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Protection of ewes against *Teladorsagia circumcincta* infection in the periparturient period by vaccination with recombinant antigens

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Graphical abstract



HIGHLIGHTS

- Immunisation of ewes to protect against teladorsagiosis in their lambs is proposed
- Immunisation of ewes with a recombinant *Teladorsagia circumcincta* vaccine induced protection
- The mechanism of vaccine-induced immunity remains elusive
- The levels of reduction in parasite egg shedding from immunised ewes was 45%

Abstract

Teladorsagiosis is a major production-limiting disease in ruminants in temperate regions throughout the world and one of the key interventions in the management of the disease is the prevention of pasture contamination with *Teladorsagia circumcincta* eggs by ewes during the periparturient relaxation in immunity which occurs in the period around lambing. Here, we describe the

immunisation of twin-bearing ewes with a *T. circumcincta* recombinant subunit vaccine and the impact that vaccination has on their immune responses and shedding of parasite eggs during a continuous *T. circumcincta* challenge period spanning late gestation and lactation. In ewes which displayed a clear periparturient relaxation in immunity, vaccination resulted in a 45% reduction in mean cumulative faecal egg count (cFEC, $p = 0.027$) compared to control (immunised with adjuvant only) ewes. Recombinant antigen-specific IgG and IgA, which bound each of the vaccine antigens, were detected in the serum of vaccinated ewes following each immunisation and in colostrum taken from vaccinated ewes post-partum whereas low levels of antigen-specific IgG were detected in serum and colostrum from control ewes. Antigen-specific IgG and IgA levels in blood collected within 48 h of birth from lambs largely reflected those in the colostrum of their ewes.

Keywords: *Teladorsagia circumcincta*, recombinant vaccine, periparturient period.

1. Introduction

The major cause of parasitic gastroenteritis (PGE) in small ruminants in temperate regions worldwide is the parasitic nematode, *Teladorsagia circumcincta*. This pathogen is acquired by ingestion of infective third stage larvae (L3) from pasture and, thereafter, the developing parasites (L3 and L4) and adult worms reside in the abomasum, where a combination of nematode-induced damage and a host inflammatory/immune responses result in a protein-losing gastropathy (Simpson, 2000). The major effect of infection with this parasite is on lamb productivity via a reduction in liveweight gain (Gibson and Everett, 1976); acquisition of infection early in life has a significant impact on lamb body fat and protein deposition as a result of a reduction in the efficiency of metabolisable energy used for growth (Coop et al., 1982). The additional cost of lamb finishing time, assuming a 10% reduction in daily weight gain resulting from PGE, is substantial and was recently estimated as £4.40 (~US\$6.30) per lamb (Wright, 2013). Protective immunity to *T. circumcincta* develops after continual exposure to the parasite over a period of weeks (Seaton et al., 1989); however, the degree of immunity acquired is dependent on the level of parasite challenge, age of the animal and its genotype (Singleton et al., 2011). In practice, immunity usually develops too late to prevent the parasite's negative effects on lamb productivity.

T. circumcincta infection is currently controlled using anthelmintics; however, resistance to Class I to III products (benzimidazoles, levamisoles and macrocyclic lactones) is rife and multi-class resistant isolates have been reported (Bartley et al., 2004; Wrigley et al., 2006). Of further concern are the field reports of resistance to one of the two new classes of anthelmintic, the aminoacetonitrile derivative monepantel (Scott et al., 2013; Mederos et al., 2014). As an alternative method of control, we have developed a recombinant vaccine for *T. circumcincta* which, when administered to 6-7 month-old lambs in two separate trials, reduced total nematode egg output by 70% and 58%, respectively (Nisbet et al., 2013). During the period of peak worm egg shedding, vaccinated lambs shed 92% and 73% fewer eggs than did adjuvant only recipient lambs, respectively.

Moreover, at post mortem, vaccinates had 75% and 56% lower adult nematode burdens than controls, respectively (Nisbet et al., 2013). Reductions in faecal egg output of this magnitude will have a substantial impact on downstream pasture contamination and could play a central role in the delivery of sustainable integrated nematode control programs.

An important component of the epidemiology of *T. circumcincta* infections is the periparturient relaxation in immunity (PPRI) to the parasite observed in ewes around lambing (Gibson, 1973). Indeed, larvae derived from eggs shed by ewes during the PPRI provide the principal source of contamination early in the grazing season and infections resulting from this transmission route lead to the deleterious effects observed in grazing lambs (Gibson, 1973, Sargison et al., 2012). Thus, by immunising ewes to reduce susceptibility to infection during the PPRI an important route of transmission of *T. circumcincta* could be blocked. Here, we describe the immunisation of breeding ewes with a *T. circumcincta* recombinant vaccine and the impact that this has on their immune responses and subsequent shedding of the parasite.

2. Materials and Methods

2.1 Recruitment of breeding ewes

Seventy-two, Texel crossbred ewes which had been reared on nematode contaminated pasture were recruited onto the study approximately 199 days prior to their estimated lambing date (“dpeld”). The ewes were divided into two equal-sized groups, based on body condition score (BCS), weight and age. BCS scores ranged from 2 to 4 in both groups and average ewe weight was 66.3 kg in both groups. Average ewe age at the start of the experiment was 3.9 years in both groups. Faecal egg count (FEC) analysis at the start of the experiment showed that most (75%) ewes were excreting low numbers of trichostrongyle nematode eggs in their faeces (Group 1 ewes: mean (\pm SEM) 5 ± 1 eggs per gram (epg); Group 2 ewes: mean 9 ± 3 epg).

