Veterinary Parasitology 306 (2022) 109722

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Differences in the protection elicited by a recombinant *Teladorsagia circumcincta* vaccine in weaned lambs of two Canarian sheep breeds

Tara Pérez-Hernández [a], Yolanda Corripio-Miyar [b], Julia N. Hernández [a,*], Cynthia Machín [a], Yania Paz-Sánchez [a], Adam D. Hayward [b], Harry W. Wright [b], Daniel R.G. Price [b], Jacqueline B. Matthews [c], Tom N. McNeilly [b], Alasdair J. Nisbet [b], Jorge F. González [a]

[a] *Instituto Universitario Sanidad Animal y Seguridad Alimentaria, Facultad de Veterinaria, Universidad de Las Palmas de Gran Canaria, Spain*
[b] *Moredun Research Institute, Edinburgh, UK*
[c] *Roslin Technologies, Edinburgh, UK*

ARTICLE INFO

Keywords:
Recombinant vaccine
Teladorsagia circumcincta
Sheep
Immunology
Genetic resistance
Weaner lambs

ABSTRACT

Gastrointestinal nematode (GIN) infections are a serious drawback on small ruminant production. Since anthelmintic resistance has extended, optimisation of alternative non-chemical control strategies has attracted interest. Recently, a prototype recombinant vaccine protected immunologically mature sheep from Texel-cross and Canaria Sheep breeds against *Teladorsagia circumcincta*. The level of protective immunity stimulated by the vaccine varied between individuals and with age. Previous studies suggest that Canaria Hair Breed (CHB) sheep is naturally resistant to GIN infection, with some evidence suggesting that this protection is present in young lambs. Here, we sought to enhance this resistance by immunising three-month-old CHB lambs with a *T. circumcincta* prototype recombinant vaccine. Following vaccination and a larval challenge period, levels of protection against *T. circumcincta* infection were compared in CHB lambs with Canaria Sheep (CS) lambs (a breed considered less resistant to GIN). Lambs from the resistant CHB breed appeared to respond more favourably to vaccination, shedding 63% fewer eggs over the sampling period than unvaccinated CHB lambs. No protection was evident in CS vaccinated lambs. At post-mortem, CHB vaccine recipients had a 68% reduction in mean total worm burden, and female worms were significantly shorter and contained fewer eggs *in utero* compared to unvaccinated CHB lambs. A higher anti-parasite IgG_2 level was detected in immunised CHB lambs compared to unvaccinated control CHB animals, with data suggesting that IgA, globular leucocytes, $CD45RA^+$, $CD4^+$ and $CD8^+$ T cells are implicated in this protective response. The development of effective immunity in vaccinated CHB lambs did not reduce lamb growth rate as immunised CHB lambs had a significantly higher average daily weight gain after challenge than their unvaccinated counterparts. Therefore, the protection of CHB lambs was enhanced by immunisation at weaning, suggesting a synergistic effect when combining vaccination with presumed genetic resistance.

Abbreviations: ADG, Average daily gain; ALN, Abomasal Lymph Node; b.w, body weight; CHB, Canaria Hair Breed; CS, Canaria Sheep; ELISA, Enzyme-linked immunosorbent assay; EPG, Eggs/g of faeces; FEC, Faecal egg counts; GIN, gastrointestinal nematodes; IFN-γ, Interferon gamma; Ig, Immunoglobulin; IL-4, Interleukin 4; IL-17A, Interleukin 17A; L3, third stage larvae; L4, fourth stage larvae; M, molar; MHC-II, Major Histocompatibility Complex II; ODI, Optical Density Index; PBS, phosphate buffered saline; SEM, standard error of the mean; SI, Stimulation Index; *T. circumcincta*, *Teladorsagia circumcincta*; Tci-APY-1, calcium-dependent apyrase-1; Tci-ASP-1, activation-associated secretory protein-1; Tci-CF-1, cathepsin F-1; Tci-ES20, 20 kDa protein of unknown function; Tci-MEP-1, astacin-like metalloproteinase-1; Tci-MIF-1, macrophage migration inhibitory factor-1; Tci-SAA-1, an homologue of a protective antigen from *Ancylostoma caninum*; Tci-TGH-2, TGF homologue.

* Correspondence to: Instituto Universitario Sanidad Animal y Seguridad Alimentaria, Facultad de Veterinaria, Universidad de Las Palmas de Gran Canaria, 35413 Arucas, Spain.

E-mail addresses: tara.perez@ulpgc.es (T. Pérez-Hernández), yolanda.corripio-miyar@moredun.ac.uk (Y. Corripio-Miyar), julia.hernandez@ulpgc.es (J.N. Hernández), cynthia.machin@ulpgc.es (C. Machín), yania.paz102@alu.ulpgc.es (Y. Paz-Sánchez), adam.hayward@moredun.ac.uk (A.D. Hayward), harrywright@hotmail.com (H.W. Wright), dan.price@moredun.ac.uk (D.R.G. Price), jacqui.matthews@roslintech.com (J.B. Matthews), tom.mcneilly@moredun.ac.uk (T.N. McNeilly), alasdair.nisbet@moredun.ac.uk (A.J. Nisbet), jorgefrancisco.gonzalez@ulpgc.es (J.F. González).

https://doi.org/10.1016/j.vetpar.2022.109722
Received 1 February 2022; Received in revised form 11 May 2022; Accepted 12 May 2022
Available online 16 May 2022

1. Introduction

Within sheep flocks, lambs are the most susceptible population to gastrointestinal nematode (GIN) infection. Generally, they are exposed to third stage larvae (L3) contaminated pasture as soon as they start grazing. However, their immune systems are still immature and therefore they are less able to control parasitic burdens. Developing a protective immune response against nematodes may require repeated contact with the parasites during the grazing season (Stear et al., 2000). Protection typically starts with a phase of hyporesponsiveness to the parasites, followed by the development of protective immunity and, finally, the expression of a mature response that prevents the development of severe disease (Greer and Hamie, 2016). Throughout the process, which has been documented as complete at around 8 months of age (Douch and Morum, 1993; Greer and Hamie, 2016), GIN can cause pathology in young individuals, resulting in economic repercussions for the sheep sector (Nieuwhof and Bishop, 2005; Lane et al., 2015; Mavrot et al., 2015). Given this, alongside the increasing emergence of anthelmintic resistance (Gilleard et al., 2021), there is a high demand for alternative methods to control GIN infections in sheep (Matthews et al., 2016).

Vaccination against GIN offers an environmentally sustainable alternative to anthelmintics but, so far, has had limited success in young lambs with reports of poor protection following immunisation in three-month-old lambs when compared to six to ten month-old animals (Kooyman et al., 2000; Vervelde et al., 2001). Indeed, it is known that younger individuals do not show evidence of immunological recognition of the parasitic antigens (McClure et al., 1998) and display weaker responses to vaccination (Winter et al., 2000; Vervelde et al., 2001), mostly ascribed to difficulties mounting secondary immune responses (McClure, 2000; Greer and Hamie, 2016; Britton et al., 2020). However, several GIN-resistant sheep breeds have shown a natural ability to control these worms at a young age (Bahirathan et al., 1996; Gruner et al., 2003; Rocha et al., 2005).

A prototype recombinant sub-unit *T. circumcincta* vaccine has been described as protective in Texel-cross lambs and in Texel-cross ewes around the periparturient period (Nisbet et al., 2013, 2016a, 2016b). It has also proven effective in 6-month-old Canaria Sheep (CS) breed, where immunisation reduced worm length and numbers of worm eggs *in utero* in vaccinates (González et al., 2019). Serum IgA, IgG_2 and abomasal globule leucocytes and $CD4^+$ T cells may be underpinning this effect (Machín et al., 2021). However, in Canaria Hair Breed (CHB) immunisation of 6-month-old sheep did not protect vaccinates, suggesting the high level of inherent resistance in CHB lambs by this age could be masking the effect of vaccination (González et al., 2019). This raises the question whether the vaccine could be protective in younger lambs of these breeds. Therefore, here we evaluated the effect of this vaccine in three-month-old CHB and CS lambs. Aspects of the immune response generated by vaccination as well as effects on lamb performance were also assessed in this study.

2. Materials and methods

2.1. Animals

The immunisation protocol was described in detail by Nisbet et al. (2013) and González et al., (2019). Three-month-old CHB (N = 24) and CS (N = 24) lambs were purchased from several farms located in Gran Canaria. Animals were treated on arrival with fenbendazole (Panacur 2.5©, Intervet, France) at the dosage recommended by the manufacturer (0.2 ml/kg b.w.) and were kept worm-free until the beginning of the trial. Lambs were fed with forage, feed and water ad libitum throughout the experiment. Within breed, animals were randomly assigned to vaccinated (CHB-VAC, CS-VAC) or control groups (CHB-Control, CS-Control), with each group containing 12 animals (N = 12). During the experiment, one animal from group CHB-VAC was euthanised for reasons not related to the experimental procedure. At the end of the trial, another animal within this group was removed due to suspected hormonal imbalance since its physical development was abnormally low (weighing 8 kg compared to the mean 26 kg of the rest of CHB lambs).

The experiment was designed according to the Spanish Legislation (RD 53/2013), approved by the Animal Welfare Ethics Committee of the Universidad de Las Palmas de Gran Canaria (OEBA_ULPGC_003_2014) and subsequently ratified by the local competent authority. Local granulomas were detected in the injection site in several lambs. They were frequently examined by the designated veterinarian until they subsided without systemic consequences.

2.2. Experimental design

The vaccine prototype, described previously by Nisbet et al. (2013), consisted of a cocktail of 8 recombinant proteins (Tci-APY-1, Tci-ASP-1, Tci-CF-1, Tci-ES20, Tci-MEP-1, Tci-MIF-1, Tci-SAA-1 Tci-TGH-2). Tci--MEP-1 was formulated with 2 M urea in phosphate buffered saline (PBS) with 5 mg Quil A (Vax Saponin, Guinness Chemical Products Ltd) while the rest of the antigens were administered as a mixture in a single injection with 5 mg of the adjuvant in PBS. The cocktail containing 50 μg of each protein was administered three times, three weeks apart, on days 0, 21 and 42 of the experiment (Nisbet et al., 2013) in two separate sites in the anterior axilla. Concurrently, the control groups received three immunisations using the same proportions and quantities of PBS/Quil A and PBS-Urea/Quil A. Starting on day 42, coinciding with the final immunisation, sheep were trickle infected orally with 2000 L3 of *T. circumcincta* (MTci2 strain, Weybridge, UK) three times a week, for four weeks (Nisbet et al., 2013).

