg count to stabilise the variance. Haematology variables were log transformed ($\ln(X)$) where appropriate. To account for repeated measures, data were analysed using the Proc MIXED procedure of SAS® (v9.1). For haematological and pepsinogen data, the model used had fixed effects for day post infection (dpi), phenotype (HighFEC or LowFEC), and their interaction, and animal as a random term. Only haematological and pepsinogen data from day 7 post infection were used as this was when the animals were parasitised. The FEC data post-infection were classified by week; data prior to day 26 were excluded as FEC did not rise above zero until after this time point. Phenotype was the only effect in the model used for the analysis of adult worm burden and fecundity.
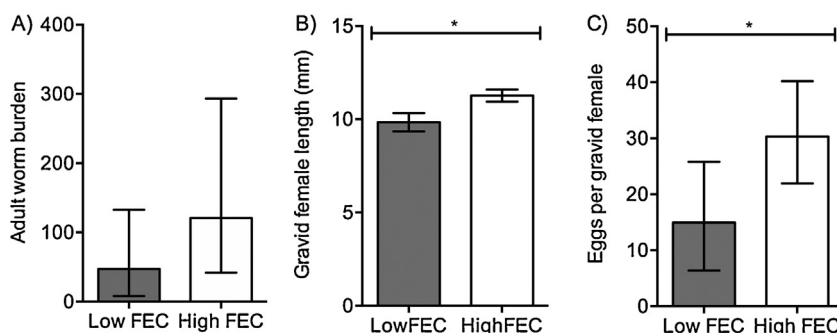
Results

FEC over the course of infection

Eggs were not observed in faeces until 28 days post infection (Fig. 1; Additional file 1). Eggs were not observed at any time during the course of infection in one LowFEC animal; in the case of a further 6 animals (1 HighFEC and 5 LowFEC) egg count never rose above 50 epg (Additional file 1). The profile of FEC was quadratic with a peak at Day 56 (week 8). The difference in FEC between HighFEC and LowFEC animals was significant ($P < 0.05$), with a highly significant phenotype by week interaction for the quadratic component of the response pattern ($P < 0.01$; Fig. 1).
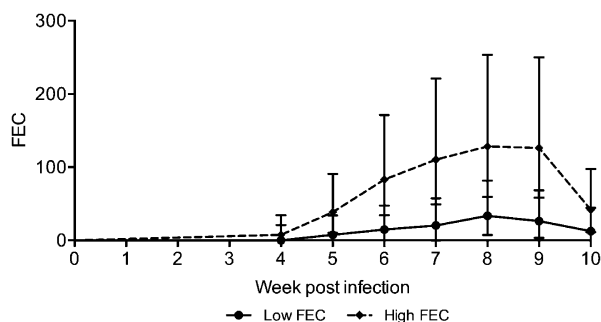
Worm burden and fecundity measurements

Average adult worm burden was lower in LowFEC animals compared to HighFEC animals although this difference was not statistically significant ($P = 0.19$; Fig. 2A).
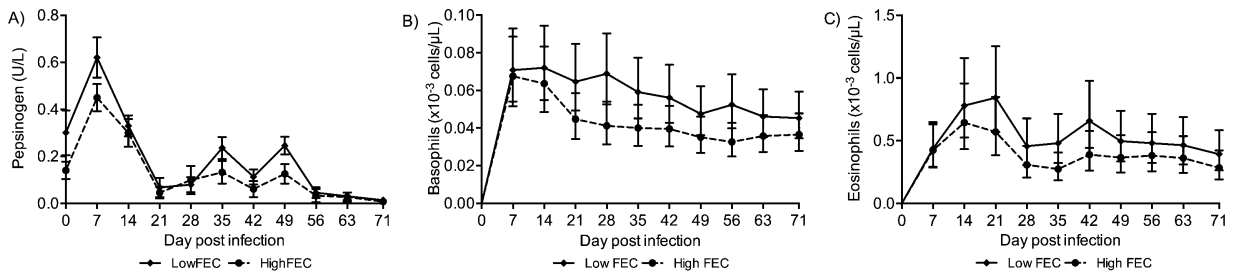

Fig. 1. Faecal egg count (FEC).
Back-transformed mean (with 95% CI) FEC in LowFEC (solid line) and High-FEC (dashed line) lambs following infection with 3×10^4 *T. circumcincta* L3 larvae. The mean FEC over the course of the infection was significantly different between the two groups ($P < 0.05$).