2.2. Preparation of recombinant vaccine

The eight recombinant proteins that constitute the sub-unit vaccine were produced as described previously (Nisbet et al., 2013). Briefly, expression of recombinant proteins, Tci-SAA-1, Tci-MIF-1, Tci-APY-1, Tci-MEP-1, Tci-TGH-2 and Tci-ASP-1, in *Escherichia coli* BL21-CodonPlus® (DE3)-RIL competent cells (Stratagene) was induced using 1 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) in liquid (Luria Bertani broth) culture. Tci-CF-1 and Tci-ES20 protein expression in *Pichia pastoris* was induced with 0.5% methanol in Buffered Complex Methanol Medium (BMM). Soluble recombinant proteins were purified from cell lysates (*E. coli*) or culture supernatant (*P. pastoris*) using HisTrap™ HP columns (GE Healthcare), then dialysed against 20 mM phosphate buffer, 0.5 M NaCl, pH 7.6. Insoluble recombinant Tci-MEP-1 was purified via nickel column affinity chromatography in the presence of 8 M urea, then dialysed against 2 M urea in 20 mM phosphate buffer, 0.5 M NaCl, pH 7.6. Protein concentrations were determined using the Pierce BCA™ (bicinchoninic acid) assay (Thermo Scientific) with bovine serum albumin (BSA) standards and the integrity of each recombinant protein assessed via SDS-PAGE.

2.3 Immunisation and feeding schedule for ewes

Thirty-six Group 1 (“Vaccinated”) ewes, on pasture, received a primary immunisation with the vaccine at 192 dpeld. Each ewe was injected subcutaneously with 400 μ g recombinant protein mix (incorporating 50 μ g each of Tci-ASP-1; Tci-MIF-1; Tci-TGH-2; Tci-APY-1; Tci-SAA-1; Tci-CF-1; Tci-ES20; Tci-MEP-1) plus a total of 10 mg Quil A (Brenntag Biosector). Seven of the proteins were soluble in phosphate buffered saline (PBS) and these were administered as a mixture in a single injection with 5 mg Quil A in PBS. Tci-MEP-1 protein was insoluble in PBS and was formulated with 2 M urea in PBS plus 5 mg Quil A. The preparations were injected separately at two sites on the neck. Two further immunizations with the same antigen preparations were administered 2 and 4 weeks after the first immunisation, at 178 dpeld and 164 dpeld, respectively. Thirty-six ewes in Group 2 (“Control”) received three immunizations with urea/PBS/Quil A (5mg Quil A in PBS in one side of the neck and

5mg Quil A in PBS with 2M urea in the other side of the neck) at the same time as vaccinated ewes. All ewes were returned to pasture following immunisation and, following synchronisation of oestrus and ovulation, were mated at 140 dpeld. At 78 dpeld, all ewes were ultrasound scanned. To avoid potential confounding effects of parity on immune function (Houdijk, 2008; Houdijk et al., 2006) only twin-bearing ewes were retained for the parasite challenge phase. Only twin-bearing ewes were used here because the increased energy demands on twin-bearing ewes, compared with single-lambing ewes, results in a more reliable induction of the PPRI (Houdijk, 2008; Houdijk et al., 2006). The twin-bearing ewes were fed on a diet with a metabolisable protein (MP) level which had previously been shown by this group to be associated with increased nematode egg shedding during late gestation and lactation, presumably associated with a PPRI (McNeilly et al., unpublished). Regulating these two factors (i.e. twin pregnancy and reduced nutrition level), was considered appropriate to limit variability in the extent of the PPRI observed between individuals. After scanning, 11 twin-bearing ewes comprised the vaccinated group and 16 twin-bearing ewes the adjuvant-only recipient group. On day 53 dpeld, all of these twin-bearing ewes were dosed *per os* with monepantel (Zolvix®, Novartis Animal Health) at the manufacturer's recommended dose rate, housed indoors and their nutritional intake gradually adjusted to 2.5% body weight per day in grass nuts (GRAZE-ON grass pellets, Northern Crop Driers) with *ad libitum* access to hay. From day 34 dpeld, the 27 ewes were individually kept in pens with sawdust bedding, with *ad libitum* access to water and feeding gradually adjusted to 4% body weight per day in grass nuts by 25 dpeld. This level of nutrition represents a marginal excess of MP in the late stages of pregnancy and during lactation, but also allows *T. circumcincta* establishment (McNeilly et al., unpublished). At 40 dpeld, all twin-bearing ewes received a fourth ('booster') immunisation with the vaccine (Group 1) or adjuvant only (Group 2) and, for a 63-day period from 40 dpeld until day 22 post-lambing ("pl"), each of these ewes was infected orally three times per week with 10,000 *T. circumcincta* third stage larvae (L3). Five of the 27 ewes (2 vaccinates; 3 controls) were removed from the study during this period for one of the following reasons: inappetance leading to low BCS (2 ewes); ewe death due to post-

parturition complications (1 ewe); failure to produce live twin lambs (1 ewe); death of one of the lambs (1 ewe). At day 45 pl all ewes were administered anthelmintic (as above) and at day 60 pl, all remaining ewes were returned to the nematode-contaminated pasture.

All animal experiments were performed under the regulations of a UK Home Office Project Licence PPL6004238; experimental design was ratified by Moredun Research Institute's Experiments and Ethics Committee (Approval E31/14).

