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Divergent humoral responses between males and females against 24 kDa excretory-secretory protein of *Haemonchus contortus* and influence of ovine β-globin polymorphism

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ABSTRACT

Lambs harboring the Hb-AA β-globin haplotype present improved cell-mediated responses and increased resistance against *Haemonchus contortus* infection. The aim of the present study was to compare the effect of sex and β-globin haplotypes on specific humoral responses and phenotypes of resistance during *H. contortus* infection in Morada Nova sheep. As expected, females displayed stronger resistance during the first and second experimental challenges. Differential systemic humoral immune responses were observed comparing sex groups, in which higher levels of specific antibodies targeting 24 kDa excretory-secretory (ES24) protein of *H. contortus* of IgG and IgM antibodies were respectively observed as predominant isotypes in males and females. The IgM levels were significantly correlated with phenotypes of resistance, evaluated by packed cell volume and fecal egg counts. To our knowledge this is the first study reporting divergent humoral responses profiles to *H. contortus* infection between male and female sheep. The impact of β-globin haplotypes was less pronounced in females compared to males. Notably, only males showed significant weight differences across haplotypes, with Hb-AA lambs being the heaviest. Additionally, Hb-AA males had significantly higher PCV (indicating better red blood cell health) and lower FEC (indicating lower parasite burden). These findings suggest a more pronounced effect of β-globin polymorphisms on *H. contortus* infection in males, potentially due to their generally weaker resistance compared to females. This study highlights the importance of sex and β-globin haplotypes in shaping immune responses to *H. contortus* infection. Specifically, IgM antibodies targeting the ES24 protein appear to play a crucial role in host-parasite interactions and may hold promise for therapeutic development.

1. Introduction

Haemonchus contortus is the most pathogenic sheep parasite, especially in tropical regions. In view of rising reports of multiple anthelmintic resistance to this gastrointestinal nematode (GIN) (Bassetto et al., 2024), alternative control measures, such as selective breeding for parasite resistance and development of immune-prophylactic management (Amarante et al., 2004; Louvandini et al., 2006) are highly required.

In this context, polymorphisms in the ovine β-globin gene were associated to altered resistance against *H. contortus* infection in experimentally infected Morada Nova lambs (Okino et al., 2021a, 2021b), and recently, similar findings were observed in naturally infected lambs, being associated with differential local cell-mediated immune responses (Okino et al., 2023).

The development of protective immunity against *H. contortus* is the result of a complex interaction among age, gender, physiological status, pregnancy, lactation, nutrition and innate and adaptive immunity in the host. Therefore, the design of effective vaccination strategies should be underpinned by deep knowledge of the host-parasite interactions,

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including developed protective immune responses and parasite biology (Nisbet et al., 2016).

Secretory-excretory products (ES) of *H. contortus* are continuously secreted antigens and were associated to the development of immune protection (Vervelde et al., 2002). The 15 and 24 kDa ES proteins from *H. contortus,* characterized as highly immunogenic, are expressed only in the parasitic stages of this nematode, which suggests a critical role in host-parasite interactions, these proteins were already successfully applied as vaccines (Nisbet et al., 2016; Schallig et al., 1994; Vervelde et al., 2002). ES products derived from *H. contortus* cultures were used as antigen in an Indirect Enzyme-Linked Immunosorbent Assay (ELISA) and successfully differentiated *H. contortus*-infected from non-infected sheep (Schallig et al., 1994). Furthermore, recombinant ES24 protein was also recognized by sera from infected sheep, indicating the presence of antigenic determinants on these recombinant proteins (Li et al., 2007; Schallig et al., 1997).

Males and females present distinct innate and adaptive immune responses, wherein females usually mount stronger responses and consequently a faster clearance of pathogens, which may forward to differential treatments, such as drugs and vaccines (Klein and Flanagan, 2016; Wesołowska, 2022). This sex difference has evolved in diverse species ranging from insects to lizards, birds and mammals (Klein and Flanagan, 2016) as major issue in the development of immune responses to infectious diseases. This sex-linked resistance was reported for some important parasitic infections, such as *Leishmania* in human (Albuquerque et al., 2021), *Trichinella spiralis* in mice (Hernández-Bello et al., 2011), *H. contortus* in sheep (Gauly M et al., 2006), and *Toxocara canis* in rats (Santos et al., 2018).

Furthermore, such sex-related differences in immune responses are primarily linked to the impact of sex hormones. Testosterone is crucial for the development of sexual traits in males, but it can also modulate the immune system, which may be linked to increased risk of infection, while oestrogens, which are main sex hormones in females, can enhance cellular and humoral immune responses in females, thus improving the resistance against infection (Wesołowska, 2022).

Humoral immunity is one of the most well conserved sex differences in immunology, and these sex-based differences contribute to variation in vaccine responses and may explain some divergences in vaccine efficacy between sexes (Fink and Klein, 2018). Higher humoral response in females compared to males is phylogenetically conserved, which suggests an adaptive advantage for reproductive success, including the passive transference of maternal immunity to progeny (Fink and Klein, 2018; Fischinger et al., 2019; Flanagan et al., 2017). On the other hand, more adverse events following vaccination and incidence of autoimmune disease are also observed in females (Fischinger et al., 2019). In this context, women receiving half dose of influenza vaccine presented improved humoral immune responses compared to men receiving full dose, which may forward the vaccination strategy, by reducing vaccine dose in women which consequently may also reducing adverse effects while still inducing high antibody levels (Engler, 2008).

Differences in the gene expression of X and Y chromosomes has also been shown to drive immunologic differences between sexes. For instance, the human X chromosome expresses 10-fold more genes than Y, and several immune related genes are located in the X chromosome or can be modulated due to oestrogen response elements, including TLR7 (receptor for viral ssRNA), CD40L (co-stimulator on T cells for interaction with CD40 on antigen-presenting cells), FOXP3 (T regulatory marker) (Fischinger et al., 2019). Similar chromosomal arrangement of these genes is also observed in sheep (https://ensembl.org - *Ovis aries* ARS-UI_Ramb_v2.0 – accessed in February 28, 2024). In addition, a larger number of microRNAs, known by modulate immune responses, are present in the human X chromosome, while the Y chromosome contains only two (Fischinger et al., 2019).

In view of absence of similar previous studies in the literature, the present study aimed to investigate systemic humoral responses specifically against ES24 protein of *H. contortus* after experimental challenge with this nematode in Morada Nova male and female lambs of different β-globin haplotypes.

