A POTENTIAL ROLE FOR MUCOSAL IgA IN MODULATING *HAEMONCHUS CONTORTUS ADULT WORM* INFECTION IN SHEEP

Hernández JN[1], Hernández A[1], Stear MJ[2], Conde-Felipe M[1], Rodríguez E[1], Piedrafita D[3*], González JF[1*]

[1]Departamento de Patología Animal, Facultad de Veterinaria, Universidad de Las Palmas de Gran Canaria. Trasmontaña s/n, Arucas, Las Palmas 35413, Spain.

[2]Institute of Biodiversity, Animal Health and Comparative Medicine, School of Veterinary Medicine, Glasgow University, Glasgow G12 8QQ, UK.

[3]School of Applied and Biomedical Sciences, Faculty of Science and Technology, Federation University VIC 3842, Australia.

***contributed equally to the work**

Corresponding author:

Dr. Jorge Francisco González

University of Las Palmas de Gran Canaria

Veterinary Faculty. Department of Animal Pathology

Arucas-Las Palmas-35413 (SPAIN)

Phone: +34 928 457242

Fax: +34 928 451142

E-mail: jorgefrancisco.gonzalez@ulpgc.es

Abstract

Haemonchus contortus (*H. contortus*) is a haematophagous parasite which causes important economic losses in small ruminants. On the island of Gran Canaria, two sheep breeds coexist which differ in their susceptibility to the infection with *H. contortus;* the resistant Canaria Hair Breed (CHB) sheep and the susceptible Canaria Sheep (CS) breed. The major target of resistance mechanisms in CHB sheep are directed to the adult parasite stage, reducing the worm burden, and decreased length and fecundity of surviving worms. Mucosal IgA (mIgA) has been shown to be an important regulator of immunity in *Haemonchus* and *Teladorsagia* infections; through correlations with larval stages where such mechanisms as antibody-dependent cell cytotoxicity and enzyme inhibition may mediate resistance. Here for the first time, we demonstrate a significant negative correlation between mIgA and adult worm length and fecundity only in the resistant CHB sheep. In contrast, and as reported in other sheep breeds, mIgA was only negatively correlated against the larval stage in the more susceptible CS breed. This study suggests mIgA may play a role in resistance to both larval and adult stages.

Keywords: Immunoglobulin-A, *Haemonchus contortus*, sheep-nematoda, worm-length, Canaria Hair Breed, Canaria Sheep

1. Introduction

Resistance to gastrointestinal nematodes is characterized by low fecal egg counts and reduced worm burdens. Egg production has been positively correlated with adult length and worm burden in *Teladorsagia circumcincta* (**Stear and Bishop, 1999; Stear *et al.*, 1995a, 1999)** and with length in *Haemonchus contortus* (**Ractliffe and Lejambre, 1971; Lacroux *et al.*, 2006**). Local mucosal IgA (mIgA) activity has been

associated with a reduction in fecundity (**Gill *et al.*, 1993; Strain and Stear, 2001; Amarante *et al.*, 2005**) and length (**Lacroux *et al.*, 2006**) in *H. contortus*. Mucosal IgA has also been shown to target the fourth larval stage of *T. circumcincta* effecting worm length and fertility (**Stear and Bishop, 1999; Stear *et al.*, 1995a, 2004**). Therefore, there is a well-established precedent in the literature, suggesting mIgA has an important modulatory role on nematode infection. However, in all these studies the negative correlations were associated between mIgA and various parasitological parameters in the larval stages, suggesting mIgA is an antibody isotype effective against early infection of the immature parasite (**Lacroux *et al.*, 2006; Stear and Bishop, 1999; Stear *et al.*, 1995a, 2004**).

On the Canary Islands, two main local breeds of sheep, Canaria sheep (CS) and Canaria Hair Breed (CHB) sheep are reared. CHB sheep are more resistance than CS to *Haemonchus contortus* infection (**González *et al.*, 2008, Piedrafita *et al.*, 2010**). The resistance mechanism of CHB sheep (which has not been previously described in other breeds of sheep) target the adult parasitic stage, modulating worm length and fecundity and may involve a mechanism dependent on abomasal γδ T cells and eosinophils (**González *et al.*, 2011**). IgA studies, suggest eosinophils and mIgA are important mediators against the immature stages of the parasite, hence, indirectly modulating length and fecundity later in worm infection of surviving parasites (**Henderson and Stear, 2006**). Given the association of eosinophils and their potential link with mIgA against larval stages, we investigated the potential modulating role of mIgA against the adult parasite stages in the resistant CHB sheep through correlative studies.

2. Material and methods

69 *2.1 Animals*

70 Eighteen CHB and nineteen CS male lambs were purchased from several farms
71 from Gran Canaria Island (Spain) at weaned (3 months old) and kept in pens (at the
72 Faculty of Veterinary Science, University of Las Palmas de Gran Canaria) until
73 experimentation when sheep were approximately one year old. The animals were fed
74 with a commercial pelleted sheep ration and water *ad libitum* throughout the total
75 experimental period. The animals were drenched on arrival with levamisole (Cyber, Fort
76 Dodge, Spain) with the recommended dose (1 ml/10 kg body weight) and remained free
77 of parasites (as determined by faecal egg counts) until experimental parasite inoculation.
78 Thirteen CHB sheep and fourteen CS were inoculated intraruminally (the rumen was
79 accessed on the left side of the animal and larvae injected with a syringe and needle),
80 with 20 000 L3 of *H. contortus* to ensure accurate delivery of larvae. Five infected
81 animals of each breed were slaughtered at 7 days and the remainder at 28 days post-
82 infection (dpi). Five uninfected animals of each breed were slaughtered as uninfected
83 controls.