However, the difference in adult worms as a proportion of total worms approached significance (99.7% for High-FEC and 84.6% for LowFEC; Kruskal-Wallis test; $P = 0.066$). Adult *T. circumcincta* females were recovered from the abomasum of 13 of the 20 lambs. The mean number of gravid female worms examined per animal was 27 (range 13–73). The within-phenotype correlation between the total number of adult *T. circumcincta* and FEC on the day of slaughter was 0.88 ($P < 0.001$). The mean (s.e.) length of female *T. circumcincta* in HighFEC and LowFEC animals was 11.3 (0.33) mm and 9.8 (0.49) mm, respectively (Fig. 2B; $P = 0.03$). Back-transformed mean values for the number of eggs per gravid female were 30 and 15 for HighFEC and LowFEC animals, respectively (Fig. 2C; $P = 0.03$). For gravid females, the correlation between length and number of eggs *in utero* on a within-animal basis was 0.33 ($P < 0.01$); the corresponding correlation for animal effects on a within-phenotype basis was 0.63 ($P < 0.01$).

Pepsinogen and haematology measurements

An increase was observed in plasma pepsinogen between day 0 and day 7 post infection in both HighFEC and LowFEC animals, with a subsequent decline (Fig. 3A). There was no effect of phenotype on plasma pepsinogen concentration or any of the haematology variables

Fig. 2. Worm burden and fecundity measurements.
Back-transformed mean (with 95% CI) worm burden (A), mean (±s.e.) length of gravid females (B) and back-transformed mean (with 95% C.I.) eggs per worm (C) measurements from LowFEC (grey bars) and HighFEC (white bars) lambs following infection with 3×10^4 *T. circumcincta* L3 larvae. (*) Indicates means differ ($P < 0.05$).

Fig. 3. Pepsinogen and haematology measurements.
Mean (±s.e.) pepsinogen (A) back-transformed mean (with 95% CI) basophil (B) and eosinophil (C) numbers in LowFEC (solid line) and HighFEC (dashed line) lambs following infection with 3×10^4 *T. circumcincta* L3 larvae. Mean pepsinogen concentration or eosinophil concentration over the course of the infection was not different between the two groups ($P=0.26$; $P=0.19$, respectively) while mean basophil concentration approached significance ($P=0.07$).

during the infection period (7–71 dpi), although the difference approached significance for the number of basophils (Fig. 3B; $P=0.07$), and the number of eosinophils was numerically higher in the LowFEC group at all time points after day 7 (Fig. 3C; $P=0.19$). Day post infection was significant for all variables ($P<0.01$) and there was no significant interaction with phenotype.

Nematode-specific antibody response

Day post infection was a significant source of variation in the concentration of CarLA-specific IgA in serum (Fig. 4A;