2. Material and methods

2.1. Experimental design

A total of 107 Morada Nova lambs, including 57 females (17 Hb-AA, 20 Hb-AB and 20 Hb-BB) and 50 males (11 Hb-AA, 26 Hb-AB and 13 Hb-BB) were used. At weaning, about 90-day-old lambs presenting natural GIN infection (96.4% *Haemonchus*) were cleared (1°Dw) by oral administration of 2.5 mg/kg of monepantel (Zolvix®, Novartis). Fourteen days after deworming (D0), the lambs were submitted to the first parasitic challenge (1°Ch) with 4000 *H. contortus* L3, as previously described (Toscano et al., 2020; Okino et al., 2021a, 2021b; Toscano et al., 2019). The flock was dewormed at D42 (2°Dw) of the 1°Ch, and the second artificial infection (2°Ch) occurred after 14 days (D56). Fecal egg counts (FEC) were assessed (Ueno and Gonçalves, 1998) at weaning, D0, D21, D28, D35, D42, D56 (D0/2), D77 (D21/2), D84 (D28/2), D91 (D35/2), and D98 (D42/2). Total blood samples were collected into EDTA vacutainer tubes and subjected to DNA extraction followed by qPCR for β-globin haplotype identification (Okino et al., 2021b) and for packed cell volume (PCV) estimation every 14 days from D0 to D98. Body weight was registered at weaning, D0, D28, D42, D56 (D0/2), D84 (D28/2), and D93 (D37/2). Plasma samples were collected at weaning, D0, D14, D56 (D0/2), and D70 (D14/2) for measurement of specific anti-*H. contortus* antibodies of IgG, IgM, and IgA isotypes.

All procedures were approved by Ethics Committee of Animal Experimentation of Embrapa Pecuaria Sudeste (process n. 04/2017) and are in accordance with national and international ethical principles and guidelines for animal experimentation.

2.2. Indirect Enzyme-linked immunosorbent assay (ELISA) for anti-ES24 of Haemonchus contortus IgG, IgM, and IgA antibody quantification

Plasma samples were submitted to measurement of specific systemic antibodies against ES24 protein of *H. contortus* of IgA, IgM, and IgG isotypes by indirect ELISA as Li et al. (2007) with the modifications described below.

Polystyrene high bind microplates (Cat. CLS3590, Corning) were coated overnight at 4 °C with 100 μL per well (6 μg/mL) of recombinant protein ES24 (manufactured by Genscript, USA) following the procedure described by Li et al. (2007) diluted in 50 mM carbonate–bicarbonate buffer pH 9.6. Plates were washed (all washing steps were performed three to six times using 0.05% Tween 20 PBS in the automatic microplate washer Model 50 TS/8V (Biotek)) and incubated with 100 μL of blocking solution (3% skimmed milk in PBS) at 37 °C for 1 h. Plates were washed and incubated with 50 μL of serum samples at dilutions of 1:100 (for IgG and IgM quantification) or 1:2 (for IgA) in blocking solution at 37 °C for 1 h. Plates were washed and incubated with 50 μL of anti-Ig ovine isotypes conjugated to horseradish peroxidase (HRP) diluted in blocking solution at 1:2000 for anti-IgG (Biorad, Cat. 5184-2504), 1:800 for anti-IgM (Biorad, Cat. AHP950P), and 1:400 for anti-IgA (Biorad, Cat. AHP949P). Plates were washed and incubated with 100 μL of SIGMAFAST OPD (Cat. P9187, Sigma) at room temperature (from 20 °C to 25 °C) for 15 min. Blocking of the enzymatic reaction was performed by the addition of 50 μL of 1 M HCl solution. All samples were tested in duplicates, and three standard controls (positive serum, negative serum, and blank) were included in each plate. During optimization of these assays, serial dilutions of recombinant protein, serum, and secondary antibodies were tested. Different blocking solutions (skimmed powder milk, bovine fetal serum, and gelatin) were also evaluated, and fetal sheep serum was included as one of the negative standard controls. Optical density (OD) at 490 nm filter was measured using the Model 800 TSI of the microplate reader (Biotek). OD values were transformed into sample/positive (S/P) values using the equation: (OD of the sample – OD

of the negative control serum)/(OD of the positive control serum – OD of the negative control serum).

2.3. Statistical analyses

Analyses were conducted in R software (R Core Team version 4.4.0, 2024). IgG, IgM, and IgA data were normalized with the log_x(.) transformation, while PCV data underwent a boxcox(.) transformation (bestNormalize package; Peterson, 2021). Cubic root transformation normalized FEC data. Repeated measures ANOVA was performed using the anova_test(.) function of the rstatix package (Kassambara, 2023), with animals as the within-subjects variable and Hb, sex, and collection date (interval) as between-subjects variables. Tukey's multiple comparison test and residual analysis were performed using the ea2(.) function with design = 7 from the easyanova package (Arnhold, 2013). Correlation coefficients (ρ) were estimated by Spearman test between phenotypic values and specific antibody isotypes levels. All the analyses were conducted using RStudio, and the significance level was set as $p < 0.05$.

3. Results

3.1. Differential humoral responses to H. contortus infection among different sexes and β-globin haplotypes

No statistically significant interaction effects were observed between β-globin haplotype, interval, and sex for the levels of IgG, IgM, and IgA. Outlier data points were identified and excluded from the analysis (5 out 527 observations for IgG, 8 for IgM, and 15 for IgA).

Increasing specific anti-*H. contortus* antibody levels after first experimental infection (D0 to D14) was observed only for IgM isotype ($p = 0.0271$). Following the second experimental infection, all immunoglobulins (IgG, IgM and IgA) showed significant increases compared to D0 (day of the first infection) at specific data points: IgG (D56), IgM (D56 and D70) and IgA (D56 and D70) (Fig. 1A).

The specific anti-*H. contortus* (ES24) antibody kinetics of IgG and IgM isotypes differed significantly between males and females after infection (Fig. 1B). Males had higher IgG levels ($p = 0.0129$), while females had higher IgM levels ($p < 0.001$) (Fig. 1B). No such differences were observed for IgA antibodies.