84 The strain of *Haemonchus contortus* **(Redmond and Knox, 2004)** used in the
85 experiment was initially donated by Drs. Knox and Bartley (Moredun Research
86 Institute, Edinburgh, Scotland) and passaged through successive inoculations in sheep at
87 the premises of the Faculty of Veterinary Science, University of Las Palmas de Gran
88 Canaria (Spain).

89 *2.2 Parasitology*

90 Faecal samples were directly taken from the rectum of sheep for egg counts by
91 the modified McMaster technique **(MAFF, 1989)**. These faeces were taken every 2 days
92 from day 15 post challenge until the end of the trial. At euthanasia, the abomasum of

each sheep were isolated and opened by cutting along the greater curvature of the abomasum, followed by abomasal contents and mucosal sample collection. Mature **(Wood *et al.*, 1995)** and immature **(Anderson, 1992)** worms were counted retrospectively from aliquots (300 ml, 10 % formaldehyde) obtained from each abomasal washing (approximately 25% of the total abomasal volume), extrapolating the result to the total volume of the abomasum. Mature adult worms were then sexually differentiated. Thirty random female worms at 7 dpi and 28 dpi of each aliquot were measured from each animal using a digital camera (ProgRes C12PLUS) coupled to an inverted microscope (Olympus CKX41) or a calibrated ocular scale (**González *et al.*, 2011**). Eggs in utero in these adult female worms were counted using a microscope at 100x after disruption of the parasite tissues using a cover slide **(Strain and Stear, 2001)**. Following the abomasal washings, the tissue was incubated at 37°C for about 30 minutes with pepsin -HCl solution [100 ml: 0.85 g NaCl; 2 ml 37% HCl; 0,8 g pepsin] to digest the tissue **(MAFF, 1989)** and the reaction was stopped with 10% formalin and larvae were counted in an aliquot of 20 ml with a magnifying glass (Wild Heerbrugg) at 160x. The summation of the larvae obtained after digestion of abomasal tissue (intramural larvae) and larvae located within the abomasal contents (luminal larvae) corresponds to the total larval count (Table 1). Larval length from thirty randomly selected larvae from the mucosa, were measured as described for adult worm length determination.

2.3 Mucus

The mucosal scrapings from each abomasum were obtained using a microscope slide and stored at -20°C immediately following collection of abomasal contents and before digestion of the abomasal tissue for each sheep. The mucus was obtained from

the abomasa in order to determine the levels of specific local IgA. The mucosal IgA (mIgA) samples were treated as follows; mucus was diluted at the rate of 2.5 ml [pH 7.1; Na_2HPO_4 0.1 M; NaCl 0.05 M; NaN_3 3 mM; PMSF (Sigma) 1 mM; EDTA 5 mM] per gram of mucus, homogenized and centrifuged at 18 000g for 30 minutes. Supernatants were then preserved at -20°C until mIgA level determination **(adapted from Amarante *et al.*, 2005)**.

2.4 Antigens for mucosal IgA (mIgA) ELISA

Larval and adult antigens were prepared as described below in order to assay stage-specific mIgA antibody levels.

Larval Antigen (LA): One million 3rd stage (L3) larvae of *H. contortus* were used to prepare somatic antigens of larval *H. contortus*. These larvae were exposed to three cycles of 20 minutes of freeze-thawing (from -80°C to room temperature) in PBS. These larvae were homogenized mechanically (Ultraturrax T8, IKA® Werke), followed by ultrasound (UP100H, Hielscher) disruption at 4°C. The homogenate was then centrifuged at 18 000g (Centrifuge MPW - 65R, MPW) for 30 minutes at 4°C and the supernatant was stored at -20 °C until use **(adapted from Lacroux *et al.*, 2006)**.

Adult Somatic Antigens (SA) of adult worms: Adult worms of *H. contortus* were collected for adult somatic antigen preparations from donor sheep with a primary infection of 7000 L3 *H. contortus*. After euthanasia, abomasal contents were collected and mixed with an agar solution (1.7% in distilled water) at 43°C and poured onto a cloth. After setting, the cloths were incubated vertically in a cuvette with PBS at 37°C for 1½ hour. Migrating motile worms that crossed the agar were collected from the bottom of the bucket and washed with successive passes in PBS to remove any traces of

agar. Finally, the somatic-adult antigens of *H. contortus* was obtained after mechanical (Ultraturrax T18, IKA® Werke) and ultrasound (UP100H, Hielscher) disruption of the adult worms in PBS at 4°C. The homogenate was centrifuged at 4500g (MPW - 65R Centrifuge, MPW) for 20 minutes at 4°C. The supernatant was stored at -20°C until use.

Protein concentration of larval and adult extracts was determined using Pierce BCA kit (Protein Assay Kit, Thermo Scientific).