2.3. Parasitology and performance evaluation

The protocol described in González et al. (2019) was followed. Briefly, rectal faecal samples were collected from the animals three times a week starting 14 days after the start of the L3 challenge period (i.e., day 56) until day 79. At the end of the experiment (day 82–85) animals were euthanised to perform post-mortem sampling of abomasal contents. Strongyle faecal egg counts (FEC) (expressed as eggs/g, EPG), worm burden, female worm length and its eggs *in utero* were determined using standard techniques (MAFF, 1989; González et al., 2019).

Lambs were weighed on days 8, 22, 62 and 77 of the experiment using a livestock scale (KERN EOS 150K50XL, KERN AND SOHN GmbH, Baligen, Germany). The average daily gain (ADG) was calculated for a period during the pre-challenge period (from day 8–22, ADG pre-infection) as well as for a period during the post-challenge period (from day 62–77, ADG post-infection).

2.4. Enzyme-linked immunosorbent assay (ELISA)

Animals were bled from the jugular vein five days before post-mortem (day 77) (Machín et al., 2021). Antibody ELISA were carried out on serum samples (1:200) to detect serum IgA (1:8000), IgG_1 and IgG_2 (1:1000) levels specific to native somatic L3, L4 and adult *T. circumcincta* antigens (5 μg/mg) (Nisbet et al., 2010; Hernández et al., 2016; Machín et al., 2021). Further details of this protocol have been thoroughly described in Machín et al. (2021). Optical densities were transformed using the optical density index (ODI) described by Strain and Stear (2001). One point zero was added to ODI values to the to prevent negative data from interfering with the statistical analysis.

2.5. Lymphocyte stimulation assays

Abomasal lymph nodes (ALN) were collected at post-mortem to obtain single cell suspensions. Lymphocyte stimulation assays were carried out by incubating ALN cells with L4 or adult *T. circumcincta* somatic antigen (5 μg/ml) according to the previously described method

(Machín et al., 2021). Negative (ALN+PBS) and positive (ALN+Concavalin A) controls were run in parallel to the samples. Proliferation was measured by the incorporation of methyl-^{3}H thymidine during the final 18 h of culture and expressed as Stimulation Index (SI) by dividing the proliferation of samples incubated with *T. circumcincta* antigen by that from PBS controls.

2.6. Cytokine ELISA

Stimulated ALN were examined through capture ELISAs to evaluate the antigen specific secretion of interferon (IFN)-γ, interleukin (IL)− 4 and IL-17A as seen in Machín et al. (2021). To quantify the cytokines of interest, samples were diluted 1:2 for IFN-γ or undiluted for IL-4 and IL-17A. All values were blank corrected, and concentrations determined from the standard curves included in all plates (Machín et al., 2021).

2.7. Phenotyping of ALN cells by flow cytometry

Single colour flow cytometry was carried out in resuscitated ALN cells using the monoclonal antibodies at pre-optimised concentrations using a MACSQuant® Analyzer 10 (Miltenyi Biotech, Germany) and were analysed using FlowJo vX for Windows 7 (Machín et al., 2021).

2.8. Histology and immunohistochemistry

Two abomasal tissue samples of the anthropiloric region were sampled to perform histological and immunohistochemical staining following previous protocols (Balic et al., 2000a; González et al., 2011; Machín et al., 2021). For each individual histological sample, cells were counted in 30 fields of 0.049 mm^2 at 400x magnification (40x) (Motic BA310E) and expressed as cells/mm^2. Eosinophils and mast cells were counted in fields adjacent to the lamina propria while globule leucocytes were counted in the luminal margin of the mucosa. In the immunohistochemistry-stained samples, positively stained cells were counted in 20 fields of 0.06 mm^2 (CD4$^+$ using Olympus CX31) or 0.049 mm^2 (CD8$^+$, γδ$^+$, CD45RA$^+$ and MHC-II$^+$ using Motic BA310E) located next to the lamina propria and luminal margin of the mucosa at 400x magnification (40x).

2.9. Statistical analysis

Statistical analysis was performed with the IBM SPSS Statistics 24.0 programme and the R version 4.0.2 (R Core Team, 2021) and figures were also produced using the R software. FEC, cumulative FEC, worm burden, length and eggs *in utero*, immunoglobulins, mean abomasal cell counts, proliferation, cytokine data and flow cytometry data were transformed, analysed and correlated as specified in Machín et al.

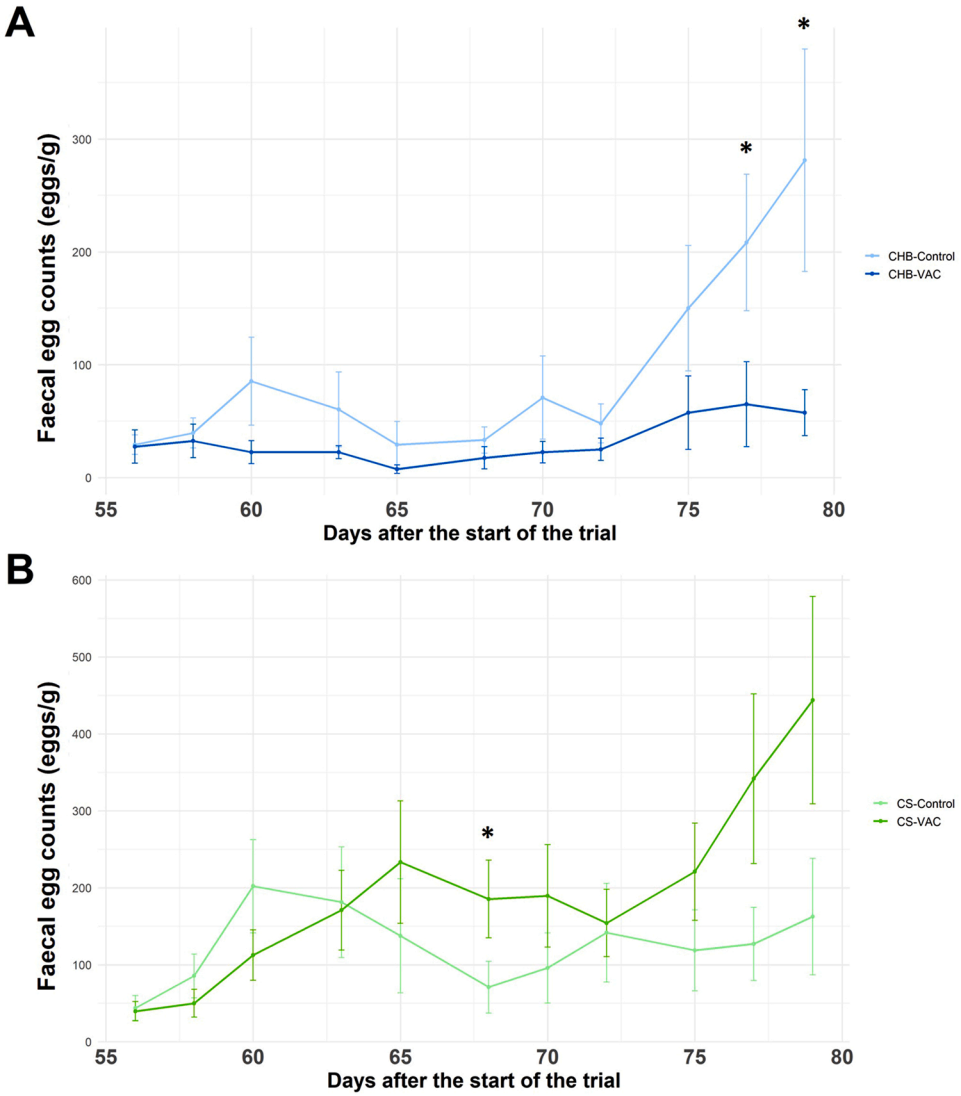

Fig. 1. Faecal egg counts after parasite challenge in two Canarian sheep breeds vaccinated against *Teladorsagia circumcincta*. Mean FEC ± SEM are shown for Canaria Hair Breed (CHB) (Panel A) and Canaria Sheep (CS) (Panel B) trickle infected with *T. circumcincta* L3 after the final vaccination. Vaccinated ("VAC") groups are represented by dark lines; adjuvant-recipient only ("Control") groups are represented by lighter lines. Asterisks indicate statistically significant differences ($p < 0.05$) between groups for a specific time-point.

(2021). Weight data were tested for normality with the Shapiro and Wilk test and were compared through general linear model. Pre- and post-infection ADG values were analysed using the non-parametric Mann-Whitney *U* test. Least Significance Difference test was used as reference. Probabilities with p value < 0.05 were considered statistically significant.

3. Results

3.1. Parasitological and performance data

Lambs from all groups began to shed *T. circumcincta* eggs by day 14 after challenge (day 56 after the first vaccination) (Fig. 1A-B). Within the CHB lamb group, FEC was lower in vaccinates than in controls throughout the experiment, reaching statistical significance on days 77 and 79 of the experiment ($p < 0.05$) (Fig. 1 A). Both vaccinated and unvaccinated CS lambs showed similar levels of egg shedding after challenge, except on day 68 when vaccinates excreted significantly more eggs than controls ($p < 0.05$) (Fig. 1B). Mean ± standard error of the mean (SEM) cumulative FECs in CHB were 711 (± 254) EPG in vaccinates and 1953 (± 632) EPG in the control group, representing an overall 63% reduction in egg excretion in vaccinated CHB lambs ($p < 0.05$). In CS, mean cumulative FECs were 4278 (± 1037) EPG in vaccinated and 2905 (± 1088) EPG in the control group. When comparing between breeds, CHB vaccinates shed significantly fewer eggs than CS vaccinates and controls ($p < 0.05$).