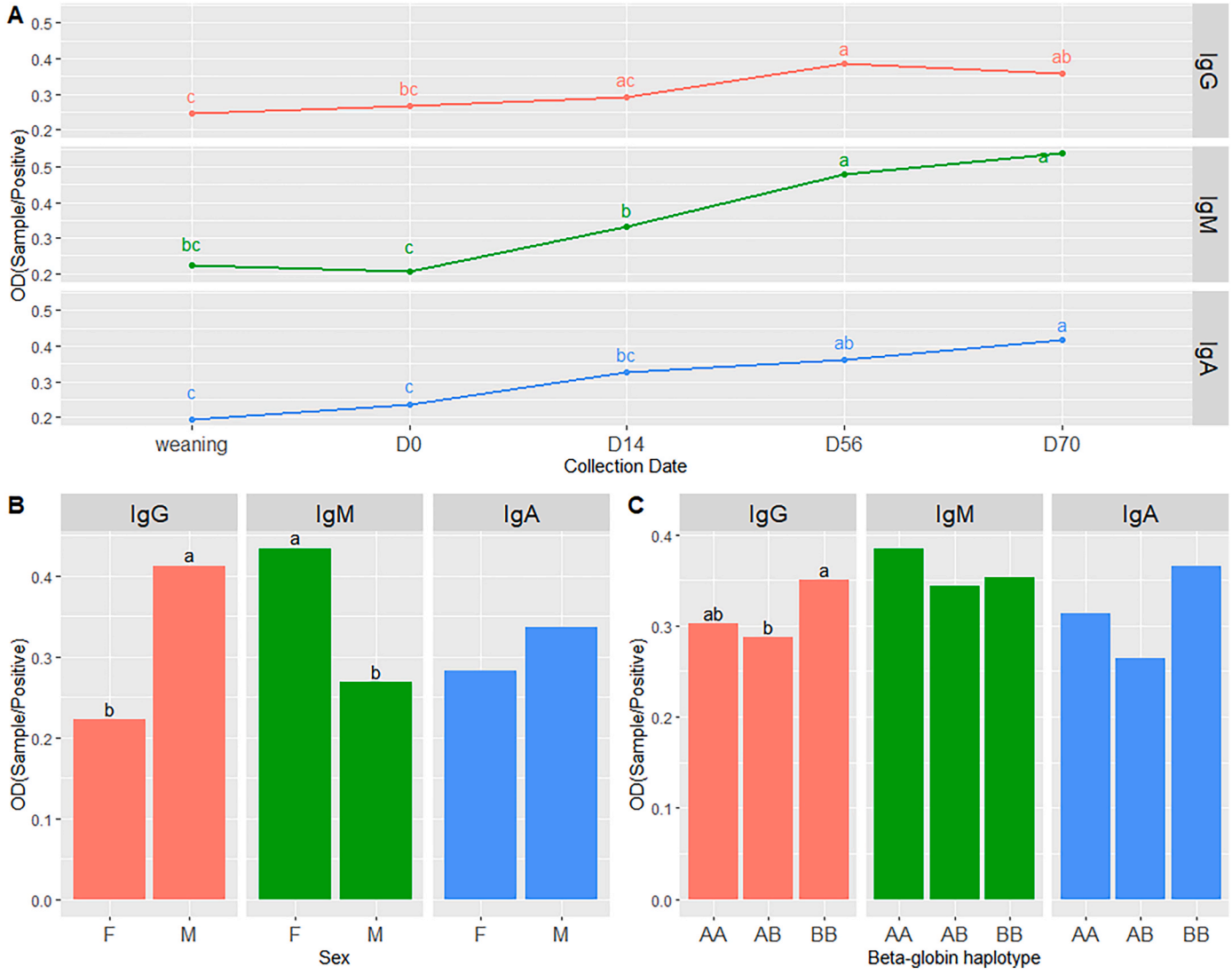

Fig. 1. Specific anti-*H. contortus* (ES24) antibody levels of IgG (red), IgM (green), and IgA (blue) isotypes in plasma collected at weaning, D0, D14, D56 (0/2), and D70 (14/2) intervals (A) from Morada Nova lambs dewormed at weaning (1°Dw) and at D42 (2°Dw) and submitted to two experimental parasitic challenges (1°Ch at D0 and 2°Ch at D56) with 4000 L_3 of *H. contortus*. Mean values in males and females (B) and in different β-globin haplotypes (C). Different letters indicate significant differences within each antibody ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Regarding β-globin haplotypes, IgG levels were significantly higher in Hb-BB animals compared to Hb-AB ($p = 0.0101$), while Hb-AA group presented no significant differences compared to Hb-AB and Hb-BB groups (Fig. 1C). No significant differences were observed for IgM and IgA levels.

3.2. *Phenotypic profiles in males and females and β-globin haplotypes after H. contortus infection*

Statistically significant interactions were observed between β-globin haplotypes and sex for weight, FEC and PCV ($p < 0.05$). Additionally, sex and time intervals interacted significantly for FEC and PCV ($p < 0.05$). Outlier data points were identified and excluded from the analysis (3 out of 749 observations for weight, 2 out 1156 for FEC and 4 out 961 for PCV).

Lambs experienced significant increase in weight at two time points during the experiment: D28 and D56 (Fig. 2). Significant sex differences were observed in weight ($p < 0.001$), with males being heavier than females. β-globin haplotype had no significant effect on weight in females. However, among males, Hb-AA sheep displayed significantly higher weight compared to both Hb-BB and Hb-AB groups ($p < 0.001$). Notably, there were no significant weight differences between Hb-BB and Hb-AB males ($p = 0.8726$).

FEC means at D0 and D56 (14 days after deworming and challenge day) were significantly lower than all other time points (Fig. 3A). Males had significantly higher FEC compared to females ($p < 0.001$) from D21 to D98 (Fig. 3A) and in all haplotypes (Fig. 3B). In females, Hb-BB presented higher FEC levels compared to Hb-AB ($p = 0.0023$) but not compared to Hb-AA group ($p = 0.0606$) (Fig. 3B). In males, Hb-AA presented significantly lower FEC levels compared to both Hb-AB and Hb-BB groups ($p < 0.001$), while Hb-AB was similar to Hb-BB ($p = 0.9606$) (Fig. 3B).