2.5 Enzyme-linked immunosorbent assay (ELISA)

The levels of mIgA were determined by enzyme-linked immunosorbent assay (ELISA). The plates (Costar 3369, Corning) were incubated with the antigen diluted in carbonate buffer (pH 9.6) for 24 hours at 4°C followed by three 5-minute-washes with PBS tween 20 (0.05 % v/v) to remove unbound antigen. Nonspecific reactions were blocked with 3% bovine serum albumin by incubating 45 minutes at 37°C, followed by washing with PBS tween 20. Samples were diluted in PBS sodium azide 0.02% (w/v) and incubated for one hour (37°C), followed by three washes. For development of the reaction, the conjugated, rabbit anti-sheep mIgA (Bethyl) in PBS was added and incubated for 45 minutes at 37°C. To remove unbound conjugate, three washes with PBS tween 20 were performed. The reaction substrate - consisting of 12.2 ml of citric acid 2.1%, 12.8 ml of sodium phosphate 2.8%, 10 mg of OPD and 35 µl of H_2O_2 was added and incubated for 10 minutes in the dark. Finally, sulfuric acid 2M was added to stop the reaction and after five minutes of incubation the plates were read at 492nm on ELISA plate reader (Multiskan Ascent). The negative control mucus came from non-inoculated animals and positive control mucus came from deliberately trickle infected

animals. Levels of IgA from mucus samples for all sheep were analysed on the same day within the same ELISA plate.

2.6 Statistical analysis

IBM SPSS Statistics software version 20 for statistical analysis was used. FEC27 data were transformed into Log_{10+1}. Parasitological data and mIgA data were analyzed using the generalized linear model (GENLIN) with a gamma distribution and the Newton-Raphson optimization technique after adding 0.1 to all values in order to avoid zero data for statistical analysis. The length of the worms was also analyzed with a GENLIN. The Spearman's correlation coefficients between parasitological data and mIgA data were used to determine the strength of the relationship. Relationships between parasite length and mIgA were also analyzed by linear regression. Probabilities of $p<0.05$ were considered statistically significant.

3. Results

3.1 Parasitology

No significant differences in any parasitological parameters (total larval burden and mean larvae length) between the breeds at 7 dpi were observed (Table 1). In contrast, at 28 dpi, CHB sheep showed a greater than 2-fold reduction in mean adult worm counts ($p<0.05$). A significant reduction was also observed in egg counts, both in utero (mean 2-fold reduction) and faeces (mean 4.5 fold reduction) at 27dpi, in CHB sheep compared to CS breed (Table 1). A significant reduction in mean worm length was also observed in CHB sheep compared with CS (Table 1).

3.2 Mucosal IgA responses

Both breeds had increasing OD values for mIgA during infection against both larval antigen (LA) and adult soluble antigen (SA) compared with uninfected control animals. When comparing between breed differences, significant differences in mean OD values for mIgA were only detected at 28 dpi; with CHB sheep having significantly higher mIgA OD values than CS against SA of adult worms (Figure 1).

3.3 Correlative Mucosal IgA responses with parasitological parameters

There was a clear trend for significant negative correlations between LA mIgA (but not SA mIgA) and intramural larval burdens and luminal larval length in CS at 7 dpi only. This was not apparent in the CHB sheep (Table 2.1. Figure 2A). In contrast, at day 28 dpi, negative and significant correlations between the specific SA mIgA (but not LA mIgA) and adult worm length and epg were observed only in the CHB sheep (Table 2.2. Figure 2B and 2C).

4. Discussion

Resistance to gastrointestinal nematodes are associated with a combination of parasitological parameters, including low fecal egg counts (FEC), reduced worm burden, stunted worms and/or lower eggs in utero in female parasites (**Ractliffe and Lejambre, 1971; Stear and Bishop, 1999; Lacroux *et al.* 2006**). Eosinophils and mucosal IgA (mIgA) have been strongly linked through genetic and correlative studies to immature parasite modulation effecting the parasite dynamics and influencing parasitological parameters (worm length/FEC/worm burden) (**Henderson and Stear,**

2006; Stear *et al.*, 1995b), presumably through impairment of larval development, by various mechanisms including ADCC (Antibody Dependent Cell Cytotoxicity) (**Rainbird *et al.*, 1998**).

Two main sheep breeds exist on the Canary Islands, CS and CHB sheep. Our previous studies suggested that the CS breed modulates parasite parameters by influencing the immature stages of the parasite, in agreement with the literature. **Balic *et al*, (2000; 2002**) have argued that L3 of *H. contortus* is the main target of the mechanisms of resistance in sheep although **Stewart (1955)** suggested that anti-adult immunity might be important in the self-cure reaction. However, CHB sheep do not appear to modulate the immature stages of the parasite and resistance mechanisms target the adult stages resulting in reduced egg production, lower worm burdens and stunted adult worms suggesting different mechanisms of parasite resistance between the breeds (**González *et al.* 2008, 2011; Piedrafita *et al.*, 2010**). Given our correlative findings of a role for γδ T cells and eosinophils modulating adult parasites (worm length and fecundity), we wondered whether mIgA could also be associated directly with adult parasite parameters. We compared both early (7 dpi) and late (28 dpi) infective stages and the CS breed was used as a comparable control for larval modulation as previous literature suggests worm modulation is directly linked with significant larval correlations in sheep breeds.