2.4 Performance, serological and parasitological measurements

Ewe and lamb weights and ewe BCS were measured weekly for all twin-bearing vaccinated and control ewes from 74 dpeld to day 45 pl. From 192 dpeld onwards blood samples were collected fortnightly. Colostrum was collected from all twin-bearing ewes at lambing and blood taken from each live lamb 24-48 h pl. Antigen-specific antibody levels (IgG and IgA) in serum and in colostrum were measured by ELISA as described in Nisbet et al., (2013). Briefly, plates (Greiner Bio-one, high binding) were coated with each recombinant antigen (50 µl per well at a concentration of 1 µg/ml for IgG and 5 µg/ml for IgA). Serum or colostrum was diluted 1:5000 for IgG and 1:10 for IgA in Tris Buffered Saline (20 mM Tris, 150 mM NaCl, pH 7.4) containing 0.1% Tween® 20 (TBST). The secondary antibody used for IgG detection was mouse monoclonal anti goat/sheep IgG-HRP conjugate (Clone GT-34, Sigma A9452), used at 1:2000 and, for IgA, mouse monoclonal anti ovine/bovine IgA, used at 1:20,000 (Clone K84,2F9, Bio-rad AbD Serotec, MCA628GA). The tertiary antibody for detection of IgA was rabbit anti-mouse IgG-HRP conjugate (Dako P0260) used at 1:1000. Faecal egg count (FEC) analysis (Christie and Jackson, 1982) was performed on all 72 ewes at 188 and 153 dpeld and on all 27 twin-bearing ewes at 76, 53 and 46 dpeld. FEC analysis was then performed three times a week on all twin-bearing ewes throughout the period of L3 infection. Cumulative FEC (cFEC) values during the trickle infection period were calculated using the trapezoid method (Taylor et al., 1997).

2.5 Statistical analyses

A random intercept linear mixed model (LMM) was fitted to ewe weight data by restricted maximum likelihood (REML) to investigate the statistical significance of the differences in mean weights between vaccinated and control ewes within the pl period. A generalized linear mixed model (GLMM) was fitted by maximum likelihood to test for differences in BCS between these groups over time, with cumulative logit link function to account for the ordinal nature of BCS and using Laplace approximations to calculate log-likelihoods. Animal identification was included in these models as a random effect. A random intercept and slope LMM with lamb identification as a random effect was fitted by REML to investigate the statistical significance of the differences in mean lamb weights over time by group.

The principal parasitological parameter of interest was ewe cFEC during the trickle infection period as this represents the likely pasture contamination to which lambs would be exposed once weaned. A negative binomial generalised linear model (NB GLM) with logarithmic link function was used to compare the mean cFEC between vaccinated and control ewes. Correlation analyses of antigen-specific antibody levels, as well as the correlations computed between these and cFEC, were performed based on the Spearman's rank correlation coefficient. The correlation coefficients were tested for statistical significance and, when multiple comparisons were conducted, the corresponding *p*-values were adjusted for false discovery rate (FDR). Statistically significant differences between antigen-specific colostrum and lamb serum antibody levels in vaccinated animals were determined using pair-wise paired Wilcoxon signed-rank tests with significance levels adjusted for FDR. All the statistical analyses were conducted using the R system for statistical computing v3.2. Statistical significance was assessed at the usual 5% significance level in all cases.

3. Results

3.1 Ewe and lamb performance and condition characteristics

Twin-bearing ewes in both vaccinated and control groups increased body weight following a similar pattern during the pre-lambing period (Fig. 1A). After lambing, ewes in both groups lost weight consistently until week 4 pl and started to increase in weight thereafter (Fig. 1A). Vaccinated ewes had higher average weights at each timepoint during lactation than ewes in the control group, ranging from 1.4 kg higher on day 8 pl to 3.3 kg higher on day 22 pl. However, no statistically significant effects of vaccination ($p = 0.678$) nor a vaccination:time interaction ($p = 0.969$) were identified for ewe weight in the pl period. The BCS of the ewes in each group were similar throughout the experiment but, by day 43 pl, the average BCS (\pm SEM) for vaccinated ewes was 3.1 (± 0.2) compared to 2.6 (± 0.2) in the control ewes. Neither vaccination ($p = 0.558$) nor time ($p = 0.910$) had a statistically significant effect on BCS pre-lambing but, post-lambing, BCS decreased over time and this effect was statistically significant ($p = 0.002$) in both vaccinated and control animals. Mean weights of lambs born to ewes in both groups increased linearly during the first 43 days pl (Fig. 1C), with lambs from the vaccinated ewes being slightly heavier on average at day 43 pl than lambs from control ewes (16.7 ± 0.8 kg for Group 1 lambs; 16.1 ± 0.5 kg for Group 2 lambs). There were no statistically significant differences in weight gain in lambs born to vaccinated ewes and those born to control ewes over this period ($p = 0.825$).

3.2 Effects of immunisation on faecal egg counts during the periparturient period

Following initiation of the L3 infection at 40 dpeld, FEC remained low in vaccinated and control groups (maximum mean FEC (\pm SEM) for any timepoint pre-lambing was 3 ± 2 eggs per gram (epg) in vaccinated ewes; 18 ± 13 epg in control ewes) until after lambing in spite of ewes receiving an infection of 30,000 L3 per week (Fig. 2). During the L3 infection period, mean FEC (\pm SEM) for both vaccinated and control ewes peaked at day 14 pl (134 ± 42 epg in vaccinated ewes; 223 ± 60 epg in control ewes) before declining (Fig. 2A). During this period, median FEC values for each group (Fig. 2B) demonstrated different patterns of egg shedding with a later peak in median FEC in vaccinated ewes than in control ewes. During the pl period, when the majority of egg shedding took