At post-mortem, CHB-VAC lambs harboured mean worm counts of 1147 (± 374) compared with 3587 (± 817) in CHB-Control lambs ($p < 0.05$). The adult female worms from CHB-VAC lambs were shorter than those from CHB-Control lambs (7.75 ± 0.1 mm and 8.31 ± 0.08 mm, respectively) ($p < 0.01$) and less prolific (11 ± 0.48 eggs *in utero* in CHB-VAC as opposed to 17 ± 0.1 in CHB-Control lambs) ($p < 0.01$). This represents a 62%, 70% and 36% reduction in worm burden, length and eggs *in utero*, respectively (Figs. 2A, 2B, 2C). Worm burden levels at post-mortem between CS vaccinates (4613 ± 652) and controls (2900 ± 644) were not statistically different ($p = 0.163$), and neither were female length (7.95 ± 0.07 vs 7.99 ± 0.07) ($p = 0.190$) or numbers of eggs *in utero* (15 ± 0.6 vs 14 ± 0.6) ($p = 0.506$) (Figs. 2A, 2B, 2C). When comparing vaccinated groups, significantly fewer worms were recovered from CHB vaccinates than CS vaccinates ($p < 0.01$) and the levels of stunting and reduction in eggs *in utero* were also significantly different ($p < 0.01$) between these groups. However, between control CHB and CS groups, statistical analysis did not find differences in worm burden ($p = 0.509$), worm length ($p = 0.530$) or eggs *in utero* ($p = 0.217$).

When comparing lamb growth during pre- and post-infection periods within breed, ADG was significantly higher in vaccinated CHB lambs than in CHB-Controls after challenge had begun ($p < 0.05$) (Fig. 3A). CS groups showed comparable ADG during both pre- and post-challenge periods (Fig. 3B).

3.2. Humoral immune response

Immunoglobulin G_2 isotype levels against L3 antigen were significantly higher in CS control groups than in CHB control groups ($p < 0.05$) (Table 1). Similarly, the same isotype against L4 *T. circumcincta* antigen was statistically elevated in vaccinated CHB lambs when comparing with the other groups ($p < 0.05$) (Table 2). IgA levels against the L4 antigen were significantly higher in control CHB than in vaccinated and control CS groups ($p < 0.05$) (Table 2). Immunoglobulin production against the adult antigen was not statistically significantly different between groups for any of the isotypes studied (Table 3).

A negative correlation between serum levels of IgA against L3 antigen and worm length was observed only in CHB vaccinated group, while the association was positive with cumulative FEC (Table 1). In addition, in sera from CS vaccinated lambs, a negative association between specific L4 serum IgG_1 against cumulative FEC was observed (Table 2). A positive correlation between IgG_1 against L4 antigen and eggs *in utero* was shown in CHB vaccinated group. Only IgG_2 against L4 *T. circumcincta* antigen was negatively and significantly associated with eggs *in utero* in CHB control group (Table 1). No statistically significant correlations were detected between immunoglobulin levels against the adult antigen and parasitological parameters (Table 3).

3.3. Cellular immune response

Proliferation of ALN cells collected at post-mortem was higher following stimulation with somatic antigens from adult when compared to L4 stages of *T. circumcincta* (L4 relative to adult estimate = −1.27 ± 0.25SE, F = 25.71, $p < 0.001$). Meanwhile, there were no effects of breed, vaccination, or any of the interactions between the three variables. Supernatants from stimulated cultures were collected to analyse IL-4 and IFN-γ secretion. Generally low levels of IFN-γ were detected in all groups, but there was a statistically supported effect of vaccination status, with vaccinated animals showing lower IFN-γ secretion (estimate = −0.81 ± 0.26SE, F = 10.02, $p = 0.002$). There was, however, no support for effects of breed or antigen, or any interactions between the variables. IL-4 secretion was influenced by both vaccine and antigen treatments, with lower IL-4 production in vaccinated animals (estimate = −1.14 ± 0.49SE, F = 5.46, $p = 0.021$) and in cells treated with media compared to adult and L4 antigens (estimate for media versus adult antigen = −2.23 ± 0.60SE; overall effect of antigen F = 7.71, $p < 0.001$). There were, however, no effects of breed, or any of the interactions. In general, there were no significant differences between breeds or vaccine treatment in the cell markers studied, except for a lower percentage of WC1 cells in the CS breed (F = 6.31, $p = 0.016$; Fig. 4).

Several cell populations were identified and enumerated in samples from the abomasal wall of the lambs (Table 4). Eosinophil counts were significantly higher in vaccinated CHB lambs than in vaccinated CS lambs and $CD4^+$ cell counts were higher in vaccinated CHB than in CHB controls ($p < 0.05$). $CD45RA^+$ cell counts were significantly higher in CHB-Control lambs in comparison with the other three experimental groups ($p < 0.05$). The other cell populations studied did not show significant differences in their counts between the four experimental groups.

Correlation studies between abomasal cell populations and parasitological parameters showed negative associations between the numbers of some of these cells and several parasitological parameters (Table 4). Eosinophil counts were negatively correlated with cumulative FEC, worm burden and fecundity in the CHB-Control lambs. Globule leucocyte counts were negatively associated with numbers of eggs *in utero* in both CHB groups and in vaccinated CS lambs. Mast cell numbers were negatively associated with parasitological variables in the CS groups only. $CD4^+$ cell numbers were significantly, and negatively, correlated with adult worm burden in vaccinated CHB lambs. Similarly, $CD8^+$ cell numbers were negatively associated with adult worm burden and cumulative FEC in vaccinated CHB lambs only. $CD45RA^+$ cell numbers were also negatively correlated with parasitological variables in both vaccinated groups. MHC-II^+ stained cell numbers were negatively associated with egg excretion and fecundity in both CHB groups but not in CS groups (Fig. 5).

4. Discussion

This study demonstrated that CHB lambs were protected against *T. circumcincta* through immunisation with a prototype recombinant subunit vaccine. Vaccinated CHB lambs were able to control egg excretion, worm burden, length of female worms and egg levels *in utero*. Anti-parasite IgA and IgG_2 levels and the presence of globular leucocytes, $CD45RA^+$, $CD4^+$ and $CD8^+$ T cells in abomasal tissue appears to be associated with this response. In contrast, no evidence of protection

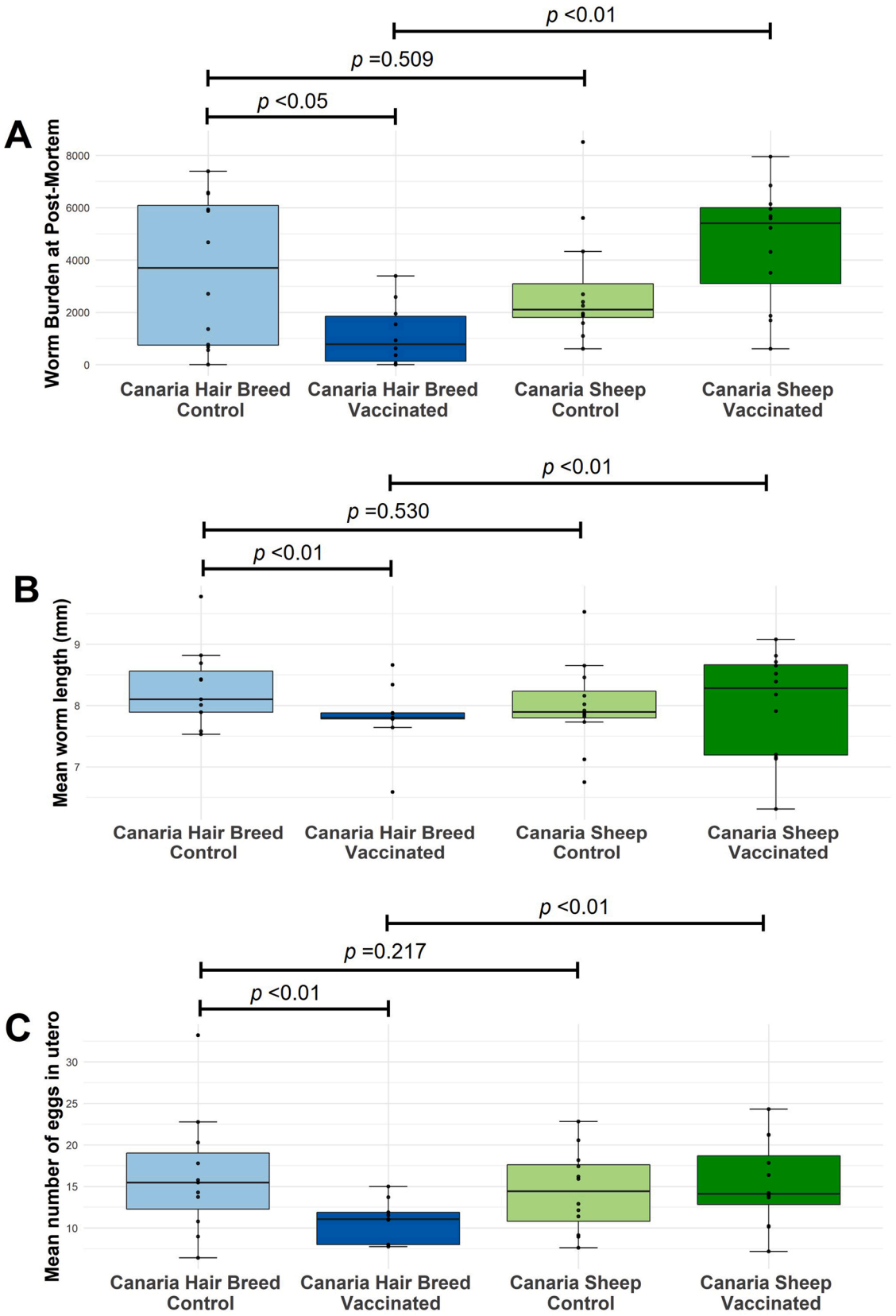

Fig. 2. Effects of immunisation against *Teladorsagia circumcincta* in two Canarian sheep breeds on worm establishment, length and fecundity. Mean worm burden (Panel A), worm length (Panel B) and eggs *in utero* (Panel C) ± SEM are shown for sheep trickle infected with *T. circumcincta* L3 after the final vaccination. Vaccinated ("VAC") groups are represented by dark boxplots; adjuvant-recipient only ("Control") groups are represented by lighter boxplots.