The mIgA levels were determined in both breeds before and after infection against both larval antigen (LA) and adult soluble antigen (SA). In both breeds higher amounts of LA and SA mIgA were detected at 7 and 28 dpi compared to uninfected

controls demonstrating a mIgA response following infection. Correlation studies were then determined between mIgA levels against L3 or adult SA with worm length, fecundity and worm burdens in both breeds to confirm whether a relationship to mIgA could be established. The suggestion of immune mediators influencing parasite development or establishment is often initially determined by attempting to establish a negative correlation between the immune parameter and a parasite factor. Mucosal IgA against larval antigens was negative and significantly associated with, worm length and burden in CS breed at 7 dpi; similarly as described by others in several breeds of sheep (**Stear and Bishop, 1999; Stear *et al.*, 1999; Strain *et al.*, 2002; Amarante *et al.*, 2005**). No such correlations were found in late infection and would agree with the hypothesis that the target in CS is the larval stage. Given negative correlations in larval length were identified against the adult stage antigens this would also suggest protective antigens within the larval stages modulating infection are not completely distinct to those of adult antigens. The literature suggests this mechanism of larval targeting involves eosinophils and parasite-specific IgA, suggesting that length may be regulated by an interaction between IgA and eosinophils (**Henderson and Stear, 2006; Mair *et al.*, 2015**), possibly through ADCC reactions (**Rainbird *et al.*, 1998**). Some studies have shown that variation in eosinophilia and IgA accounted for more of the variation in adult worm length than either trait alone (**Henderson and Stear, 2006**). Human, mouse and rat eosinophils differ in the receptors for IgA that they possess (**Decot *et al.* 2005**). The situation in sheep is currently unknown (**Henderson and Stear, 2006**) but the existence of suitable IgA receptors on eosinophils in sheep should be explored.

No significant correlations in CHB sheep between anti-mIgA to larval antigens and length and FEC were identified at any parasitic stage, further emphasizing disparate

immune responses and potential differential recognition of immune reactivity to parasitic antigens between the breeds. Reductions in adult worm burdens independent of larval establishment have also been observed in other indigenous breeds (**Aumont *et al.*, 2003; Beriajaya and Copeman, 2006**) and differences in parasite fecundity between breeds without corresponding differences in worm burden have been reported in some of these breeds (**Zajac *et al.*, 1990; Gauly *et al.*, 2002; Hielscher *et al.*, 2006**). Our strong negative correlations between adult SA mIgA and FEC or worm length may yet suggest mIgA could play an unidentified role in worm fecundity and/or worm length. There was also a clear negative trend of CHB sheep anti-mIgA antibodies against adult stages with worm burden at 28 dpi; albeit this was not statistically significant. Given our previous findings of higher eosinophil numbers in CHB sheep when adult parasites are present which are not evident at this period in CS (**González *et al.*, 2011**), and the high correlation between mIgA and worm parameters in CHB sheep in this study, it is tempting to speculate a similar mechanism involving eosinophils and mIgA (as described for larval parasites) could play a role in adult parasite modulation. In this case, this modulation may be mediated by γδ T cells (**González *et al.*, 2011)** rather than CD4+ T cells as described for larval parasites (**Balic *et al.*, 2002**). However, such a mechanism requires validation and is beyond the scope of this study. These results firmly support the interest in poorly characterized local breeds, as an important resource and emphasis's natural models to study relevant disease resistance mechanisms to parasitic infection (**Piedrafita *et al.*, 2010**).

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References

Amarante, A.F.T., Bricarello, P.A., Huntley, J.F., Mazzolin, L.P., Gomes, J.C., 2005. Relationship of abomasal histology and parasite-specific immunoglobulin A with the resistance to *Haemonchus contortus* infection in three breeds of sheep. Vet. Parasitol. 128, 99–107.

Anderson, R.C., 1992. Nematodes parasites of vertebrates. Wallingford, UK. CAB International.

Aumont, G., Gruner, L., Hostache, G., 2003. Comparison of the resistance to sympatric and allopatric isolates of *Haemonchus contortus* of Black Belly sheep in Guadalupe (FWI) and of INRA401 sheep in France. Vet. Parasitol. 116, 139-150.

Balic, A., Bowles, V.M., Meeusen, E.N.T., 2000. 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., 2002. Mechanisms of immunity to *Haemonchus contortus* infection in sheep. Parasite Immunol. 24, 39-46.

Beriajaya, Copeman, D.B., 2006. *Haemonchus contortus* and *Trichostrongylus colubriformis* in pen-trials with Javanese thin tail sheep and Kacang cross Etawah goats. Vet. Parasitol. 135, 315-323.

Decot, V., Woerly, G., Loyens, M., Loiseau, S., Quatannens, B., Capron, M., Dombrowicz, D., 2005. Heterogeneity of Expression of IgA Receptors by Human, Mouse and Rat eosinophils. J. Immunol. 174, 628-635.

Gauly, M., Kraus, M., Vervelde, L., van Leeuwenb, M.A.W., Erhardt, G., 2002. Estimating genetic differences in natural resistance in Rhön and Merinoland sheep following experimental *Haemonchus contortus* infection. Vet. Parasitol. 106, 55-67

Gill, H.S., Gray, G.D., Watson, D.L., Husband, A.J., 1993. Isotype-specific antibody responses to *Haemonchus contortus* in genetically resistant sheep. Parasite Immunol. 15, 61-67.