place in both vaccinated and control groups, a subset of three control ewes did not display an egg shedding pattern typical of the PPRI, as determined by their FEC values: The average FEC (\pm SEM) for control ewes which did display a PPRI-induced increase in FEC level across this period was 131 ± 21 epg ($n = 11$), whereas the average FEC in the subset of the three atypical ewes was 7 ± 4 epg ($n = 3$). In addition, the average FECs for each of these three ewes post-lambing, during sustained infection, were lower than the FECs observed in these individuals when they were sampled at the end of the previous grazing season on contaminated pasture, demonstrating that they had maintained immunity during the experimental parasite challenge period. These three ewes each had cFEC values of < 500 epg and contributed to the large difference between minimum and maximum cFEC values in the control group to which the NB GLM was fitted (maximum to minimum cFEC value range for the control group = 5003, compared to 2769 in vaccinated group). Two ewes in the vaccinated group also had cFEC values of < 500 epg but it was not known whether this was the result of maintained natural immunity or vaccine-induced immunity. Thus, when all ewes were included in the cFEC analysis, there was no statistically-significant effect of vaccination on cFEC (mean cFEC vaccinated ewes 1345 epg; control ewes 2418 epg; $p = 0.157$, Fig. 3A). To ensure that only animals from the control and vaccinated groups which had displayed a clear periparturient relaxation in immunity were included in the final FEC analyses, the ewes which had cFEC values of < 500 epg (2 from the vaccinated group and 3 from the control group) were excluded from the final NB GLM analysis. This analysis indicated a statistically significant ($p = 0.027$) difference between the two groups, represented by a 45% lower mean cFEC in the vaccinated group (Fig. 3B).

3.3 Serological analyses of immune responses to vaccination and parasite exposure

ELISA experiments were performed to establish serum and colostrum IgG and IgA levels to each recombinant antigen in vaccinated and control ewes and in their lambs (Fig. 4 and Supplementary data Fig. S1). In vaccinated ewes, antigen-specific serum IgG and IgA responses were induced after the first three immunisations (Supplementary data Fig. S1). The levels of IgA and IgG

varied between antigens, with highest levels of IgG and IgA detected to Tci-ASP-1. The levels decreased again (particularly IgG), then, after the fourth vaccination, antigen specific antibody levels increased (Supplementary data Fig. S1A and C). In control animals, IgG levels remained low throughout the vaccination and infection periods (Supplementary data Fig. S1B). Serum antigen-specific IgA levels in control ewes increased to some of the antigens during grazing on contaminated pasture and again during the trickle infection period, but not to the extent of that observed in the vaccinates (Supplementary data Fig. S1D). Recombinant antigen-specific IgG and IgA were also detected in colostrum taken from vaccinated ewes immediately post partum (Fig. 4). Low levels of antigen-specific IgG were detected in colostrum from control ewes (Fig. 4A); however, in contrast, the levels of antigen-specific IgA detected in colostrum from control ewes were similar to those measured in the vaccinated animals (Fig. 4B). In vaccinated ewes, statistically significant positive correlations were identified between serum (taken 13 days prior to lambing) and colostrum IgG levels to Tci-ASP-1 ($0.82, p = 0.008$) and Tci-MIF-1 ($0.86, p = 0.008$) but no statistically significant correlations were observed between levels of any antigen-specific IgA in the ewe serum at 13 days prior to lambing and in the colostrum. In control ewes, statistically significant positive correlations were observed between levels of antigen-specific IgA in the ewe serum 13 days prior to lambing and in the colostrum for Tci-MEP-1 ($0.65, p = 0.032$) and Tci-CF-1 ($0.64, p = 0.032$). Antigen-specific IgG and IgA levels in blood collected within 48 h of birth from lambs largely reflected those in the colostrum of ewes (cf. Figs 4A and B and Supplementary data Fig. S2A and B).

Thirteen days prior to lambing represented the timepoint approximately mid-way in the L3 infection protocol and the point at which serum IgG and IgA levels against each antigen peaked following the fourth immunisation and parasite challenge. This timepoint was chosen for correlation analyses between antigen-specific antibody levels and the final cFEC in each ewe. No statistically significant relationships were observed between antigen-specific IgG or IgA levels in vaccinated ewes and cFEC for any of the antigens analysed.

4. Discussion

Here, immunisation of ewes with a *T. circumcincta* recombinant vaccine prior to pregnancy, followed by vaccine boosting during pregnancy, was shown to reduce faecal egg shedding from ewes in which a periparturient relaxation in immunity occurs. One of the key control strategies for reducing the impact of PGE on lamb production and welfare is the reduction of exposure to the causative parasites (Githigia et al., 2001). Using FEC data obtained over a 4-year period from a Scottish Blackface flock on an upland farm, Singleton et al., (2011) developed a model of *T. circumcincta* infection which incorporated the effects of immunity on parasite establishment and fecundity. The model, which accurately reproduced observed measurements, demonstrated that larval contamination of the pasture was primarily derived from the large numbers of eggs excreted by ewes during the PPRI period (Singleton et al., 2011). The PPRI is thought to impact parasite transmission by the resumed development of inhibited fourth stage larvae in previously infected ewes, augmented by further ingestion/ development of new infections derived from overwintered L3 on pasture (Gibson, 1973). Suppression of this periparturient source of infection is currently addressed in practice by the administration of anthelmintic to ewes around lambing (Taylor et al., 1993; Sargison et al., 2012); however, this is likely to increase selection for anthelmintic resistance as the lambing treatment is applied when *refugia* levels of the nematodes on pasture are relatively low (Sargison et al., 2012). There are further concerns regarding the presence of anthelmintics with persistent activity in milk, which will achieve sub-optimal concentrations in lambs and hence select for resistance in parasites that they subsequently ingest (Leathwick et al., 2015).

Nematode egg excretion around lambing has also been shown to be reduced in ewes by increasing the quantity and quality of dietary protein offered in the diet. Mechanistically, this is thought to work through balancing metabolisable protein resources for immune function and gestation/lactation (Donaldson et al., 2001; Houdijk et al., 2001, 2003; Sakas et al., 2012). However, the impact of nutritional supplementation on the PPRI in nematode infections varies amongst

different breeds of sheep (Kidane et al., 2010). Reducing energy demands in the periparturient period (for example, in ewes with a single rather than twin lambs) can also reduce the effect of the PPRI on nematode egg shedding (Houdijk, 2008; Houdijk et al., 2006). Both increasing the levels of nutrition and reducing energy demands by limiting lamb numbers imposes a substantial economic cost on sheep farmers, with vaccination potentially offering a more economical alternative.