Similarly to FEC results, PCV was highest at D0 and D56 (day of challenge) and remained stable at D14 and D70 (Fig. 4A). However, PCV declined significantly 28 days after infection (D28 and D84). Females consistently had higher PCV levels throughout the experiment, especially after the first (D28 and D42) and second (D70 to D98) infections. β-globin haplotype also influenced PCV in both sexes, with a more pronounced effect in males, in which Hb-AA individuals presented significantly higher means compared to the other groups ($p < 0.001$). In females, Hb-AA and Hb-AB were similar ($p = 0.8753$), but both groups presented significantly higher means compared to Hb-BB ($p = 0.0015$

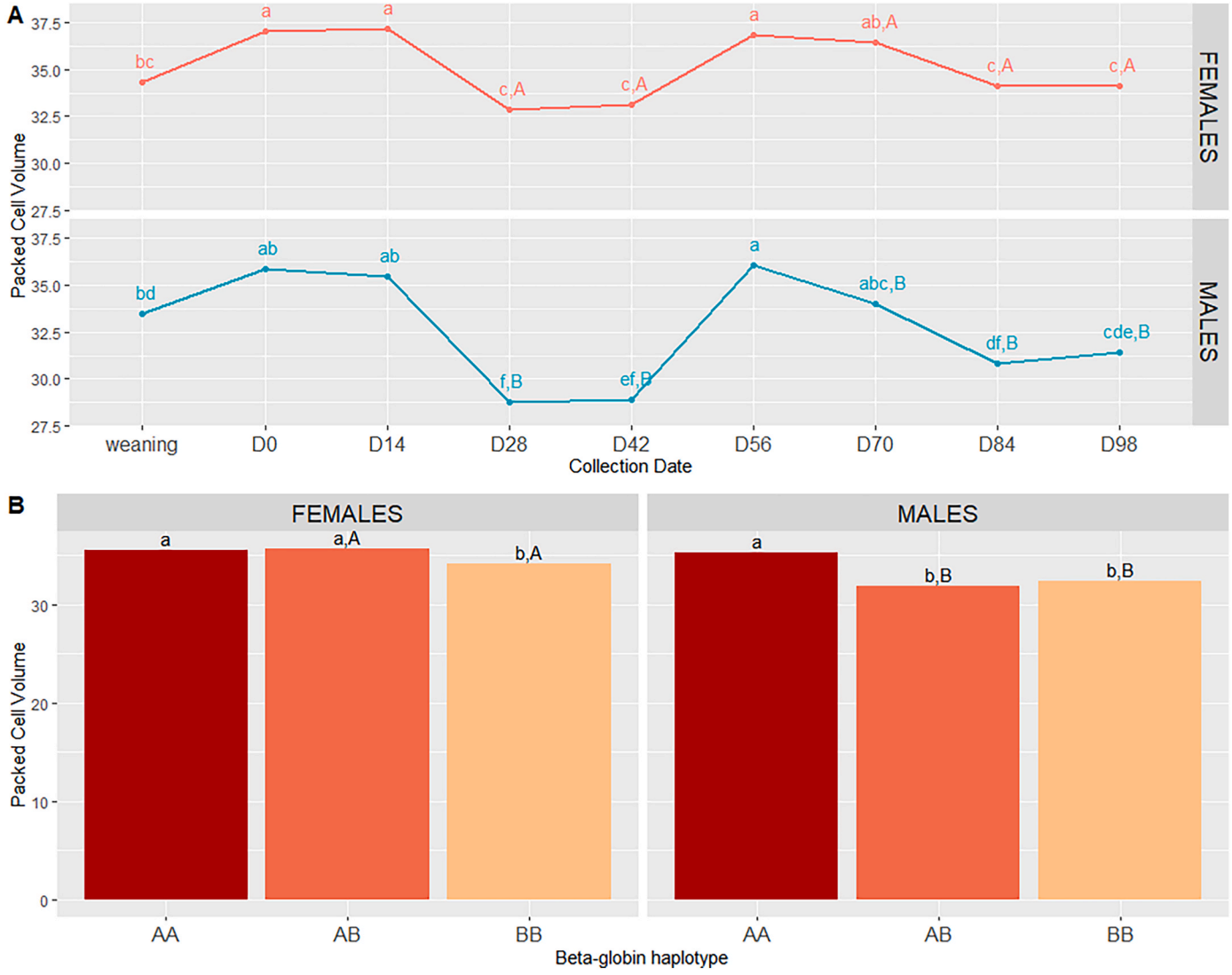

Fig. 2. Packed cell volume means evaluated at different experimental intervals and sex (A) in Morada Nova lambs dewormed at weaning (1°Dw) and at D42 (2°Dw) and submitted to two experimental parasitic challenges (1°Ch at D0 and 2°Ch at D56) with 4000 L_3 of *H. contortus*. Mean values for males and females with different β-globin haplotypes (B). Different lowercase letters indicate significant differences ($p < 0.05$) among time intervals within sex and haplotypes within sex, while different uppercase letters indicate significant differences ($p < 0.05$) between sexes within time interval and within haplotype.