González, J.F., Hernández, A., Molina, J.M., Fernández, A., Raadsma, H.W., Meeuseen, E.N.T., Piedrafita, D., 2008. Comparative experimental *Haemonchus*

328 *contortus* infection of two sheep breeds native to the Canary Islands. Vet. Parasitol. 153,
329 374-378.

330

331 **González, J.F., Hernández, A., Meeuseen, E.N.T., Rodríguez, F., Molina, J.M.,**
332 **Jaber, J.R., Raadsma, H.W., Piedrafita, D., 2011.** Fecundity in adult *Haemonchus*
333 *contortus* parasites is correlated with abomasal tissue eosinophils and γδ T cells in
334 resistant Canaria Hair Breed sheep. Vet. Parasitol. 153, 374-378.

335

336 **Henderson, N.G., Stear, M.J., 2006.** Eosinophil and IgA responses in sheep infected
337 with *Teladorsagia circumcincta*. Vet. Immunol. Immunopathol. 112, 62-66.

338

339 **Hielscher, A., Brandt, H., Erhardt, G., Gauly, M., 2006.** Heterosis analysis of
340 *Haemonchus contortus* resistance and production traits in Rhoen sheep, Merino Land
341 sheep and cross breed lambs. Vet. Parasitol. 141, 279-284.

342

343 **Lacroux, C., Nguyen, T.H.C., Andreoletti, O., Prevot, F., Grisez, C., Bergeaud, J.P.,**
344 **Gruner, L., Brunel, J.C., Francois, D., Dorchies, P., Jacquiet, P., 2006.** *Haemonchus*
345 *contortus* (Nematoda: Trichostrongylidae) infection in lambs elicits an unequivocal Th2
346 immune response. Vet. Res. 37, 607–622.

347

348 **MAFF (Ministry of Agriculture, Fisheries and Food), 1989.** Manual of Veterinary
349 Parasitological Laboratory Techniques. London. H.M.S.O.

Mair, C., Matthews, L., de Cisneros, J.P., Stefan, T., Stear, M.J., 2015. Multitrait indices to predict worm length and number in sheep with natural, mixed predominatly *Teladorsagia circumcincta* infection. Parasitology,142, 773-782.

Piedrafita, D., Raadsma, H., González, J., Meeusen, E., 2010. Increased production through parasite control: can ancient breeds of sheep teach us new lessons. Trends Parasitol. 26, 568-573.

Ractliffe, L.H., Lejambre, L.F., 1971. Increase of rate of egg production with growth in some intestinal nematodes of sheep and horses. Int. J. Parasitol. 1, 153-156.

Rainbird, M.A., MacMillan, D., Meeusen, E.N.T., 1998. Eosinophil-mediated killing of *Haemonchus contortus* larvae: effect of eosinophil activation and role of antibody, complement and interleukin-5. Parasite Immunol., 20, 93-103.

Redmond D.L., Knox D.P., 2004. Protection studies in sheep using affinity-purified and recombinant cysteine proteinases of adult *Haemonchus contortus*. Vaccine 22, 4252-4261.

Stear, M.J., Bishop, S.C., 1999. The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta*. Int. J. Parasitol. 29, 777-780.

Stear, M.J., Bishop, S.C., Doligalska, M, Duncan, J.L., Holmes, P.H., Irvine, J., McCririe, L., McKellar, Q.A., Sinski, E., Murray, M., 1995a. 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., Bairden, K., Duncan, J.L., Gettinby, G., Mckellar, Q.A., Murray, M. Wallace, D.S., 1995b. The distribution of faecal nematode egg counts in Scottish Blackface lambs following natural, predominantly *Ostertagia circumcincta* infection. Parasitology 110, 573-581.

Stear, M. J., Strain, S., Bishop, S.C., 1999. Mechanisms underlying resistance to nematode infection. Int. J. Parasitol. 29, 51-56.

Stear, M.J., Bairden, K., Innocent, G.T., Mitchell, S., Strain, S.A.J., Bishop, S.C., 2004. The relationship between IgA activity against fourth-stage larvae and density-dependent effects on the number of fourth-stage larvae of *Teladorsagia circumcincta* in naturally infected sheep. Parasitology, 129, 1-7.

Stewart, D.F., 1955. 'Self-cure' in nematode infestations of sheep. Nature 176, 1273-1274.

Strain, S.A., Stear, M.J., 2001. The influence of protein supplementation on the immune response to *Haemonchus contortus*. Parasite Immunol. 23, 523–531.

Strain, S.A.J., Bishop, S.C., Henderson, N.G., Kerr, A., Mckellar, Q.A., Mitchell, S., Stear, M.J., 2002. The genetic control of IgA activity against *Teladorsagia circumcincta* and its association with parasite resistance in naturally infected sheep. Parasitology 124, 545-552.

Wood, I.B., Amaral, N.K., Bairden, K., Duncan, J.L., Kassai, T., Malone, J.B., Pankavich, J.A., Reinecke, R.K., Slocombe, O., Taylor, S.M., Vercrysse, J., 1995. World Association for the advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of antihelmintics in ruminants (bovine, ovine, caprine). Vet. Parasitol.58, 181-213.

Zajac, A.M., Krakowka, S., Herd, R.P., McClure, K.E., 1990. Experimental *Haemonchus contortus* infection in three breeds of sheep. Vet. Parasitol. 36, 221-235.