$P<0.0001$). The LowFEC animals had significantly higher CarLA-specific IgA levels in serum over the course of infection, but particularly at 7 and 14 dpi (Fig. 4A; $P=0.04$), and in mucosa at slaughter (Fig. 4B; $P=0.04$), than their High-FEC counterparts. LowFEC animals also had significantly higher anti *T. circumcincta* IgA levels in serum over the course of infection ($P=0.02$) than HighFEC animals; there was a clear peak at day 7 post infection (Fig. 4C). Levels of anti *T. circumcincta* IgA in abomasal mucosa were also higher in LowFEC animals at slaughter (day 71) but this difference was not statistically significant ($P=0.3$) while levels of mucosal IgG approached significance ($P=0.07$).
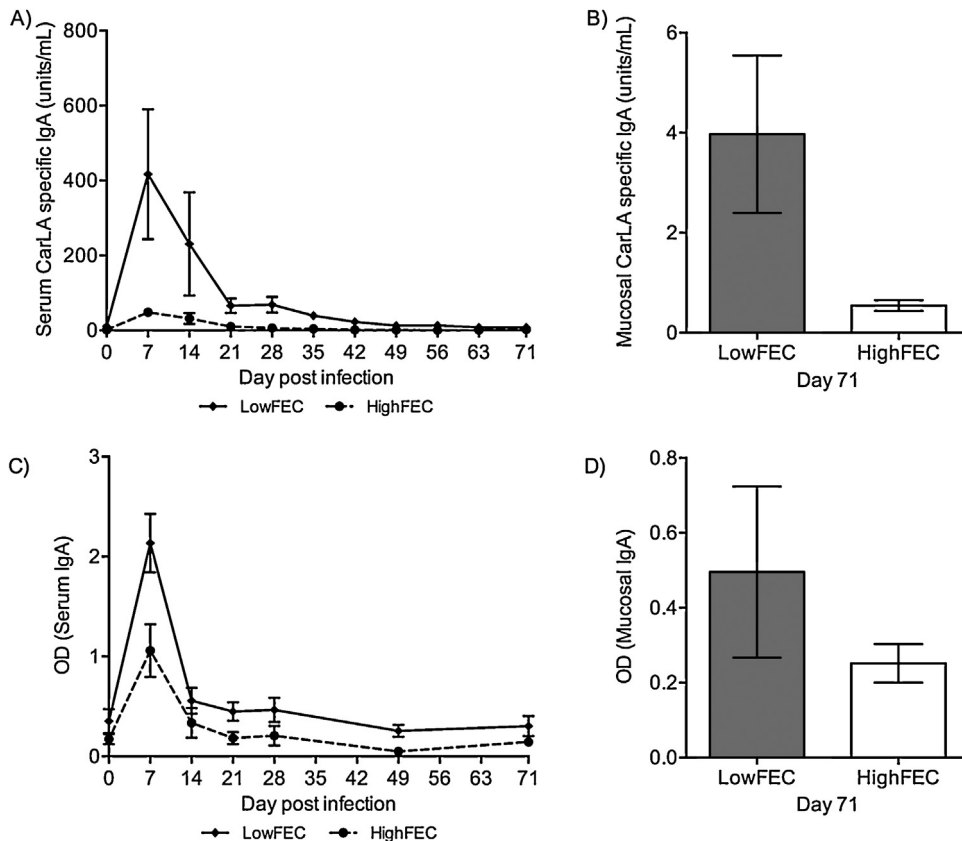
Fig. 4. Nematode-specific antibody levels.
Mean (±s.e.) CarLA- (A, B) and *T. circumcincta*-specific (C, D) IgA antibody levels in serum and abomasal mucosa, respectively of LowFEC and HighFEC lambs following infection with 3×10^4 *T. circumcincta* L3 larvae. Serum and mucosal CarLA-specific IgA (A, B) and serum anti *T. circumcincta* IgA (C) were significantly different between the HighFEC and LowFEC groups ($P<0.05$).

There was also no significant difference between the High-FEC and LowFEC groups for the level of anti *T. circumcincta* IgG in serum. However, day post infection was a significant source of variation for this variable; the levels increased from challenge until 21 days post infection in both groups, after which they declined.

Discussion

An experimental infection with *T. circumcincta* confirmed the existence of repeatable within-breed variation in the ability of Scottish Blackface lambs to resist gastrointestinal nematode infection. Selected LowFEC animals displayed lower FEC throughout the course of infection than their HighFEC contemporaries, validating FEC measurements associated with natural infection as a reliable method of identifying resistant animals.