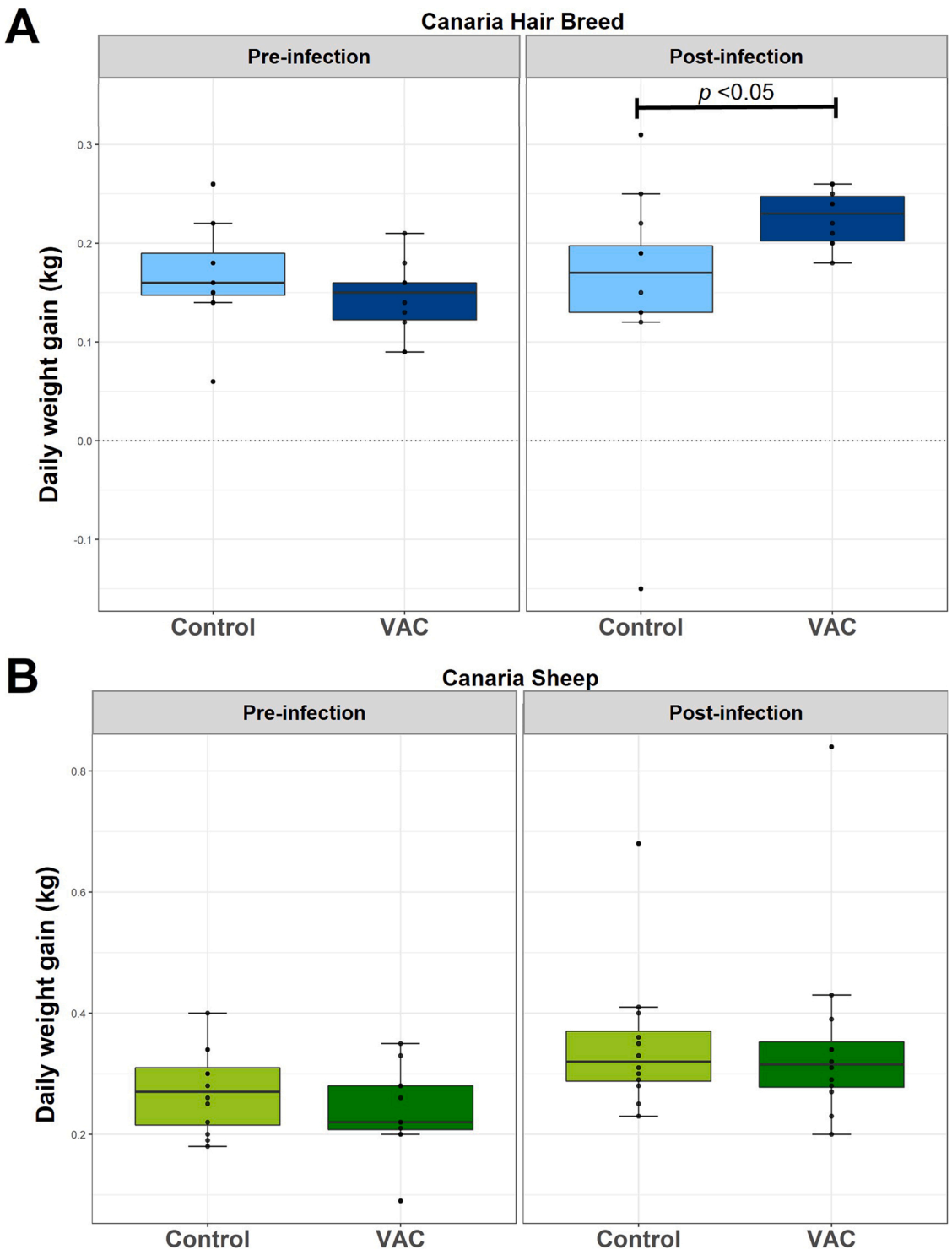

Fig. 3. Effects of immunisation against *Teladorsagia circumcincta* in two Canarian sheep breeds on production performance. Daily weight gain ± SEM for Canaria Hair Breed (CHB) (Panel A) and Canaria Sheep (CS) (Panel B) are shown before and after trickle infection with *T. circumcincta* L3 after the final vaccination. Vaccinated ("VAC") groups are represented by dark boxplots; adjuvant-recipient only ("Control") groups are represented by lighter boxplots.

was determined in CS vaccinated lambs.

Within the flock, young lambs are more likely to suffer severe GIN infections, partly because at the time they start grazing and contacting with parasites their immune system may be relatively immature and therefore less able to control the nematode burden (Manton et al., 1962; Smith et al., 1985; Barger, 1988; Colditz et al., 1996; McClure et al., 1998; Vervelde et al., 2001). Consequently, by vaccinating lambs against GIN at an early age, young animals could be better protected against larval challenge and overcome the production effect of parasitism, preserving animal health and welfare, all of which are the priority goals of immunisation. However, a lack of response to immunisation has been recurrently observed with different vaccine prototypes against several GIN in young lambs (Bitakaramire, 1966; Urquhart et al., 1966; Smith and Angus, 1980; Smith et al., 1985; Kooyman et al., 2000; Vervelde et al., 2001; Nisbet et al., 2016b). An exception to this paradigm is when hidden antigens, such as H11 and

Table 1
Levels of serum immunoglobulins against L3 *Teladorsagia circumcincta* antigen in Canaria Hair Breed (CHB) and Canaria Sheep (CS) after challenge and vaccination against *T. circumcincta* and correlation with parasitological variables. IgA, IgG_1 and IgG_2 levels against L3 antigen are shown as mean of Optical Density Index (ODI) ± SEM.

L3			Correlation			
Isotype	Group	Mean ODI ± SEM	Cumulative FEC	Worm burden	Worm length	Eggs in utero
IgA	CHB-VAC	1.459 ± 0.126[a]	0.659*	0.491	-0.667*	-0.517
	CHB-Control	1.546 ± 0.189[a]	0.119	-0.140	-0.100	-0.318
	CS-VAC	1.867 ± 0.184[a]	-0.196	-0.308	0.014	-0.084
	CS-Control	1.899 ± 0.159[a]	0.071	-0.056	-0.105	-0.371
IgG_1	CHB-VAC	1.200 ± 0.101[a]	0.207	0.200	-0.05	0.15
	CHB-Control	1.078 ± 0.043[a]	-0.274	-0.539	-0.405	-0.597
	CS-VAC	1.175 ± 0.089[a]	-0.284	-0.175	0.147	-0.046
	CS-Control	1.265 ± 0.012[a]	-0.418	-0.434	-0.189	-0.301
IgG_2	CHB-VAC	1.212 ± 0.079[ab]	0.507	0.432	-0.126	-0.017
	CHB-Control	1.097 ± 0.042[a]	0.042	0.091	-0.023	-0.191
	CS-VAC	1.278 ± 0.120[ab]	-0.305	-0.392	0.231	-0.056
	CS-Control	1.413 ± 0.138[b]	-0.426	-0.315	-0.224	-0.203

Statistically significantly differences ($p < 0.05$) between groups for a specific isotype and antigen are represented with different letters. Significant correlations are represented with "*" at $p < 0.05$.

Table 2
Levels of serum immunoglobulins against L4 *Teladorsagia circumcincta* antigen in Canaria Hair Breed (CHB) and Canaria Sheep (CS) after challenge and vaccination against *T. circumcincta* and correlation with parasitological variables. IgA, IgG_1 and IgG2 levels against L4 antigen are shown as mean of Optical Density Index (ODI) ± SEM.

L4			Correlation			
Isotype	Group	Mean ODI ± SEM	Cumulative FEC	Worm burden	Worm length	Eggs in utero
IgA	CHB-VAC	1.393 ± 0.106[ab]	0.056	-0.285	0.050	-0.200
	CHB-Control	1.639 ± 0.205[a]	0.221	0.126	-0.027	-0.391
	CS-VAC	1.239 ± 0.063[b]	-0.100	0.361	0.168	0.312
	CS-Control	1.162 ± 0.071[b]	-0.262	-0.392	0.140	-0.147
IgG_1	CHB-VAC	1.322 ± 0.082[a]	-0.270	-0.018	0.500	0.767*
	CHB-Control	1.158 ± 0.023[a]	-0.544	-0.501	-0.068	-0.314
	CS-VAC	1.324 ± 0.091[a]	-0.725**	-0.531	-0.126	-0.406
	CS-Control	1.206 ± 0.089[a]	0.121	0.140	0.21	-0.140
IgG_2	CHB-VAC	1.37 ± 0.085[a]	0.145	0.036	0.117	0.109
	CHB-Control	1.042 ± 0.026[b]	-0.357	-0.406	-0.573	-0.682*
	CS-VAC	1.053 ± 0.081[b]	-0.557	-0.483	-0.049	-0.329
	CS-Control	0.980 ± 0.112[b]	0.504	0.615	0.566	0.238

Statistically significantly differences ($p < 0.05$) between groups for a specific isotype and antigen are represented with different letters. Significant correlations are represented with "*" at $p < 0.05$.

Table 3
Levels of serum immunoglobulins against adult *Teladorsagia circumcincta* antigen in Canaria Hair Breed (CHB) and Canaria Sheep (CS) after challenge and vaccination against *T. circumcincta* and correlation with parasitological variables. IgA, IgG_1 and IgG_2 levels against adult antigen are shown as mean of Optical Density Index (ODI) ± SEM.