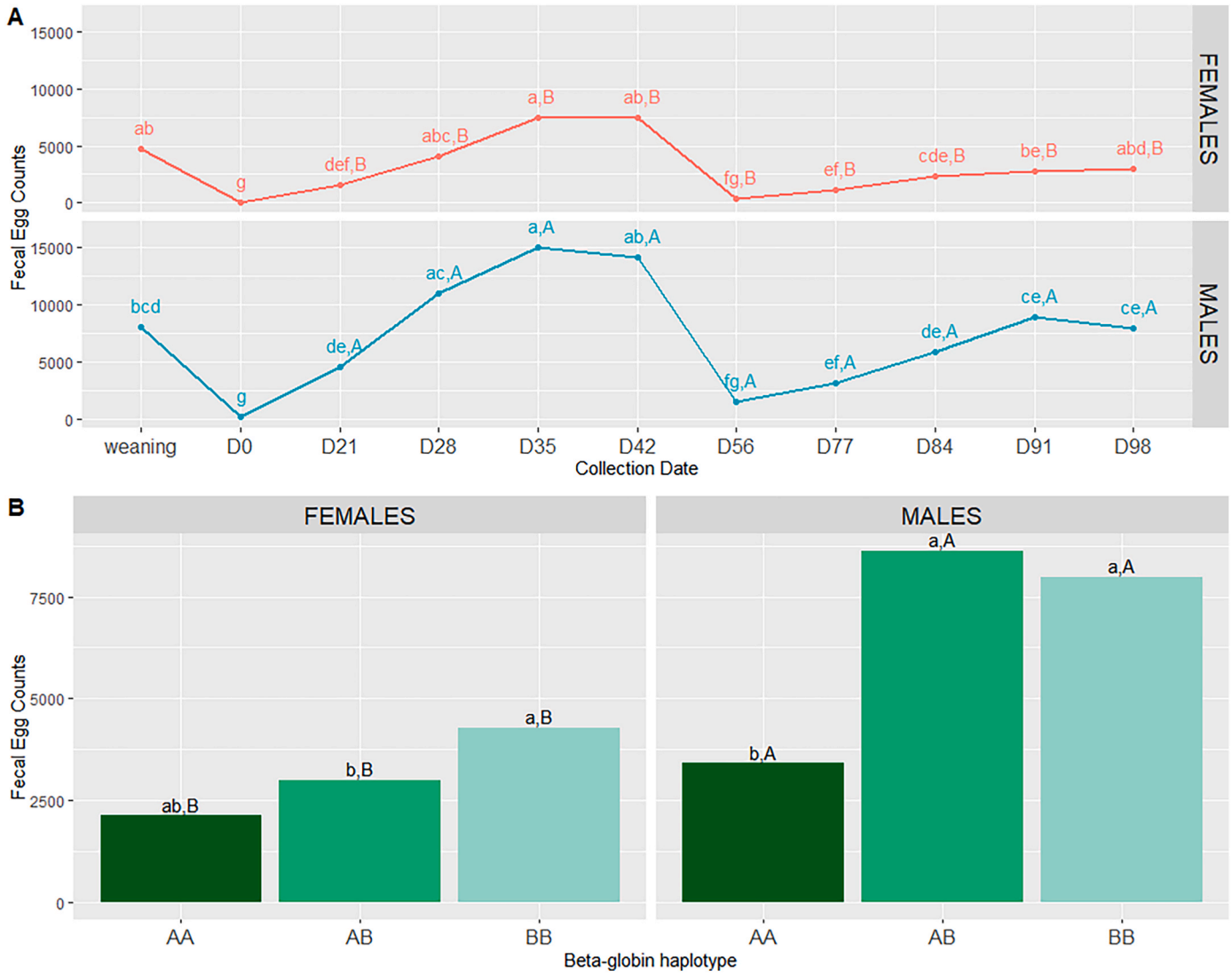

Fig. 3. Fecal egg count means evaluated at different experimental intervals and sex (A) in Morada Nova lambs dewormed at weaning (1°Dw) and at D42 (2°Dw) and submitted to two experimental parasitic challenges (1°Ch at D0 and 2°Ch at D56) with 4000 L_3 of *H. contortus*. Mean values for males and females with different β-globin haplotypes (B). Different lowercase letters indicate significant differences ($p < 0.05$) among time intervals within sex and haplotypes within sex, while different uppercase letters indicate significant differences ($p < 0.05$) between sexes within time interval and within haplotype.

and $p = 0.0001$, respectively).

3.3. Correlation between phenotypes and specific antibody isotype levels

There were no significant correlations between specific anti-*H. contortus* (ES24) antibodies of IgG and IgA isotypes and FEC or PCV levels (Tables 1 and 2).

On the other hand, the specific anti-*H. contortus* (ES24) IgM levels from all evaluated intervals (D0 to D70) were negatively correlated ($p < 0.001$) with FEC from D21 to D42 and from D77 to D98. Stronger negative correlation ($\rho > -0.4$) was observed between IgM levels from D14 and D70 and FEC from D35, D42, D91, and D98 (Table 1).

The IgM antibodies measured on D14 and D70 were positively correlated with PCV levels from all intervals (excepted IgM_D14 and PCV_D0), with stronger positive correlation ($\rho > 0.4$) to PCV on D42 and D98. The IgM levels from D0 and D56 were also significantly correlated with PCV on D28, D42, D84, and D98 (Table 2).

4. Discussion

As expected, females presented improved resistance against *H. contortus* infection during the first and second experimental challenges, based on significantly lower fecal egg counts and higher packed cell volumes compared to males. Higher prevalence and intensity of helminth infections have been found in males compared to female hosts, including birds, rodents, ungulates, and humans (Wesołowska, 2022). Sex hormones, sex chromosomes, microbiome, and immune regulation contribute to sex differences in helminth-host interactions and the immune responses activated (Wesołowska, 2022). Likewise, the development of Th2 responses (required for worm expulsion) in a sex-biased model of *Trichuris muris* infection in mice was mediated by oestradiol, while dehydroepiandrosterone suppressed Th2 immunity (Hepworth et al., 2010). Despite the increased resistance in females being frequently described as associated to hormonal regulation, the immunological mechanisms behind this sex bias remains poorly understood.

In the present study, differential systemic humoral immune responses were elicited after *H. contortus* infection in males compared to females, resulting in higher levels of specific anti-*H. contortus* (ES24) of the IgG isotype in males and of the IgM isotype in females from D14 to D70 after experimental infection. To our knowledge this is the first study to report divergent profiles of humoral responses to *H. contortus* infection between male and female sheep. Mice knockout for IgM displayed