Table 1: Parasitological parameters (± SEM) measured from samples collected from CS and CHB sheep at 7 and 28 days post- infection. Zero values were transformed to 0.1 prior to gamma distribution analyses and were analysed with a generalized linear mixed model. The arrows represent a fold increase (‘) or

decrease (“) or no change (=) of the parasitological data comparisons between CHB sheep and CS.

Table 2.1 Spearman correlation coefficients between IgA mucosal OD values (against LA and SA) and parasitological variables in CS and CHB sheep at 7 dpi

Table 2.2 Spearman correlation coefficients between IgA mucosal OD values (against LA and SA) and parasitological variables in CS and CHB sheep at 28 dpi

Figure 1: Optical density (OD) values for mucosal IgA against (A) larval antigen (LA) and (B) somatic antigen (SA) of adult worms**.**

Figure 2: Relationship between mucosal IgA and select parasitological parameters in CS and CHB sheep. (A) Larval length was negatively associated with mucosal IgA in CS breed ($p < 0.05$) but not in CHB sheep. (B) Female worm length was significantly negatively associated with mucosal IgA in CHB sheep ($p<0.05$) but not in CS. (C) Faecal egg counts at 27 dpi were significantly negatively correlated with mucosal IgA in CHB sheep but not in CS.

Days Post Infection	Parasitological Data	CHB	CS	Mean Fold change (CS/CHB)
7dpi	**Total larvae count (% of infective dose)**	6825 ± 887 (34.1 %); n= 5	5691 ± 890 (28.5 %); n=5	0.83 (“)
	Larval length (mm)	4.5 ± 0.040	4.5 ± 0.068	1 (=)
28dpi	**Adults counts (% of infective dose)**	1926 ± 600* [G] (9.63 %); n=8	4666 ± 1164* [G] (23.3 %); n=9	2.42 (‘)
	FEC27	5443 ± 2664*[G]	24778 ± 6263*[G]	4.55 (‘)
	Adult length (mm)	15.4 ± 0.122** [L]	17.0 ± 0.117** [L]	1.13 (‘)
	Eggs in utero	104 ± 5**[G]	199 ± 8**[G]	1.9 (‘)

*Shows differences between breeds (p<0.05); **Shows differences between breeds (pd0.01); [G] gamma distribution; [L]Normal distribution; FEC27, dpi, days post-infection; faecal egg counts at day 27 post-infection; n= animals per group.

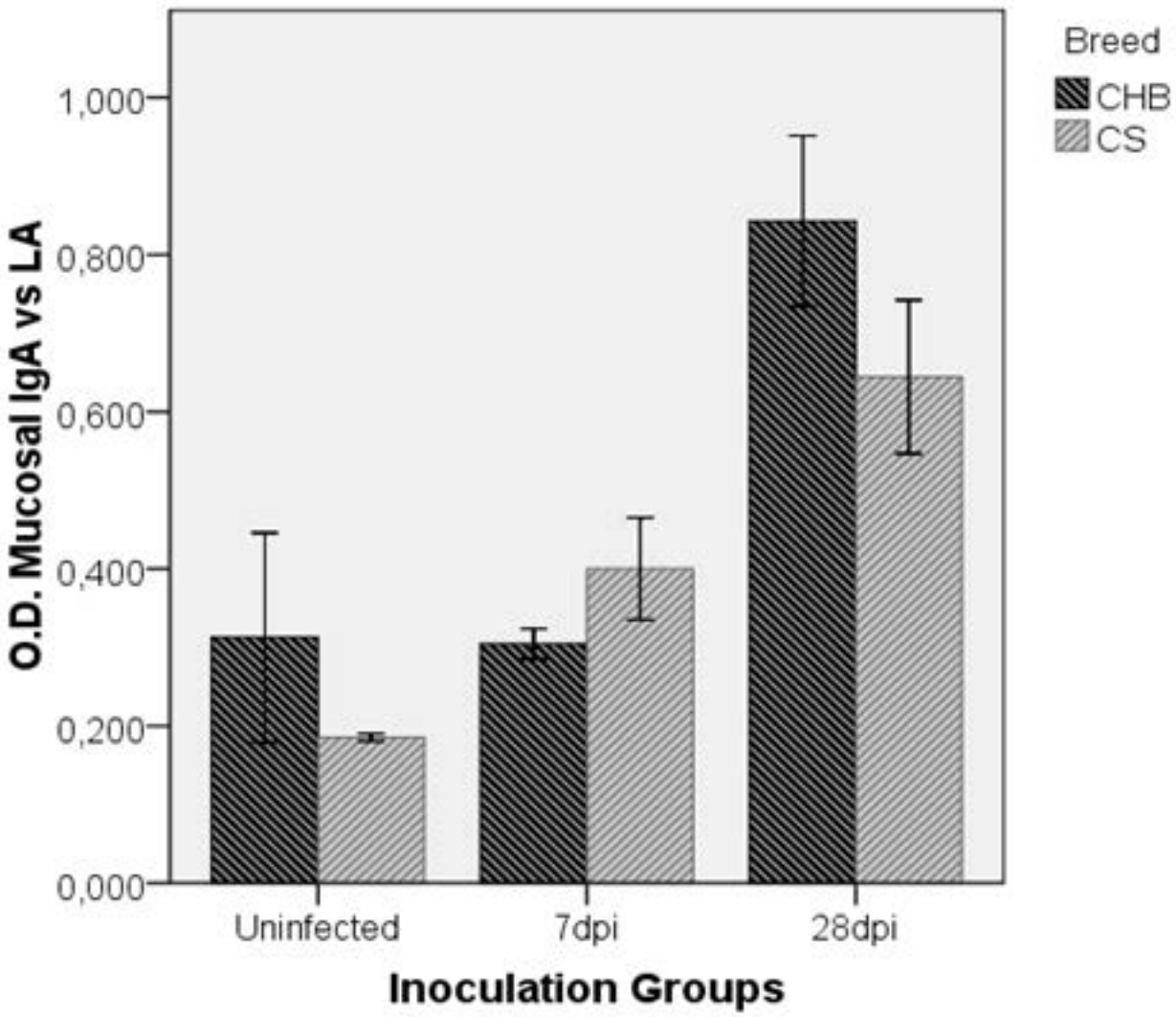
		Breed	Total Larvae	Intramural Larvae	Luminal Larvae	Luminal larvae Length
7dpi	**LA mIgA**	CHB	0.3	0.1	0.3	0.1
		CS	-0.7	-0.9*	-0.4	-0.9*
	SA mIgA	CHB	0.462	0.205	0.462	0.205
		CS	-0.5	-0.6	-0.1	-0.9*