AD			Correlation			
Isotype	Group	Mean ODI ± SEM	Cumulative FEC	Worm burden	Worm length	Eggs in utero
IgA	CHB-VAC	1.252 ± 0.059[a]	0.496	0.455	-0.217	-0.050
	CHB-Control	1.284 ± 0.130[a]	0.123	-0.133	-0.451	-0.556
	CS-VAC	1.330 ± 0.081[a]	-0.381	-0.217	-0.200	-0.214
	CS-Control	1.307 ± 0.057[a]	-0.366	-0.347	-0.098	-0.060
IgG_1	CHB-VAC	1.078 ± 0.040[a]	0.370	0.539	-0.083	0.350
	CHB-Control	1.050 ± 0.031[a]	-0.118	-0.462	-0.159	-0.369
	CS-VAC	1.091 ± 0.038[a]	-0.473	-0.490	-0.091	-0.357
	CS-Control	1.068 ± 0.042[a]	-0.007	0.056	0.049	-0.385
IgG_2	CHB-VAC	1.029 ± 0.014[a]	0.527	0.576	-0.317	-0.300
	CHB-Control	1.018 ± 0.011[a]	0.119	-0.133	-0.255	-0.045
	CS-VAC	1.020 ± 0.018[a]	-0.423	-0.119	-0.249	-0.322
	CS-Control	1.038 ± 0.035[a]	-0.203	-0.056	-0.200	-0.396

Statistically significantly differences ($p < 0.05$) between groups for a specific isotype and antigen are represented with different letters. Significant correlations are represented with "*" at $p < 0.05$.

H-gal-GP from *H. contortus* have been used in vaccines, inducing high levels of circulating IgG in a range of ages of lambs (Broomfield et al., 2020; Kebeta et al., 2021). However, as this type of antigens are normally not exposed to the immune system, a natural boost from exposure to the parasites in the field does not occur (Kooyman et al., 2000). Hence, lambs need around 3 priming doses and booster vaccinations every 6 weeks to maintain acceptable levels of protection (Kebeta et al., 2020).

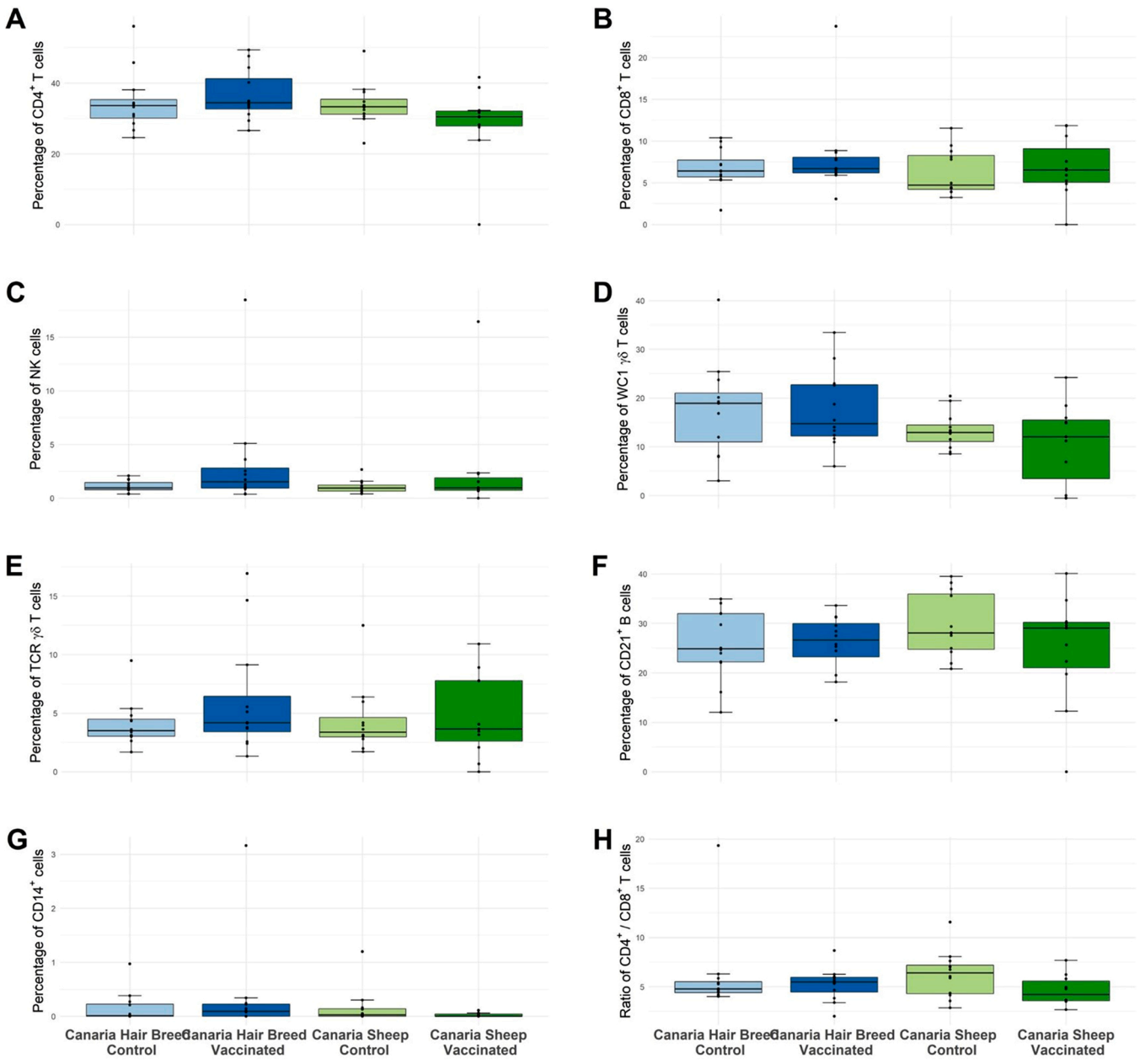

Fig. 4. Phenotypic profile of abomasal lymph node cells in two Canarian sheep breeds after challenge and vaccination against *Teladorsagia circumcincta*. The expression of $CD4^+$, $CD8^+$ and $\gamma\delta^+$ T cells, NK, $CD21^+$ B cells and $CD14^+$ (monocytes and macrophages) cells is represented. Results are shown as boxplots with IQR ± SEM. Vaccinated ("VAC") groups are represented by dark boxplots; adjuvant-recipient only ("Control") groups are represented by lighter boxplots.

The age of lambs can be key when it comes to vaccine efficacy, as low responses observed in young lambs to some immunogens can be due to the animals not being fully immunocompetent. In which case, vaccination can be impacted by the inability of an immature immune system to translate vaccination into effective immunity. Young lambs develop weak adaptive immune responses (Manton et al., 1962; Smith et al., 1985; Barger, 1988; Colditz et al., 1996; McClure et al., 1998; Vervelde et al., 2001) with low $CD4^+$ and $CD8^+$ counts and immunoglobulin levels (Watson et al., 1994; Colditz et al., 1996), although populations of γδ T cells and B cells in peripheral blood are similar to mature sheep (Colditz et al., 1996). This suggests that while there is an adequate pool of B cells in young lambs, a relative deficiency in T cell help may result in less efficient induction of adaptive immune responses and, consequently, protection would be negatively impacted. This would be consistent with our recent study of the abomasal transcriptome of Texel-cross lambs immunised with the same *T. circumcincta* vaccine used in this study, in which protective immune pathways associated with T cell activation and polarisation were present in 6-month but not 3-month-old lambs (Liu et al., 2021). Likewise, great individual and inter-breed variation in immunity, which may be more related with metabolic than chronological lamb age, has been observed (Greer and Hamie, 2016).

Not all sheep breeds are equally susceptible to GIN (Piedrafita et al., 2010). Several breeds have shown ability to control these worms at a relatively young age (Bahirathan et al., 1996; Gruner et al., 2003; Rocha et al., 2005) and it is possible that some breeds may respond earlier to vaccines (Piedrafita et al., 2010). The results of our study are in agreement with these reports, as protection from the impacts of GIN infection in CHB lambs, a breed previously described as having some levels of resistance to *H. contortus* (González et al., 2008) and *T. circumcincta* (González et al., 2019), was enhanced through vaccination at weaning, indicating synergy between genetic and vaccine-induced protection.

Table 4
Immune cell counts in the abomasal wall in Canaria Hair Breed (CHB) and Canaria Sheep (CS) after challenge and vaccination against *Teladorsagia circumcincta* and correlation with parasitological variables. Mean (cells/mm^2) ± SEM in abomasal tissue are shown.

			Correlation			
Cell population	Group	Mean (cells/mm^2) ± SEM	Cumulative FEC	Worm burden	Worm length	Eggs in utero
Eosinophils	CHB-VAC	119.18 ± 27.89^a	-0.075	-0.079	0.267	0.067
	CHB-Control	91.21 ± 11.73^{ab}	-0.669*	-0.580*	-0.027	-0.655*
	CS-VAC	60.15 ± 19.41^b	-0.161	-0.469	-0.042	-0.301
	CS-Control	81.92 ± 18.44^{ab}	-0.369	-0.252	0.028	-0.189
Globule Leucocytes	CHB-VAC	243.4 ± 57.08^a	0.458	0.079	-0.233	-0.767*
	CHB-Control	213.15 ± 73.68^a	-0.347	-0.217	-0.236	-0.727*
	CS-VAC	164.11 ± 58.99^a	-0.592*	-0.559	-0.385	-0.580*
	CS-Control	132.25 ± 36.68^a	-0.489	-0.238	-0.462	0.091
Mast cells	CHB-VAC	30.06 ± 8.73^a	0.634*	0.515	-0.333	-0.650
	CHB-Control	40.98 ± 12.54^a	0.151	0.273	-0.173	-0.218
	CS-VAC	40.81 ± 15.06^a	-0.744**	-0.444	-0.465	-0.479
	CS-Control	18.87 ± 4.47^a	-0.707*	-0.609*	-0.266	-0.298
CD4$^+$	CHB-VAC	69.25 ± 22.38^a	-0.376	-0.673*	0.583	0.283
	CHB-Control	26.33 ± 3.74^b	0.178	0.091	0.297	-0.006
	CS-VAC	60.87 ± 13.75^{ab}	-0.525	-0.308	-0.322	-0.217
	CS-Control	54.48 ± 12.91^{ab}	-0.170	-0.182	-0.343	0.189
CD8$^+$	CHB-VAC	109.18 ± 22.37^a	-0.857**	-0.867**	0.567	0.500
	CHB-Control	79.76 ± 31.72^a	0.039	-0.350	-0.036	0.027
	CS-VAC	68.79 ± 14.87^a	0.361	0.119	-0.063	0.098
	CS-Control	68.67 ± 11.77^a	-0.213	-0.315	-0.035	0.217
γδ$^+$	CHB-VAC	28.16 ± 6.91^a	-0.462	-0.620	0.251	0.059
	CHB-Control	13.95 ± 3.83^a	0.174	0.165	0.278	0.155
	CS-VAC	27.42 ± 9.71^a	-0.039	0.175	0.014	0.280
	CS-Control	22.15 ± 4.26^a	-0.675*	-0.480	-0.529	-0.497
CD45RA$^+$	CHB-VAC	10.45 ± 2.96^b	-0.032	-0.141	-0.770*	-0.728*
	CHB-Control	23.08 ± 5.44^a	0.186	0.301	0.282	0.045
	CS-VAC	7.31 ± 2.67^b	-0.552	-0.593*	-0.470	-0.474
	CS-Control	7.90 ± 1.88^b	-0.170	-0.056	-0.399	0.182
MHC-II$^+$	CHB-VAC	40.82 ± 21.88^a	-0.584	-0.477	0.623	0.563
	CHB-Control	21.01 ± 9.88^a	-0.758**	-0.547	-0.205	-0.683*
	CS-VAC	64.46 ± 22.59^a	-0.060	-0.203	0.357	-0.007
	CS-Control	44.39 ± 13.09^a	-0.277	-0.070	-0.413	0.238

Statistically significantly differences ($p < 0.05$) between groups for a specific cell population are represented with different letters. Significant correlations are represented with "*" at $p < 0.05$ and "**" at $p < 0.01$.