Several ewes in the experimental groups here had low FEC throughout the trial in spite of mothering twin lambs and being subjected to a substantial L3 challenge (cf. Kidane et al., 2009). It is assumed that they maintained a low FEC in the post partum period as they were able to regulate adult parasite numbers better than ewes which had higher levels of nematode egg shedding. When ewes with cFEC of < 500 epg in both vaccinated and control groups were removed from the final analysis, a significant difference was observed in cFEC between vaccinates and controls (Fig. 3B) demonstrating that immunisation had an impact on ewe egg shedding in the post partum period. The mechanism of the vaccine's effect is unclear as no statistically-significant relationships were observed between antigen-specific IgG or IgA levels in vaccinated ewes and FEC for any of the antigens analysed. The work presented here did not analyse cellular responses and current work in our group aims to elucidate the mechanism of vaccine efficacy to improve the impact of the vaccine.

Immunisation of pregnant ewes to control pasture contamination with nematode eggs has previously been attempted in cross-bred Bergamacia ewes grazing pastures contaminated with *Haemonchus contortus* (Bassetto et al., 2014). In this South American study, ewes were immunised with a vaccine containing *Haemonchus* native integral gut membrane proteins enriched for H-gal-GP and H11 (Barbervax®, WormVax Ltd) approximately 1 month before parturition. This immunisation regime induced low antigen-specific antibody titres and did not have a significant impact on anaemia or *Haemonchus* FEC compared to non-vaccinated ewes (Bassetto et al., 2014). For this reason, in the protocol here, ewes received immunisations before pregnancy, with a single booster immunisation during pregnancy. This protocol induced high levels of antigen-specific IgG in the ewes prior to

parturition (Supplementary data, Fig. S1A) but, unlike the *Haemonchus* vaccine system where antigen specific IgG levels are known to correlate with immunity (Munn et al., 1997; Smith et al., 1999), a role for vaccine-induced serum IgG in immunity to *T. circumcincta* has not been established (Nisbet et al., 2013). Several studies have indicated that local immune responses, in particular IgA, play a role in the regulation of *T. circumcincta* larval growth and development in resistant sheep (Stear et al., 2004; Smith et al., 2009). Following booster vaccination here, antigen-specific serum IgA levels increased and, for some antigens, remained elevated in late gestation and lactation (Supplementary data, Fig. S1C). Thus, an involvement of this isotype in vaccine-induced immunity at mucosal surfaces cannot be ruled out.

Analysis of the antigen-specific IgA levels also confirmed increased antigen-specific IgA in the sera and colostrum of control ewes during the L3 infection period (Fig. 4B and Supplementary data, Fig. S1D) and that levels of Tci-CF-1 specific IgA in control ewe serum prior to lambing and colostrum at the point of lambing were higher than IgA levels to the other antigens. This may reflect higher numbers of worms establishing in control ewes during this period and thus the elevated IgA levels may be secondary to release of Tci-CF-1 into the sheep's digestive system from developing and adult worms as Tci-CF-1 is their major secretory antigen (Redmond et al., 2006). This is supported by previously-published studies; IgA in ewe milk is almost exclusively gut-derived and is transported to the mammary tissue via plasma (Jeffcoate et al., 1992), so Tci-CF-1-specific IgA in both serum and colostrum could be the result of excretory/secretory (ES) Tci-CF-1 from *T. circumcincta* in the abomasum of the control ewes. Previously, it has been shown that *T. circumcincta* L4 ES-specific plasma IgA (but not IgG or IgM) levels increase in infected ewes prior to lambing (Jeffcoate et al., 1992). Similarly, in the present study, control ewes had elevated antigen-specific IgA, but not IgG, levels prior to lambing (Supplementary data, Fig. S1D)

In conclusion, the efficacy of a recombinant *T. circumcincta* vaccine was demonstrated in periparturient ewes and the effect of the vaccine was to reduce nematode egg shedding. A vaccine

based on this prototype could play an important role in reducing challenge to lambs in practice, affording them protection from the clinical and production effects of teladorsagiosis. The use of both vaccination and of the selective use of ewes which are able to resist infection during the periparturient period (see Fig. 3A) could have a major impact on the epidemiology of the parasite. Furthermore, vaccination could play an important part in the integrated management of this parasite by replacing the need to treat ewes with anthelmintic when refugia levels in the environment are low and also to reduce the impact of exposure of the nematodes to sub-optimal levels of anthelmintic transferred to lambs in the milk (cf. Dever and Kahn (2015); Leathwick et al., (2015)).

Note: Supplementary data are associated with this article.