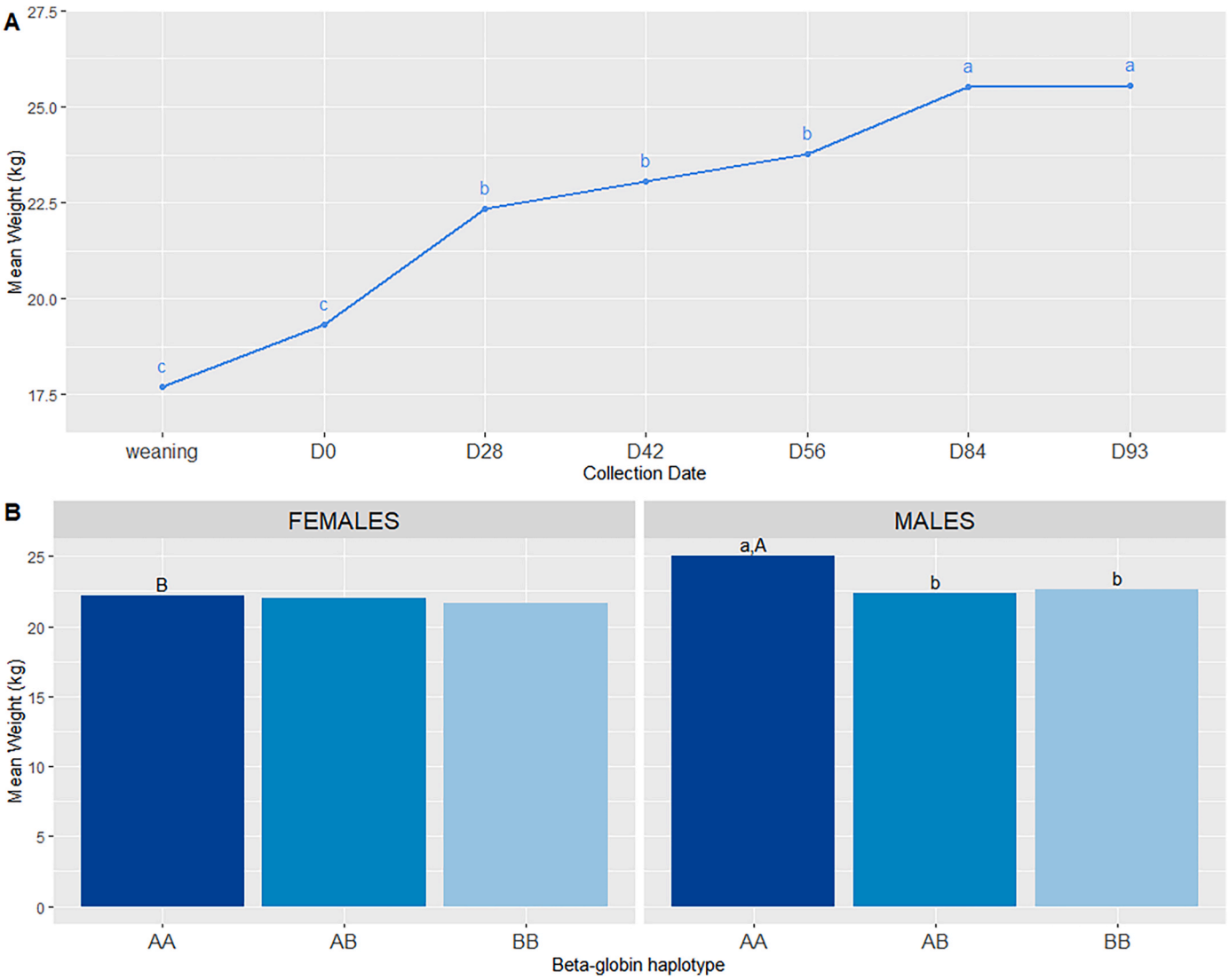

Fig. 4. Weight means evaluated at different experimental intervals (A) in Morada Nova lambs dewormed at weaning (1°Dw) and at D42 (2°Dw) and submitted to two experimental parasitic challenges (1°Ch at D0 and 2°Ch at D56) with 4000 L_3 of *H. contortus*. Mean values for males and females with different β-globin haplotypes (B). Different lowercase letters indicate significant differences ($p < 0.05$) among time intervals and haplotypes within sex, while different uppercase letters indicate significant differences ($p < 0.05$) between sexes within haplotype.

exacerbated disease, mortality, and parasitaemia compared to wild-type controls when experimentally challenged with *Plasmodium chabaudi AS*. Additionally, specific IgM (rather than natural IgM) played an important role in the limitation of parasite replication during asexual erythrocytic infection (Couper et al., 2005). IgM is highly effective for neutralizing and agglutinating pathogens, and to activate the classical complement pathway with 1000-fold higher avidity than IgG, due to its pentameric structure (Cooper, 1985). Passive transference of monoclonal IgM antibody specific for *Strongyloides ratti* HSP60, which is a ES protein actively secreted by helminths, protects mice against infection challenge due to improved complement C3b fixation to IgM coated larvae (Nouir et al., 2012). In another study using *S. stercoralis* in a murine model, effective passive immunity was transferred to naïve mice, and the IgM isotype fraction was able to confer protection, whereas IgG was not, and the depletion of IgE and IgA appeared to have no effect on L3 killing in immunized mice (Brigandi et al., 1996). In the same experiment, IgM was the only isotype that bound to the L3 surface *in vivo* and was correlated with high C3 levels, suggesting IgM-mediated classical pathway of C3 fixation on the L3 surface. These findings demonstrated that IgM-mediated killing of L3 was dependent on cell contact, presence of granulocytes, and complement activation. Similar findings were obtained in previous *in vitro* study (Abraham et al., 1995). Additionally, it was shown that protective antibody isotypes bound to different regions in *S. stercoralis* L3 in mice, therefore, despite both IgM and IgG isotypes are protective against *S. stercoralis* larvae, they recognize different antigens and utilize different killing mechanisms (Ligas et al., 2003). However, in an experimental filarial infection study, IgM was the only isotype that reacted strongly with the surface of *Brugia* L3, and activated peritoneal exudate cells adhered to L3 only in the presence of specific anti-filaria serum or purified IgM fraction, but this attachment was not reduced by heated-inactivated serum, suggesting complement independent activity (Rajan et al., 2005).

Seroepidemiological investigations were performed in a hyperendemic region for malaria, in which specific IgG levels presented the same evolution of parasite loads, while IgM antibodies increased when IgG and parasite levels began to decline (Boudin et al., 1993). In the same study, three different groups were characterized: nonimmune children (with one or more malaria attacks), partially immune children (asymptomatic with high parasitemia loads) and immunoprotected adults, and the IgM levels were lower in nonimmune children compared to the other groups. Besides, a negative correlation was found between parasite loads and specific IgM levels (Boudin et al., 1993). In our study,

Table 1
Correlation coefficients (ρ) and p-values between specific anti-*H. contortus* (ES24) antibody levels of different isotypes (IgG, IgM, and IgA) and fecal egg counts (FEC) in different intervals post experimental infection with *H. contortus* in Morada Nova lambs.