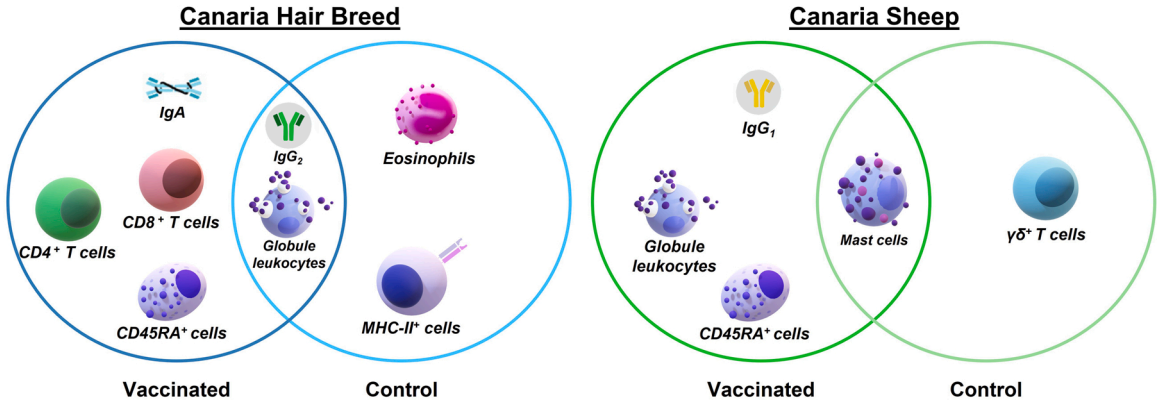

Fig. 5. Graphic representation of the possible immune mechanisms implicated in the response for Canaria Hair Breed and Canaria Sheep trickle infected with *Teladorsagia circumcincta* L3 after the final vaccination. Humoral and cellular elements associated with protection were represented within a circle for each group (dark lines for vaccinates and lighter lines for controls). Elements included in the overlapped surface between vaccinates and controls indicate it is shared by both groups.

The vaccine cocktail used had a high impact in this experimental *T. circumcincta* study in 3-month-old CHB lambs. Worm establishment, growth and fecundity were impaired in vaccinated CHB lambs. Previous vaccination trials using this vaccine in older Texel-crossbred have also shown reductions in worm number and FEC (Nisbet et al., 2013) and use of this vaccine in 6-month-old CS lambs impacted worm length and fecundity but not on worm burden (González et al., 2019). Although vaccinated 3-month-old Texel-crossbred lambs were protected in comparison to their non-vaccinated counterparts, FEC and worm burdens were higher than in older (6 month old) Texel-cross vaccinated lambs (Liu et al., 2021). Interestingly, parasitological data (FEC levels, worm counts) in 3-month-old vaccinated CHB lambs was very similar to that recorded in 6-month-old vaccinated CHB lambs and substantially lower than in vaccinated six-month-old CS lambs, when animals were exposed to an identical experimental infection (González et al., 2019).

Lambs of 3–4 months are able to develop similar immune response mechanisms to older sheep, although quantitatively, these are lower than 10–12 months lambs, at which point immunity is considered mature (Smith et al., 1985; McRae et al., 2015). Sheep immune responses against *T. circumcincta* initially impact worm length and, later,

worm burden (McRae et al., 2015). However, in the work described here, both worm length and worm burden were impacted by day 40 post-infection in vaccinated CHB lambs, suggesting a strong and effective immune response to immunisation.

Interestingly, in 6-month-old CHB lambs, vaccination did not add to the, already substantial impact of natural breed resistance to *T. circumcincta*, (González et al., 2019) (Fig. 6). However, the data presented here demonstrate that, at weaning, vaccination of CHB lambs can significantly boost natural breed resistance to control worm numbers and enhance weight gain in this breed. It is notable, however, that abomasal lymph node lymphocyte responses to parasite antigens in all lamb groups were low, with some animals not responding at all, and no differences in cell population counts were observed across groups. This still suggests certain level of immaturity in the immune response of this age of lamb, in contrast to lambs of 6 months (Machín et al., 2021).

Although lymphocytes did not proliferate in vitro, all groups produced IL-4 when they were cultured with parasitic antigens. IL-4 is a key cytokine in GIN control (Venturina et al., 2013) in addition to GL activation (McRae et al., 2015). A similar response was observed in a previous vaccination trial with CHB/CS lambs of six months of age. Local lymph node lymphocytes cultured in the presence of parasite antigens produced IL-4 independently of vaccination status or breed (Machín et al., 2021). Interestingly, lymphocytes from older lambs also produced IFN-γ, particularly in CHB (Machín et al., 2021), while only the control group of three-month-old CHB lambs produced this cytokine in the present work. Lower production of IFN-γ by blood lymphocytes has also been previously described in young lambs (Watson et al., 1994; Colditz et al., 1996).

Similarly to a previous vaccination trial with 6-month-old CS lambs, no major differences in cell populations in the abomasum between vaccinates and controls were observed, which is in contrast with trials in other breeds using vaccines for related worms such as *H. contortus* (Kooyman et al., 2000). The only exceptions were the elevated eosinophil and $CD4^+$ counts in vaccinated CHB lambs compared to vaccinated CS lambs and control CHB, respectively. Globular leucocytes have been associated with the control of *T. circumcincta* in sheep with acquired resistance (Gruner et al., 2004; McRae et al., 2015; Albuquerque et al., 2019), manifested as rapid larval rejection (Balic et al., 2000b). This cell type was negatively associated with parasitological data in the work here, independently of vaccination status or breed and a similar response was observed in older animals (Machín et al., 2021), suggesting that this response is independent of age in these breeds.

The levels of some other cell types were also negatively associated with parasitological parameters and some of these potential immune responses might be associated with breed resistance. For instance, $MHC\text{-}II^+$ cells in the abomasum were only negatively associated with parasitology in CHB controls, suggesting recruitment of antigen presenting cells is important for protection in this breed. Also, $CD4^+$ and B cells (CD45RA) were associated with protection here; other authors have described increases in these cell types with another prototype vaccine against *H. contortus* in goats (Zhao et al., 2012). The vaccine may have induced similar response in both breeds, but it was more effective in the CHB lambs and, perhaps, this difference could be unravelled with a more detailed study of the response. $CD8^+$ could be a critical cell type in protection because it was only negatively associated with worm burden and cumulative FEC in the vaccinated CHB group. $CD8^+$ cells were not associated with protection in six-month-old vaccinated CS lambs in a previous study but they were present at elevated levels in six-month-old CHB lambs (Machín et al., 2021). $CD8^+$ cells are involved in type-1 responses (Spellberg and Edwards, 2001) and, in terms of natural immunity, have been traditionally linked with susceptibility to GIN (Gill et al., 2000; Pernthaner et al., 2005; Craig et al., 2014; McRae et al., 2015), with type 2 responses associated with protection. However, in this study, $CD8^+$ cells may play a role in successful vaccination as early type 1 responses have been shown to be predictive of vaccine-induced protection in older Texel cross-bred lambs vaccinated with this *T. circumcincta* vaccine (Liu et al., 2021). In fact, recent studies have found upregulation of transcripts encoding proteins associated with type 1 response in small ruminants resistant to GIN at 35 days after challenge (Aboshady et al., 2020) and it is possible that a mixed, or sequential, type1/type 2 response could be optimal instead of the type-2-biased response, traditionally accepted as being protective. In addition, a successful vaccine incorporating E/S antigens of GIN developed both type 1 and type 2 responses (Vervelde et al., 2001).