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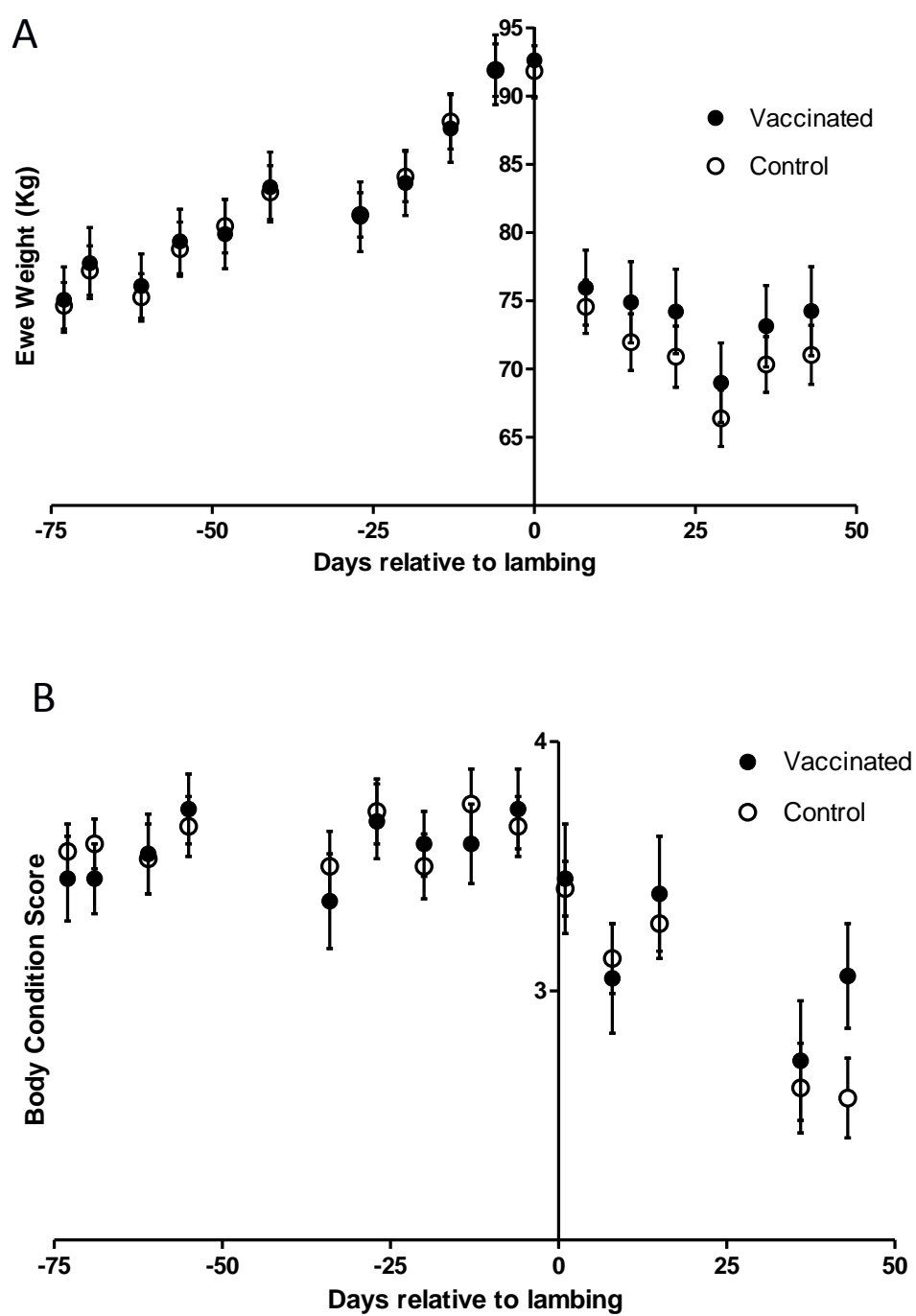
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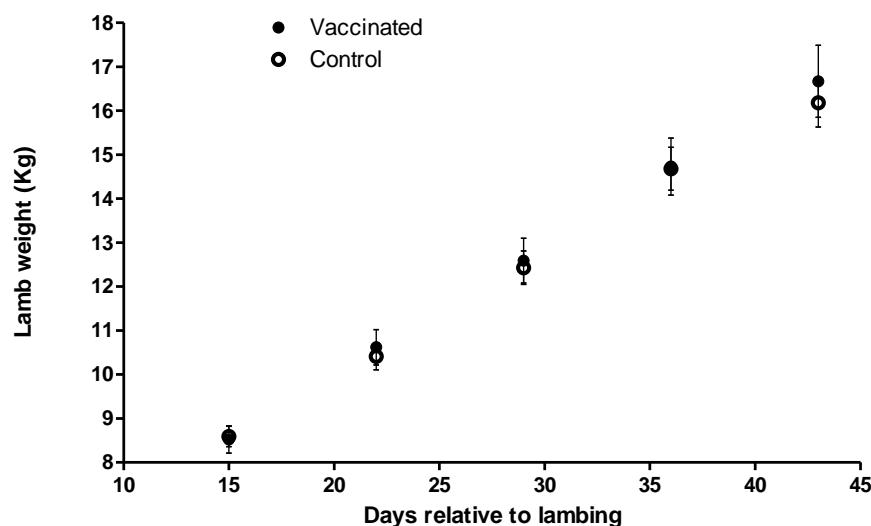
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Figure legends

Fig. 1. Influence of immunisation against *Teladorsagia circumcincta* on production characteristics of ewes and lambs during the periparturient period