Variation among hosts in nematode egg output reflects variation in the adult worm burden or variation in the average fecundity of each worm, or some combination of both (Stear et al., 1996). Results from the study by Good et al. (2006), involving Texel and Suffolk animals, showed that the breed difference in FEC was a reflection of a difference in worm burden, rather than in average worm fecundity. However, evidence from previous studies of Scottish Blackface animals has shown that variability in FEC is largely due to differences in average worm fecundity (Stear et al., 1996), although adult worm burden was also a contributing factor (Stear et al., 1995b). Results from a study by Stear et al. (1996) indicate that the acquired immune response in Scottish Blackface lambs develops in two stages. Firstly, lambs develop a specific local IgA response, resulting in a reduction in worm fecundity. Subsequently, in association with the production of globule leukocytes in the abomasal mucosa, an effective hypersensitivity response is developed that regulates worm burden, in conjunction with reduced fecundity of the worms that do establish (Stear et al., 1995b, 1996).

It has been widely reported that IgA mediates the suppression of gastrointestinal nematode growth and fecundity during infection with *T. circumcincta* (Beraldi et al., 2008; Martínez-Valladares et al., 2005; Strain et al., 2002), with a strong IgA response often observed against 4th-stage larvae (Stear et al., 1995b). Most of the IgA, the isotype closely associated with intestinal mucosal immune responses, in sheep blood derives from the mucosal surface of the gastrointestinal tract (Sheldrake et al., 1984), and blood and mucosal IgA levels are correlated (Henderson and Stear, 2006; Martínez-Valladares et al., 2005). IgA produced in the gastrointestinal tract can either bind to parasites and parasite secretions, or be transferred to the blood via the lymphatic system. The major determinants of blood IgA level are, therefore, mucosal IgA activity and the interaction between worm mass (burden and size) and mucosal IgA activity (De Cisneros et al., 2014). In the present study serum IgA concentrations indicate that resistant animals had larger quantities of unbound IgA entering the bloodstream, particularly at day 7. This could potentially be due to excess IgA production or the result of an increase in free IgA due to low worm numbers or reduced worm length.

While CarLA is purified from exsheathed *Trichostrongylus colubriformis* L3 larvae, there is an epitope on the CarLA molecule that is common to CarLA from a wide range of gastrointestinal nematode species (Harrison et al., 2003a). This epitope is hidden when CarLA is present on the L3 larvae but is available for detection by the immune system once CarLA is released when L3 larvae moult to become L4 stage larvae. The response detected in the samples of serum and mucosa is most likely to be to this epitope. Indeed, challenge of sheep with a developed immunity to *T. circumcincta* has been shown to induce a local antibody response to a molecule with very similar properties to CarLA (Balic et al., 2003). Animals identified as having 'high levels' of salivary anti-CarLA IgA have been shown to have lower (20–30%) FEC during a mixed-species infection than animals with low or undetectable titres (Shaw et al., 2012). While the anti-CarLA IgA assayed by Shaw et al. (2012) was in saliva rather than in blood serum or mucosa, these results are consistent with the findings in the present study. The serum IgA response to CarLA was found to mirror the response to *T. circumcincta* antigen from L3 larvae, indicating that the CarLA test could be used as a proxy if *T. circumcincta* antigen was unavailable.

Although there were differences in IgA between the HighFEC and LowFEC groups, neither plasma pepsinogen nor any of the haematology variables could differentiate the groups. While pepsinogen levels have previously been reported to be an indicator of ostertagiosis in lambs (Balic et al., 2000; Davies et al., 2005; Lawton et al., 1996), another study of within-breed differences in gastrointestinal nematode resistance found that it is not significantly different between lambs with consistently high FEC and those whose FEC was consistently low (Stear et al., 1995a). Therefore, plasma pepsinogen concentration alone may be an unreliable indicator of ostertagiosis in the individual animal (Lawton et al., 1996). No significant effect of phenotype was observed for any of the haematology variables. In a previous study involving Scottish Blackface lambs an association was found between peripheral eosinophil concentration and both FEC (Stear et al., 2002) and worm burden (Beraldi et al., 2008), with resistant (lower FEC) animals having higher circulating eosinophils. In this study, peripheral eosinophil concentrations were not significantly different between HighFEC and LowFEC lambs. However, the relationship between peripheral blood eosinophilia and tissue eosinophilia is reasonably weak, with only a proportion of eosinophils found in the blood moving into the abomasal mucosa (Henderson and Stear, 2006). The lack of a statistically significant difference between HighFEC and LowFEC groups in peripheral eosinophilia may therefore not be reflective of eosinophil levels in the abomasal mucosa, the site of infection.