*Shows differences between breeds ($p<0.05$); LA, larval antigen; SA, somatic antigen; total larvae = larval tissue (intramural) counts + mucosal (luminal) counts.

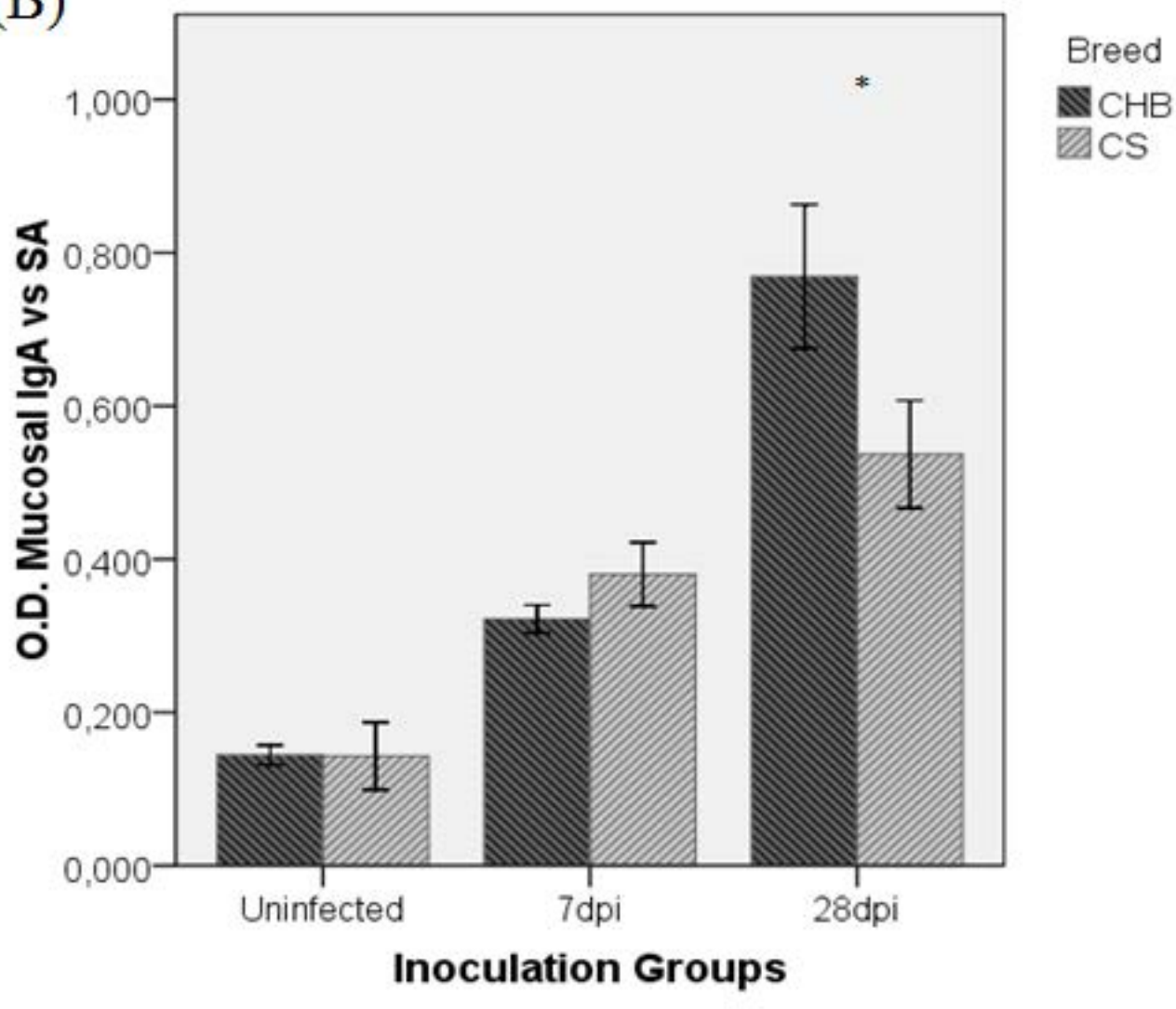
		Breed	Total Worms	Length	EIU	FEC
28dpi	**LA mIgA**	CHB	-0.371	-0.657	-0.6	-0.543
		CS	0.048	0.333	0.024	-0.4
	SA mIgA	CHB	-0.771	-0.943**	-0.657	-0.829*
		CS	-0.119	0.167	-0.262	-0.417