	FEC									
	First experimental challenge					Second experimental challenge				
Ig levels	D0	D21	D28	D35	D42	D56 (D0/2)	D77 (D21/2)	D84 (D28/2)	D91 (D35/2)	D98 (D42/2)
IgG_D0	ρ = −0.0528 (p > 0. 05)	ρ = −0.1393 (p > 0. 05)	ρ = −0.0092 (p > 0. 05)	ρ = −0.0335 (p > 0. 05)	ρ = 0.032 (p > 0. 05)	ρ = −0.0528 (p > 0. 05)	ρ = −0.1393 (p > 0. 05)	ρ = −0.0092 (p > 0. 05)	ρ = −0.0335 (p > 0. 05)	ρ = 0.0308 (p > 0. 05)
IgG_D14	ρ = 0.0514 (p > 0. 05)	ρ = −0.0568 (p > 0. 05)	ρ = 0.0776 (p > 0. 05)	ρ = −0.0015 (p > 0. 05)	ρ = 0.0334 (p > 0. 05)	ρ = 0.0514 (p > 0. 05)	ρ = −0.0568 (p > 0. 05)	ρ = 0.0776 (p > 0. 05)	ρ = −0.0015 (p > 0. 05)	ρ = 0.0334 (p > 0. 05)
IgG_D56	ρ = −0.0528 (p > 0. 05)	ρ = −0.1393 (p > 0. 05)	ρ = −0.0092 (p > 0. 05)	ρ = −0.0335 (p > 0. 05)	ρ = 0.032 (p > 0. 05)	ρ = −0.0528 (p > 0. 05)	ρ = −0.1393 (p > 0. 05)	ρ = −0.0092 (p > 0. 05)	ρ = −0.0335 (p > 0. 05)	ρ = 0.0321 (p > 0. 05)
IgG_D70	ρ = 0.0515 (p > 0. 05)	ρ = −0.0583 (p > 0. 05)	ρ = 0.0776 (p > 0. 05)	ρ = −0.0015 (p > 0. 05)	ρ = 0.0334 (p > 0. 05)	ρ = 0.0515 (p > 0. 05)	ρ = −0.0568 (p > 0. 05)	ρ = 0.0776 (p > 0. 05)	ρ = −0.0015 (p > 0. 05)	ρ = 0.0334 (p > 0. 05)
IgM_D0	ρ = 0.0195 (p > 0. 05)	**ρ= -0.2787 (p=0.0036)**	**ρ= -0.2506 (p=0.0095)**	**ρ= -0.3037 (p=0.0016)**	**ρ= -0.2934 (p=0.0023)**	ρ = 0.0195 (p > 0. 05)	**ρ= -0.2787 (p =0.0036)**	**ρ= -0.2506 (p =0.0095)**	**ρ= -0.3037 (p =0.0016)**	**ρ= -0.2934 (p =0.0023)**
IgM_D14	ρ = −0.1811 (p > 0. 05)	**ρ= -0.3581 (p=0.0001)**	**ρ= -0.3192 (p =0.0008)**	**ρ= -0.4268 (p =5.591e-06)**	**ρ= -0.4269 (p=5.01e-06)**	ρ = −0.1811 (p > 0. 05)	**ρ= -0.3581 (p=0.0001)**	**ρ= -0.3192 (p=0.0008)**	**ρ= -0.4268 (p=5.591e-06)**	**ρ= -0.4269 (p =5.01e-06)**
IgM_D56	ρ = 0.0195 (p > 0. 05)	**ρ= -0.2787 (p=0.0036)**	**ρ= -0.2506 (p =0.0095)**	**ρ= -0.3037 (p=0.0016)**	**ρ=-0.2934 (p=0.0022)**	ρ = 0.0195 (p > 0. 05)	**ρ= -0.2787 (p=0.0036)**	**ρ= -0.2506 (p=0.0095)**	**ρ=-0.3037 (p=0.0016)**	**ρ=-0.2934 (p =0.0022)**
IgM_D70	ρ = −0.1811 (p > 0. 05)	**ρ= -0.3581 (p =0.0001)**	**ρ= -0.3192 (p=0.0008)**	**ρ= -0.4268 (p =5.591e-06)**	**ρ= -0.4269 (p =5.015e-06)**	ρ = −0.1811 (p > 0. 05)	**ρ= -0.3581 (p =0.0001)**	**ρ= -0.3192 (p=0.0008)**	**ρ= -0.4268 (p=5.591e-06)**	**ρ= -0.4269 (p=5.01e-06)**
IgA_D0	ρ = −0.0962 (p > 0. 05)	ρ = −0.1772 (p > 0. 05)	ρ = −0.1045 (p > 0. 05)	ρ = −0.0766 (p > 0. 05)	ρ = −0.0465 (p > 0. 05)	ρ = −0.0962 (p > 0. 05)	ρ = −0.1772 (p > 0. 05)	ρ = −0.1045 (p > 0. 05)	ρ = −0.0766 (p > 0. 05)	ρ = −0.0465 (p > 0. 05)
IgA_D14	ρ = 0.0692 (p > 0. 05)	ρ = −0.037 (p > 0. 05)	ρ = −0.0179 (p > 0. 05)	ρ = −0.044 (p > 0. 05)	ρ = 0.0044 (p > 0. 05)	ρ = 0.0692 (p > 0. 05)	ρ = −0.037 (p > 0. 05)	ρ = −0.0179 (p > 0. 05)	ρ = −0.0441 (p > 0. 05)	ρ = 0.0044 (p > 0. 05)
IgA_D56	ρ = −0.0962 (p > 0. 05)	ρ = −0.1772 (p > 0. 05)	ρ = −0.1045 (p > 0. 05)	ρ = −0.0766 (p > 0. 05)	ρ = −0.0465 (p > 0. 05)	ρ = −0.0962 (p > 0. 05)	ρ = −0.1772 (p > 0. 05)	ρ = −0.1045 (p > 0. 05)	ρ = −0.0767 (p > 0. 05)	ρ = −0.0465 (p > 0. 05)
IgA_D70	ρ = 0.0692 (p > 0. 05)	ρ = −0.037 (p > 0. 05)	ρ = −0.0179 (p > 0. 05)	ρ = −0.044 (p > 0. 05)	ρ = 0.0044 (p > 0. 05)	ρ = 0.0692 (p > 0. 05)	ρ = −0.037 (p > 0. 05)	ρ = −0.0179 (p > 0. 05)	ρ = −0.0441 (p > 0. 05)	ρ = 0.0044 (p > 0. 05)

Bold letters highlight the significant correlation coefficients ($p < 0.05$).

similar findings were observed, since moderated correlation coefficients were observed between IgM levels and FEC (negative correlation) or PCV (positive correlation). These findings point out for important protective role of specific IgM titers against ES24 protein of *H. contortus* infection that may forward alternative therapeutic management of haemonchosis, as previously observed for *Strongyloides* in mice (Brigandi et al., 1996; Nouir et al., 2012).

In this context, some previous studies evaluating ES proteins, reinforce potential use of IgM specific for ES24 protein of *H. contortus* as passive treatment of this parasite infection. ES proteins extracted from adult *H. contortus* were demonstrated to be very antigenic, particularly the 15 and 24 kDa proteins presented strong secondary immune response (Schallig et al., 1994). These ES15 and ES24 proteins were also further successfully applied as vaccines against *H. contortus* (Nisbet et al., 2016; Schallig et al., 1997; Vervelde et al., 2002). While another *H. contortus* ES protein (α/β-hydrolase domain) was demonstrated to be immunomodulatory by interacting with T cells, and when applied as vaccine, presented reduction in the FEC and worm burden in goats (Lu et al., 2021). In this last study, passive immunization with anti-*H. contortus* specific for recombinant α/β-hydrolase ES protein antibodies of IgG isotype conferred substantial protection to challenge (Lu et al., 2021).