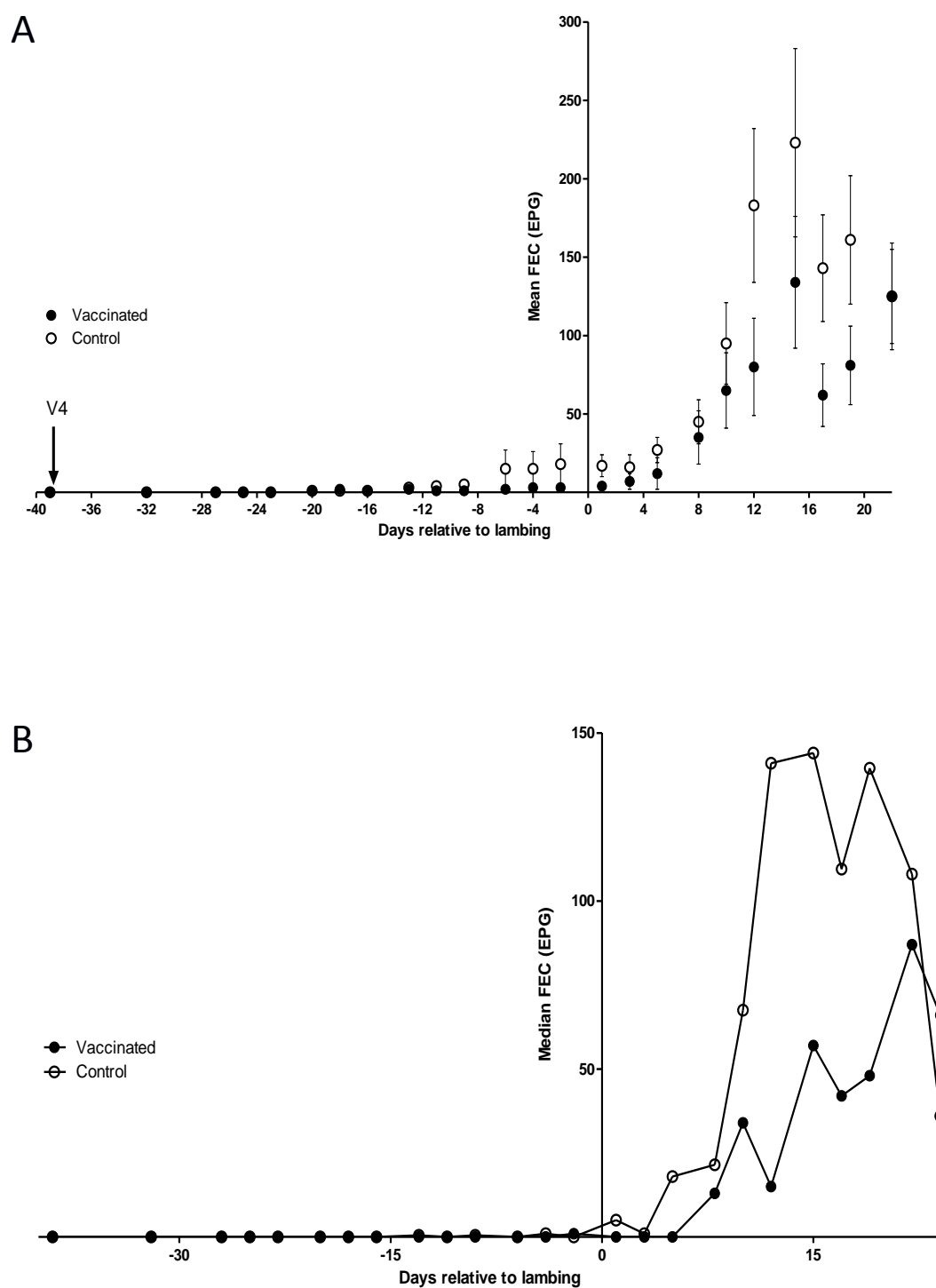


C



Weights (Panel A) and Body Condition Scores (Panel B) of ewes immunised with a *T. circumcincta* recombinant vaccine during parasite challenge throughout the periparturient period. Weights of lambs (Panel C) from birth. Data shown are means (\pm SEM). For vaccinated ewes, $n = 11$ (day -74 to day 6 pl), then $n = 10$ until day 15 pl, and $n = 9$ until the end of the experiment. For control ewes $n = 16$ (day -74 to day 12 pl), then $n = 15$ until day 17 pl, $n = 14$ from day 17 pl until day 43 pl, and $n = 13$ from day 43 pl until the end of the experiment. For lambs from vaccinated ewes, $n = 20$ on day 15 pl, $n = 18$ thereafter; lambs from control ewes $n = 30$ on day 15, thereafter $n = 28$ until day 43, when $n = 27$.

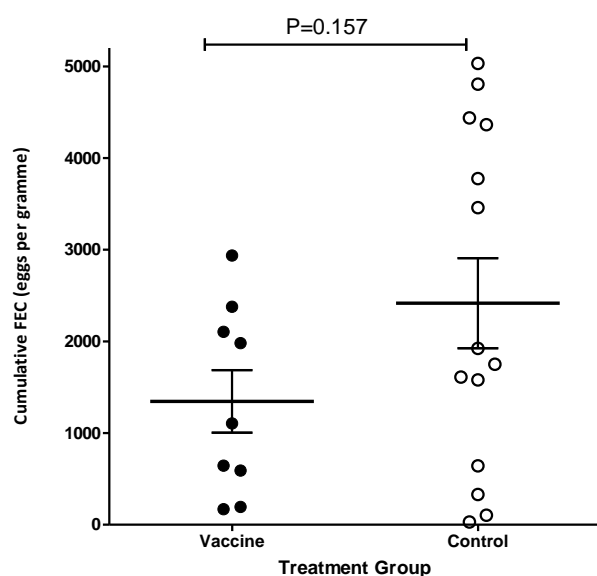
Fig. 2. Influence of immunisation against *Teladorsagia circumcincta* on faecal egg counts of ewes during the periparturient period