A negative association between *T. circumcincta*-specific IgA and worm length was observed in vaccinated CHB lambs and *T. circumcincta*-specific IgG_2 may be also involved in protection because higher levels of this isotype were detected in vaccinated CHB than in the other groups. Both immunoglobulins showed similar patterns in successfully vaccinated six- month-old CS lambs (Machín et al., 2021). Besides, vaccinated cross-bred Texel lambs produced higher total IgG against *T. circumcincta* L4 ES antigens than control animals (Nisbet et al., 2013). These immunoglobulins have been considered relevant in protection of sheep against GIN infections through natural immunity (Stear et al., 1995; Pernthaner et al., 2005; McRae et al., 2015). Worm-specific IgG_2 has also been associated with protection in sheep with a vaccine against *H. contortus* (Knox et al., 2005). Using different immunogens against *H. contortus,* IgG_1 and IgA were associated with protection, and lower production was observed in younger lambs that responded less well to the vaccine (Vervelde et al., 2001). Generally, lower levels of immunoglobulins can be detected in younger lambs than in mature lambs (Smith et al., 1985; Watson and Gill, 1991; Watson et al., 1994). It is

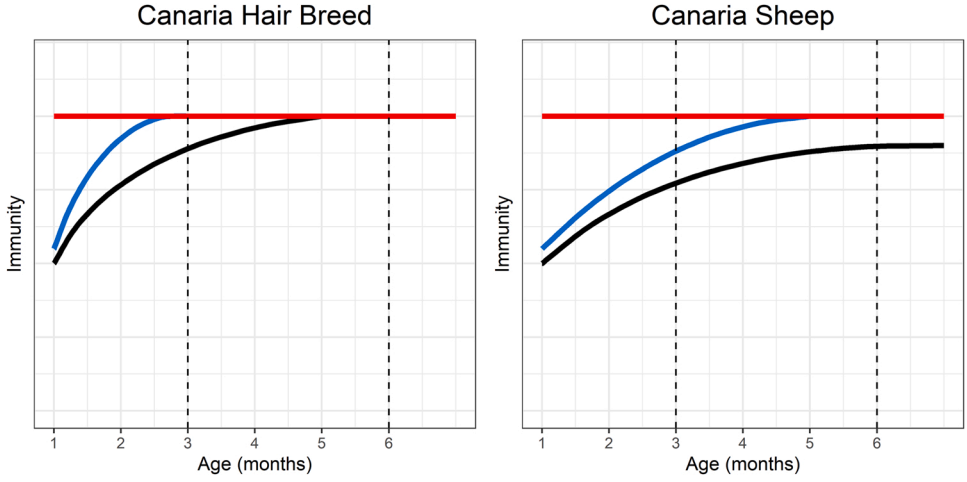

Fig. 6. Schematic representation of immunity dynamics in 3 and 6-month-old Canaria Hair Breed (CHB)(left panel) and Canaria Sheep lambs (CS)(right panel) in two vaccination trials with a prototype recombinant sub-unit *Teladorsagia circumcincta* vaccine and after challenge with *T. circumcincta*. 3-month-old CHB lambs demonstrated detectable levels of protective immunity (horizontal red line) when vaccinated (light blue line) in comparison with controls (dark blue line) whereas at 6 months the effect of immunisation was not evident. In contrast, in CS lambs, 3-month-old vaccinates (light blue line) were not protected by the vaccine when compared to controls (dark blue line) whilst vaccination proved protective by 6 months of age.

possible the CHB lambs were able to produce enough immunoglobulins at this younger age.

Finally, daily weight gain was higher in vaccinated CHB lambs than in control CHB lambs in this trial. Protection against *H. contortus* with the commercial vaccine Barbervax® -composed of two native integral membrane proteins isolated from the intestinal brush border of adult worms- has not been associated with weight gain in lambs in other trials (Kebeta et al., 2020, 2021) in contrast with what is described here. This is particularly intriguing because, even though the vaccine induced a protective immune response, it did not have (in the context of this relatively short time frame) a negative production cost. There has been concern about the "metabolic cost" of the development of a protective response against worms in young lambs due to the energy and protein required to develop an effective response (Greer, 2008; Greer and Hamie, 2016). Both negative -favourable- and positive -unfavourable- associations between lamb weight and worm burden have been observed in different trials. However, lamb growth is generally stunted while developing an immune response (Kimambo et al., 1988; Greer and Hamie, 2016). In the work presented here, the vaccine did not impair growth in the CHB lambs.

In conclusion, the combination of genetic resistance and an effective vaccine, could be a good example of integrated control strategy for *T. circumcincta*. Several immunoglobulins and cells may be involved in the effective response of 3-month-old CHB lambs. In future, a more detailed study of the response to the vaccine in lambs could unravel the most relevant mechanisms in protection to improve this vaccine prototype.

CRediT authorship contribution statement

Tara Pérez-Hernández: Formal analysis, Investigation, Writing – original draft, Visualization. **Yolanda Corripio-Miyar**: Formal analysis, Investigation, Writing – original draft, Visualization. **Julia N. Hernández**: Investigation, Writing – original draft, Supervision. **Cynthia Machín**: Investigation. **Yania Paz-Sánchez**: Investigation. **Adam D. Hayward**: Formal analysis, Writing – review & editing. **Harry W. Wright**: Investigation. **Daniel R.G. Price**: Investigation. **Jacqueline B. Matthews**: Conceptualization, Methodology, Supervision, Project administration, Funding acquisition. **Tom N. McNeilly**: Conceptualization, Methodology, Writing – review & editing, Supervision. **Alasdair J. Nisbet**: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. **Jorge F. González**: Conceptualization, Methodology, Writing – original draft, Supervision, Project administration.

Acknowledgements

The authors would like to express gratitude to Piedrafita and Meeusen (Federation University, Australia) for providing some of the antibodies used here. They also acknowledge Alison Morrison, Moredun Research Institute for the production of *T. circumcincta* larvae. This project was funded by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 635408 ('PARAGONE: vaccines for animal parasites'), European Commission. Tara Pérez-Hernández was sponsored by "Universidad de Las Palmas de Gran Canaria" and "Cabildo Insular de Gran Canaria" as a Ph.D. student in the ULPGC Predoctoral Training Program, Spain. Cynthia Machín was the recipient of a fellowship funded by "Agencia Canaria de Investigación, Innovación y Sociedad de la Información de la Consejería de Economía, Industria, Comercio y Conocimiento" (ACIISI) and European Social Fund (ESF) Integrated Operational Programme for the Canary Islands 2014–2020, axis 3, priority theme 74 (85%), Spain and European Commission. Additionally, she was also supported by "Fundación Canaria Universitaria de Las Palmas (FULP)" and "Obra Social La Caixa", Spain. Dr. Hernández was initially sponsored by PARAGONE and later on by "Agencia Canaria de Investigación, Innovación y Sociedad de la Información de la Consejería de Economía, Industria, Comercio y Conocimiento" (ACIISI) through the grant "ControlPara" (ProID2017010109), Spain.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jacqueline Matthews has patent #EP2812023A1 SHEEP NEMATODE VACCINE pending to Moredun Research Institute. Alasdair Nisbet has patent #EP2812023A1 SHEEP NEMATODE VACCINE pending to Moredun Research Institute.

References

Aboshady, H.M., Mandonnet, N., Félicité, Y., Hira, J., Fourcot, A., Barbier, C., Johansson, A.M., Jonas, E., Bambou, J.-C., 2020. Dynamic transcriptomic changes of goat abomasal mucosa in response to Haemonchus contortus infection. Vet. Res. 51, 44.

Albuquerque, A.C.A., Bassetto, C.C., Almeida, F.A., Hildersley, K.A., McNeilly, T.N., Britton, C., Amarante, A.F.T., 2019. Differences in immune responses to Haemonchus contortus infection in the susceptible Ile de France and the resistant Santa Ines sheep under different anthelmintic treatments regimens. Vet. Res. 50, 104.

Bahirathan, M., Miller, J.E., Barras, S.R., Kearney, M.T., 1996. Susceptibility of Suffolk and Gulf Coast Native suckling lambs to naturally acquired strongylate nematode infection. Vet. Parasitol. 65, 259–268.

Balic, A., Bowles, V.M., Meeusen, E.N.T., 2000a. Cellular profiles in the abomasal mucosa and lymph node during primary infection with *Haemonchus contortus* in sheep. Vet. Immunol. Immunopathol. 75, 109–120.

Balic, A., Bowles, V.M., Meeusen, E.N.T., 2000b. The immunobiology of gastrointestinal nematode infections in ruminants. Adv. Parasitol. 45, 181–241.

Barger, I.A., 1988. Resistance of young lambs to *Haemonchus contortus* infection, and its loss following anthelmintic treatment. Int. J. Parasitol. 18, 1107–1109.

Bitakaramire, P.K., 1966. Studies on immunity to *Haemonchus contortus* infection: the elimination of a challenge infection by immune sheep. Parasitology 56, 619–622.

Britton, C., Emery, D.L., McNeilly, T.N., Nisbet, A.J., Stear, M.J., 2020. The potential for vaccines against scour worms of small ruminants. Int. J. Parasitol. 50, 533–553.

Broomfield, M.A., Doyle, E.K., Kahn, L.P., Smith, W.D., Walkden-Brown, S.W., 2020. A simplified Barbervax® vaccination regimen in lambs to evoke immunological protection to Haemonchus contortus. Vet. Parasitol. 287, 109243.

Colditz, I.G., Watson, D.L., Gray, G.D., Eady, S.J., 1996. Some relationships between age, immune responsiveness and resistance to parasites in ruminants. Int. J. Parasitol. 26, 869–877.

Craig, N.M., Smith, D.W., Pate, J.A., Morrison, I.W., Knight, P.A., 2014. Local cytokine transcription in naïve and previously infected sheep and lambs following challenge with Teladorsagia circumcincta. BMC Vet. Res. 10, 87.

Douch, P., Morum, P., 1993. The effect of age on the response of Romney sheep to gastrointestinal nematodes during grazing. Int. J. Parasitol. 23, 651–655.

Gill, H.S., Altmann, K., Cross, M.L., Husband, A.J., 2000. Induction of T helper 1- and T helper 2-type immune responses during *Haemonchus contortus* infection in sheep. Immunology 99, 458–463.

Gilleard, J.S., Kotze, A.C., Leathwick, D., Nisbet, A.J., McNeilly, T.N., Besier, B., 2021. A journey through 50 years of research relevant to the control of gastrointestinal nematodes in ruminant livestock and thoughts on future directions. Int. J. Parasitol. 51, 1133–1151.

González, J.F., Hernández, Á., Molina, J.M., Fernández, A., Raadsma, H.W., Meeusen, E. N.T., Piedrafita, D., 2008. Comparative experimental *Haemonchus contortus* infection of two sheep breeds native to the Canary Islands. Vet. Parasitol. 153, 374–378.

González, J.F., Hernández, Á., Meeusen, E.N.T., Rodríguez, F., Molina, J.M., Jaber, J.R., Raadsma, H.W., Piedrafita, D., 2011. Fecundity in adult *Haemonchus contortus* parasites is correlated with abomasal tissue eosinophils and γδ T cells in resistant Canaria Hair Breed sheep. Vet. Parasitol. 178, 286–292.