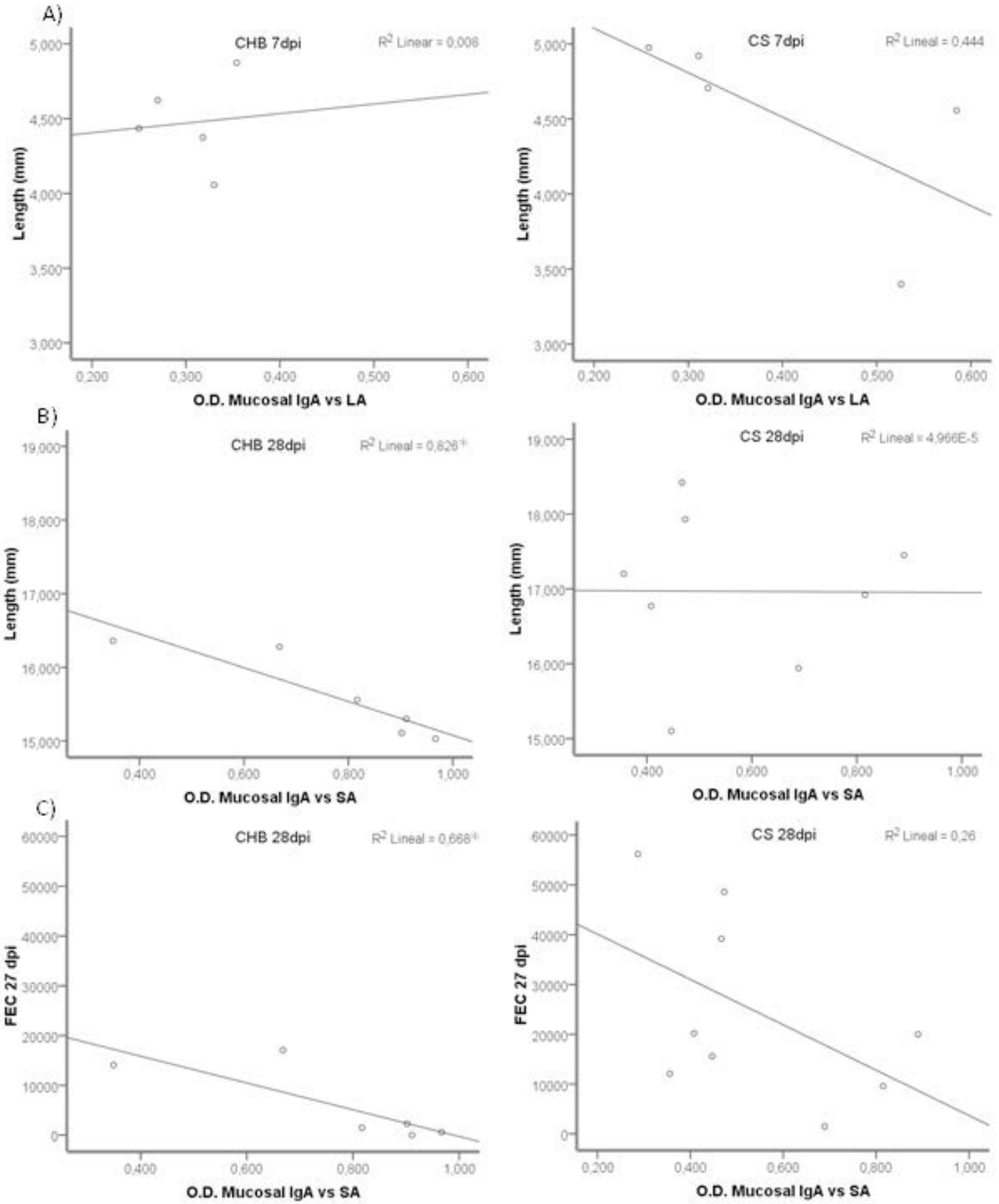
*Shows differences between breeds ($p<0.05$) ** Shows differences between breeds ($p<0.01$). LA, larval antigen; SA, somatic antigen; EIU= Eggs in utero; FEC, faecal egg counts.

(A)


Breed
CHB
CS
1,000
0,800
0,600
0,400
0,200
0,000
O.D. Mucosal IgA vs LA
Uninfected
7dpi
28dpi
Inoculation Groups

(B)


Breed
CHB
CS
*
1,000
0,800
0,600
0,400
0,200
0,000
O.D. Mucosal IgA vs SA
Uninfected
7dpi
28dpi
Inoculation Groups


A)
CHB 7dpi
R² Linear = 0,008
CS 7dpi
R² Lineal = 0,444
Length (mm)
O.D. Mucosal IgA vs LA
B)
CHB 28dpi
R² Lineal = 0,828
CS 28dpi
R² Lineal = 4,966E-5
Length (mm)
O.D. Mucosal IgA vs SA
C)
CHB 28dpi
R² Lineal = 0,668
CS 28dpi
R² Lineal = 0,26
FEC 27 dpi
O.D. Mucosal IgA vs SA