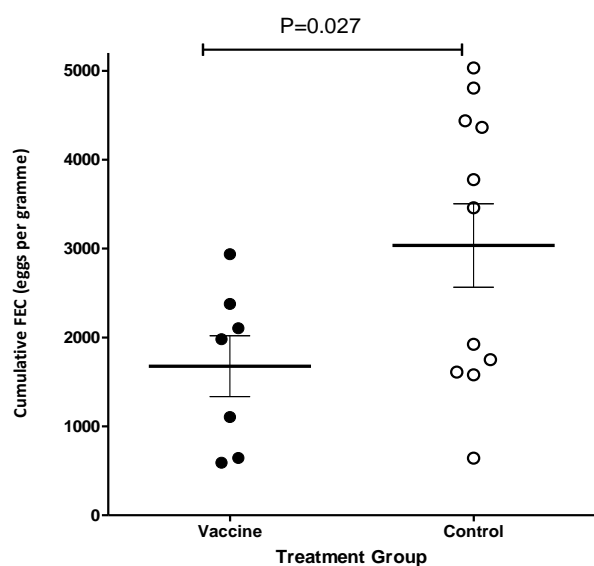
Mean \pm SEM (Panel A) and median (Panel B) faecal egg counts of ewes immunised with a *T. circumcincta* recombinant vaccine during parasite challenge in the periparturient period. “V4” on Panel A represents the date of the fourth (booster) vaccination and initiation of the L3 challenge. For vaccinated ewes, n = 11 (day -74 to day 6 pl), then n = 10 until day 15 pl, and n = 9 until the end of the trickle infection period. For control ewes, n = 16 (day -74 to day 12 pl), then n = 15 until day 17 pl, and n = 14 from day 17 pl until the end of the trickle infection period.

Fig. 3. Cumulative faecal egg counts, across a 63-day L3 infection period, of ewes immunised with a *Teladorsagia circumcincta* recombinant vaccine.

A



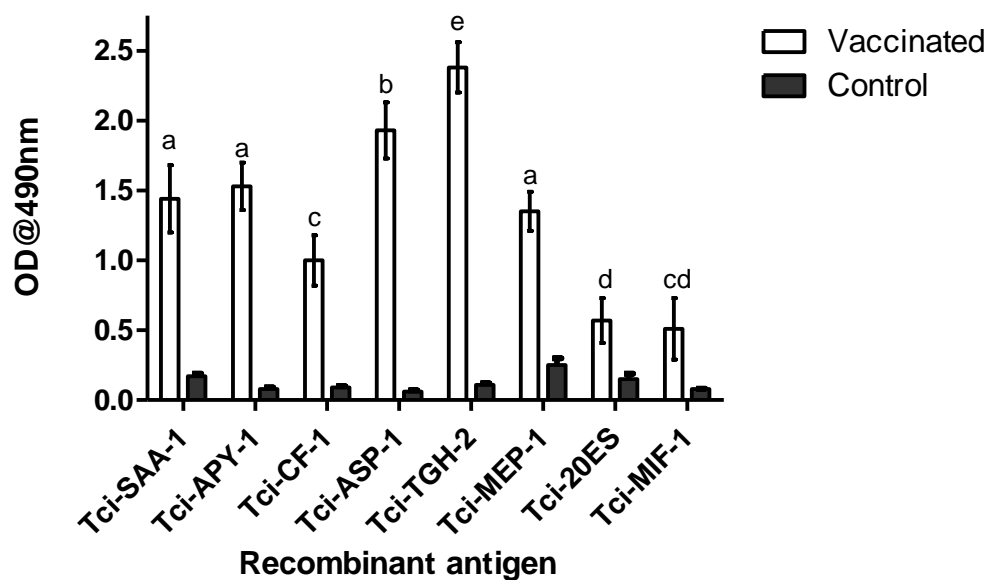
B



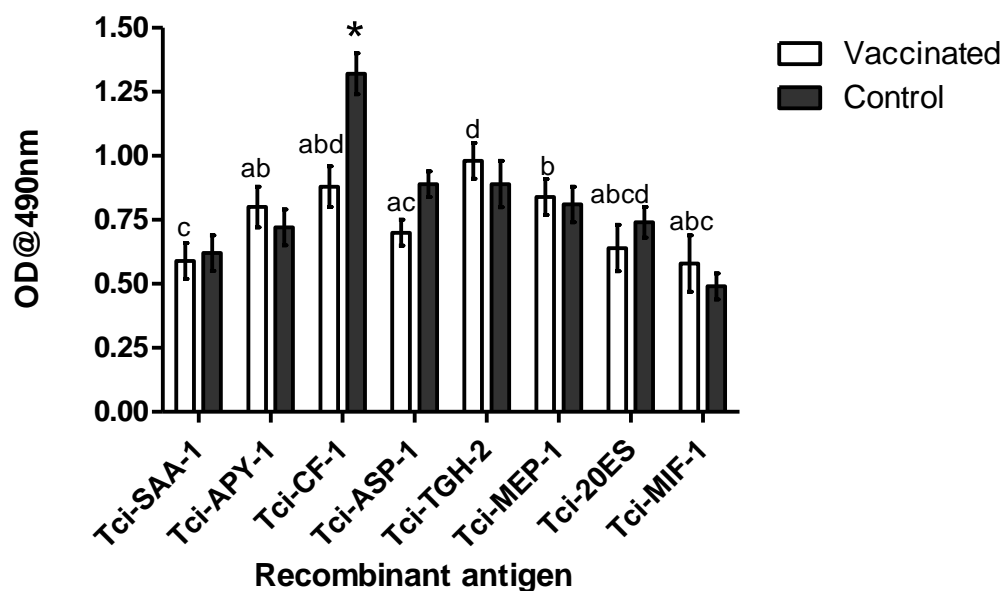
Data shown are for all ewes (Panel A) and the subset which demonstrated a periparturient relaxation in immunity (Panel B). Bars are means \pm SEM.

Fig. 4. Antigen-specific colostrum antibody levels in ewes vaccinated with the *Teladorsagia circumcincta* recombinant antigen cocktail and in control (adjuvant-only) ewes.

A



B



Panel A represents IgG and Panel B represents IgA. Each bar represents the mean normalized level \pm SEM (n=11 for vaccinated, n = 16 for control). Bars of vaccinated groups annotated with the same letter mean that the corresponding antigen-specific antibody levels are not statistically significantly different. In the control ewes the values for Tci-CF-1-specific IgA were statistically significantly (maximum $p < 0.008$) higher than those for the other antigens, marked with an asterisk.