González, J.F., Hernández, J.N., Machín, C., Pérez-Hernández, T., Wright, H.W., Corripio-Miyar, Y., Price, D.R.G., Matthews, J.B., McNeilly, T.N., Nisbet, A.J., 2019. Impacts of breed type and vaccination on Teladorsagia circumcincta infection in native sheep in Gran Canaria. Vet. Res. 50, 29.

Greer, A.W., 2008. Trade-offs and benefits: implications of promoting a strong immunity to gastrointestinal parasites in sheep. Parasite Immunol. 30, 123–132.

Greer, A.W., Hamie, J.C., 2016. Relative maturity and the development of immunity to gastrointestinal nematodes in sheep: an overlooked paradigm? Parasite Immunol. 38, 263–272.

Gruner, L., Aumont, G., Getachew, T., Brunel, J.C., Pery, C., Cognié, Y., Guérin, Y., 2003. Experimental infection of Black Belly and INRA 401 straight and crossbred sheep with trichostrongyle nematode parasites. Vet. Parasitol. 116, 239–249.

Gruner, L., Cortet, J., Sauvé, C., Hoste, H., 2004. Regulation of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* worm populations by grazing sheep with differing resistance status. Vet. Res. 35, 91–101.

Hernández, J.N., Hernández, A., Stear, M.J., Conde-Felipe, M., Rodríguez, E., Piedrafita, D., González, J.F., 2016. Potential role for mucosal IgA in modulating *Haemonchus contortus* adult worm infection in sheep. Vet. Parasitol. 223, 153–158.

Kebeta, M.M., Hine, B.C., Walkden-Brown, S.W., Kahn, L.P., Doyle, E.K., 2020. Evaluation of Barbervax® vaccination for lambing Merino ewes. Vet. Parasitol. 283, 109187.
Kebeta, M.M., Hine, B.C., Walkden-Brown, S.W., Kahn, L.P., Doyle, E.K., 2021. Protective efficacy of Barbervax® in Merino weaner sheep trickle infected with five doses of Haemonchus contortus infective larvae. Vet. Parasitol. 292, 109386.
Kimambo, A.E., MacRae, J.C., Walker, A., Watt, C.F., Coop, R.L., 1988. Effect of prolonged subclinical infection with *Trichostrongylus colubriformis* on the performance and nitrogen metabolism of growing lambs. Vet. Parasitol. 28, 191–203.
Knox, D.P., Smith, S.K., Redmond, D.L., Smith, W.D., 2005. Protection induced by vaccinating sheep with a thiol-binding extract of *Haemonchus contortus* membranes is associated with its protease components. Parasite Immunol. 27, 121–126.
Kooyman, Schallig, Leeuwen, Van, Mackellar, Huntley, Cornelissen, Vervelde, 2000. Protection in lambs vaccinated with *Haemonchus contortus* antigens is age related, and correlates with IgE rather than IgG1 antibody. Parasite Immunol. 22, 13–20.
Lane, J., Jubb, T., Shephard, R., Webb-Ware, J., Fordyce, G., 2015. Priority list of endemic disease for red meat industries in Australia. Sydney, Australia.
Liu, W., McNeilly, T.N., Mitchell, M., Burgess, S.T., Matthews, J.B., Babayan, S.A., 2021. The right time and place: time-and age-dependent vaccine-enhanced mucosal immunity to parasite infection 2. bioRxiv. https://doi.org/10.1101/2021.04.28.441781.
Machín, C., Corripio-Miyar, Y., Hernández, J.N., Pérez-Hernández, T., Hayward, A.D., Wright, H.W., Price, D.R.G., Matthews, J.B., McNeilly, T.N., Nisbet, A.J., González, J. F., 2021. Cellular and humoral immune responses associated with protection in sheep vaccinated against *Teladorsagia circumcincta*. Vet. Res. 52, 89.
MAFF, 1989. Manual of veterinary parasitological laboratory techniques. H. M. Stationery Off., London.
Manton, V.J.A., Peacock, R., Poynter, D., Silverman, P.H., Terry, R.J., 1962. The influence of age on naturally acquired resistance to *Haemonchus contortus* in lambs. Res. Vet. Sci. 3, 308–314.
Matthews, J.B., Geldhof, P., Tzelos, T., Claerebout, E., 2016. Progress in the development of subunit vaccines for gastrointestinal nematodes of ruminants. Parasite Immunol. 38, 744–753.
Mavrot, F., Hertzberg, H., Torgerson, P., 2015. Effect of gastro-intestinal nematode infection on sheep performance: a systematic review and meta-analysis. Parasite Vectors 8, 557.
McClure, S.J., Emery, D.L., Bendixsen, T., Davey, R.J., 1998. Attempts to generate immunity against *Trichostrongylus colubriformis* and *Haemonchus contortus* in young lambs by vaccination with viable parasites. Int. J. Parasitol. 28, 739–746.
McClure, S., 2000. Sheep Immunity To Gastrointestinal Nematode Parasites – Review 2000.
McRae, K.M., Stear, M.J., Good, B., Keane, O.M., 2015. The host immune response to gastrointestinal nematode infection in sheep. Parasite Immunol. 37, 605–613.
Nieuwhof, G.J., Bishop, S.C., 2005. Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. Anim. Sci. 81, 23–29.
Nisbet, A.J., Smith, S.K., Armstrong, S., Meikle, L.I., Wildblood, L.A., Beynon, R.J., Matthews, J.B., 2010. *Teladorsagia circumcincta*: activation-associated secreted proteins in excretory/secretory products of fourth stage larvae are targets of early IgA responses in infected sheep. Exp. Parasitol. 125, 329–337.
Nisbet, A.J., McNeilly, T.N., Wildblood, L.A., Morrison, A.A., Bartley, D.J., Bartley, Y., Longhi, C., McKendrick, I.J., Palarea-Albaladejo, J., Matthews, J.B., 2013. Successful immunization against a parasitic nematode by vaccination with recombinant proteins. Vaccine 31, 4017–4023.
Nisbet, A.J., McNeilly, T.N., Greer, A.W., Bartley, Y., Oliver, E.M., Smith, S., Palarea-Albaladejo, J., Matthews, J.B., 2016a. Protection of ewes against *Teladorsagia circumcincta* infection in the periparturient period by vaccination with recombinant antigens. Vet. Parasitol. 228, 130–136.
Nisbet, A.J., Meeusen, E.N., González, J.F., Piedrafita, D.M., 2016b. Immunity to *Haemonchus contortus* and vaccine development. Adv. Parasitol. 93, 353–396.
Pernthaner, A., Cole, S.A., Morrison, L., Hein, W.R., 2005. Increased expression of interleukin-5 (IL-5), IL-13, and tumor necrosis factor alpha genes in intestinal lymph cells of sheep selected for enhanced resistance to nematodes during infection with *Trichostrongylus colubriformis*. Infect. Immun. 73, 2175–2183.
Piedrafita, D., Raadsma, H.W., Gonzalez, J., Meeusen, E., 2010. Increased production through parasite control: can ancient breeds of sheep teach us new lessons? Trends Parasitol. 26, 568–573.
R Core Team, 2021. R: a language and environment for statistical computing.
Rocha, R.A., Amarante, A.F.T., Bricarello, P.A., 2005. Resistance of Santa Inês and Ile de France suckling lambs to gastrointestinal nematode infections. Rev. Bras. Parasitol. Vet. 14, 17–20.
Smith, W.D., Angus, K.W., 1980. *Haemonchus contortus*: attempts to immunise lambs with irradiated larvae. Res. Vet. Sci. 29, 45–50.
Smith, W.D., Jackson, F., Jackson, E., Williams, J., 1985. Age immunity to *Ostertagia circumcincta*: comparison of the local immune responses of 4 and a half and 10-month-old lambs. J. Comp. Pathol. 95, 235–245.
Spellberg, B., Edwards, J.E., 2001. Type 1/Type 2 immunity in infectious diseases. Clin. Infect. Dis. 32, 76–102.
Stear, M.J., Bishop, S.C., Doligalska, M., Duncan, J.L., Holmes, P.H., Irvine, J., McCririe, L., McKellar, Q.A., Sinski, E., Mu, M., 1995. Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. Parasite Immunol. 17, 643–652.
Stear, M.J., Mitchell, S., Strain, S., Bishop, S.C., McKellar, Q.A., 2000. The influence of age on the variation among sheep in susceptibility to natural nematode infection. Vet. Parasitol. 89, 31–36.
Strain, S.A.J., Stear, M.J., 2001. The influence of protein supplementation on the immune response to *Haemonchus contortus*. Parasite Immunol. 23, 527–531.
Urquhart, G.M., Jarrett, W.F., Jennings, F.W., McIntyre, W.I., Mulligan, W., 1966. Immunity to *Haemonchus contortus* infection: relationship between age and successful vaccination with irradiated larvae. Am. J. Vet. Res. 27, 1645–1648.
Venturina, V.M., Gossner, A.G., Hopkins, J., 2013. The immunology and genetics of resistance of sheep to Teladorsagia circumcincta. Vet. Res. Commun. 37, 171–181.
Vervelde, L., Kooyman, F.N.J., Van Leeuwen, M.A.W., Schallig, H.D.F.H., Mackellar, A., Huntley, J.F., Cornelissen, A.W.C.A., 2001. Age-related protective immunity after vaccination with *Haemonchus contortus* excretory/secretory proteins. Parasite Immunol. 23, 419–426.
Watson, D.L., Gill, H.S., 1991. Post natal ontogeny of immunological responsiveness in Merino sheep. Res. Vet. Sci. 51, 88–93.
Watson, D.L., Colditz, I.G., Andrew, M., Gill, H.S., Altmann, K.G., 1994. Age-dependent immune response in Merino sheep. Res. Vet. Sci. 57, 152–158.
Winter, M.D., Wright, C., Lee, D.L., 2000. Vaccination of young lambs against infection with *Nematodirus battus* using gamma irradiated larvae. Int. J. Parasitol. 30, 1173–1176.
Zhao, G.W., Yan, R.F., Muleke, C.I., Sun, Y.M., Xu, L.X., Li, X.R., 2012. Vaccination of goats with DNA vaccines encoding H11 and IL-2 induces partial protection against *Haemonchus contortus* infection. Vet. J. 191, 94–100.