Adult worm burden at the time of slaughter was not significantly different between the HighFEC and LowFEC groups in the present study. Total worm numbers were low for both groups probably because the animals were not re-infected during the course of the study. From the FEC data it appears that egg production was beginning to tail off, which could be a consequence of a reduction in worm number or in the number of eggs produced per worm. Therefore slaughter at 71 days post infection may have been too late

to fully capture the peak values for the number of adult worms.

It has been observed in experimental infections that there is a skew towards the immature life stages (L4) of *T. circumcincta* in resistant animals (Gruner et al., 2003; Kemper et al., 2010). While this study used a single infection as opposed to trickle infections, immature life stages were observed among the worms recovered from three of the LowFEC animals and one of the HighFEC animals. L4 larvae were observed in one LowFEC animal, indicating that hypobiosis had occurred. This animal had the highest levels of both anti *T. circumcincta* IgA and anti-CarLA IgA in the mucosal samples. Previous studies have shown that the number of inhibited larvae is positively associated with the magnitude of the local IgA response to 4th-stage larvae (Stear et al., 1995b). While the antigen used in all three ELISA assays in this study was generated from L3 larvae, a significant correlation ($r = 0.68$) has been reported between the IgA response to both L3 and L4 antigen (Stear et al., 1995b). The presence of inhibited larvae is not surprising as at the time of infection the animals were over 9 months old, by which time protective immunity is becoming established (Abbott et al., 2009; Vlassoff et al., 2001). This may also account for the very low FEC values observed over the course of infection. Female lambs also have a stronger immune response than males, who are less resistant to the establishment of infection (Barger, 1993; Gulland and Fox, 1992).

Adult female *T. circumcincta* were shorter and less fecund in the LowFEC (resistant) compared with HighFEC hosts. The correlation observed between worm length and the number of eggs *in utero* (0.63) was lower than a previous estimate (0.97) for *T. circumcincta* (Stear et al., 1995b). The lower correlation observed in the present study may be a result of the length of the infection period, given the evidence from FEC data that the infection had begun to wane prior to slaughter. While there was no significant difference in adult worm burden between the HighFEC and LowFEC animals, the correlation between the number of mature females and FEC recorded on the day of slaughter indicates that adult worm burden probably played a role in the observed differences in FEC between the HighFEC and LowFEC groups.

In summary, we have developed an animal selection model that can be used to identify Scottish Blackface lambs that differ in resistance to GIN. Resistant (low FEC) animals displayed consistently lower FEC throughout the course of an experimental infection with *T. circumcincta*. The difference in FEC was largely the result of a corresponding difference in worm fecundity, with adult female worms from resistant animals being shorter and less fecund. While there was no significant difference in adult worm burden between the resistant and susceptible animals, there was a correlation between the number of mature females and FEC on day of slaughter. The anti-nematode response was mediated, at least in part, by IgA, with resistant animals having significantly higher levels of anti *T. circumcincta* IgA in serum throughout the infection. Taken together, these results indicate that lower FEC in resistant Scottish Blackface lambs is primarily a result of reduced worm fecundity; although a lower adult worm burden may also play a role.

Competing interests

Authors' contributions

OMK, BG and JPH conceived of the study. KMM, OMK, BG, and JPH designed the experiments. BG, AG and KMM collected samples. KMM carried out the experiments and drafted the manuscript. JPH and KMM analysed the data. All authors read and approved the final manuscript.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetpar.2014.10.023.

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