Regarding the effect of β-globin polymorphisms in responses developed after *H. contortus* infection, male lambs of the Hb-AA β-globin haplotype presented significantly higher weight compared to other haplotypes, while no significant differences were observed for females. Improved resistance against *H. contortus* infection was also pronounced in Hb-AA males, presenting higher PCV (indicating better red blood cell health) and lower FEC (indicating lower parasite burden) values during experimental infections, which probably lead to the higher weight gain observed in this group. Among females, the Hb-BB presented higher FEC and lower PCV means compared to both Hb-AA and Hb-AB groups. Similar profiles of all specific immunoglobulin kinetics (IgG, IgM and IgA) were observed among female groups, while significantly higher IgG levels were found in the Hb-BB males compared to Hb-AB.

Taken together these findings directly indicate the need of a differential management between sex, with males requiring more attention and monitoring in the farm routine.

The findings of the current study pointed out to an increased impact of β-globin polymorphisms on *H. contortus* infection in male compared to female sheep. This increased effect in sheep males is probably due to their well-known lower general resistance to infections compared to females, wherein specific anti-*H. contortus* (ES24) IgM antibody levels seem to have an important role on the containment of infection in hosts, and this antibody isotype have the potential to be applied in therapeutic management of this parasite infection.

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Table 2
Correlation coefficients (ρ) and p-values between specific anti-*H. contortus* (ES24) antibody levels of different isotypes (IgG, IgM, and IgA) and packed cell volume (PCV) in different intervals post experimental infection with *H. contortus* in Morada Nova lambs.

	PCV							
	First experimental challenge				Second experimental challenge			
Ig levels	D0	D14	D28	D42	D56 (D0/2)	D70 (D14/2)	D84 (D28/2)	D98 (D42/2)
IgG_D0	ρ = 0.0553 (p > 0. 05)	ρ = −0.0916 (p > 0. 05)	ρ = 0.0466 (p > 0. 05)	ρ = −0.0456 (p > 0. 05)	ρ = 0.0553 (p > 0. 05)	ρ = −0.0916 (p > 0. 05)	ρ = 0.0496 (p > 0. 05)	ρ = −0.0456 (p > 0. 05)
IgG_D14	ρ = 0.0518 (p > 0. 05)	ρ = −0.0396 (p > 0. 05)	ρ = −0.0633 (p > 0. 05)	ρ = 0.016 (p > 0. 05)	ρ = 0.0517 (p > 0. 05)	ρ = −0.0396 (p > 0. 05)	ρ = −0.0633 (p > 0. 05)	ρ = 0.016 (p > 0. 05)
IgG_D56	ρ = 0.0553 (p > 0. 05)	ρ = −0.0916 (p > 0. 05)	ρ = 0.0466 (p > 0. 05)	ρ = −0.0456 (p > 0. 05)	ρ = 0.0553 (p > 0. 05)	ρ = −0.0916 (p > 0. 05)	ρ = 0.0466 (p > 0. 05)	ρ = −0.0465 (p > 0. 05)
IgG_D70	ρ = 0.0518 (p > 0. 05)	ρ = −0.0396 (p > 0. 05)	ρ = −0.0633 (p > 0. 05)	ρ = 0.016 (p > 0. 05)	ρ = 0.0517 (p > 0. 05)	ρ = −0.0396 (p > 0. 05)	ρ = −0.0633 (p > 0. 05)	ρ = 0.016 (p > 0. 05)
IgM_D0	ρ = 0.1285 (p > 0. 05)	ρ = 0.1029 (p > 0. 05)	**ρ= 0.2384 (p=0.0133)**	**ρ= 0.3371 (p=0.0004)**	ρ = 0.1285 (p > 0. 05)	ρ = 0.1029 (p > 0. 05)	**ρ= 0.2384 (p=0.0133)**	**ρ= 0.3371 (p=0.0004)**
IgM_D14	ρ = 0.2177 (p > 0. 05)	**ρ= 0.2774 (p=0.0038)**	**ρ= 0.3435 (p=0.0002)**	**ρ= 0.4233 (p=5.541e-06)**	**ρ= 0.2177 (p=0.0242)**	**ρ= 0.2774 (p=0.0038)**	**ρ= 0.3435 (p=0.0002)**	**ρ= 0.4233 (p=5.541e-06)**
IgM_D56	ρ = 0.1285 (p > 0. 05)	ρ = 0.1029 (p > 0. 05)	**ρ= 0.2384 (p=0.0133)**	**ρ=0.3371 (p=0.0003)**	ρ = 0.1285 (p > 0. 05)	ρ = 0.1029 (p > 0. 05)	**ρ= 0.2384 (p=0.0133)**	**ρ=0.3371 (p=0.0003)**
IgM_D70	**ρ= 0.2177 (p=0.0242)**	**ρ= 0.2774 (p=0.0038)**	**ρ= 0.3435 (p=0.0002)**	**ρ= 0.4233 (p=5.541e-06)**	**ρ= 0.2177 (p=0.0242)**	**ρ= 0.2774 (p=0.0038)**	**ρ= 0.3435 (p=0.0002)**	**ρ= 0.4233 (p=5.541e-06)**
IgA_D0	ρ = 0.1736 (p > 0. 05)	ρ = −0.0145 (p > 0. 05)	ρ = 0.089 (p > 0. 05)	ρ = 0.0382 (p > 0. 05)	ρ = 0.1736 (p > 0. 05)	ρ = −0.0145 (p > 0. 05)	ρ = 0.089 (p > 0. 05)	ρ = 0.0382 (p > 0. 05)
IgA_D14	ρ = 0.1556 (p > 0. 05)	ρ = −0.0967 (p > 0. 05)	ρ = −0.0076 (p > 0. 05)	ρ = 0.0163 (p > 0. 05)	ρ = 0.1556 (p > 0. 05)	ρ = −0.0967 (p > 0. 05)	ρ = −0.0076 (p > 0. 05)	ρ = 0.0163 (p > 0. 05)
IgA_D56	ρ = 0.1736 (p > 0. 05)	ρ = −0.0145 (p > 0. 05)	ρ = 0.089 (p > 0. 05)	ρ = 0.0382 (p > 0. 05)	ρ = 0.1736 (p > 0. 05)	ρ = −0.0145 (p > 0. 05)	ρ = 0.089 (p > 0. 05)	ρ = 0.0382 (p > 0. 05)
IgA_D70	ρ = 0.1556 (p > 0. 05)	ρ = −0.0967 (p > 0. 05)	ρ = −0.0076 (p > 0. 05)	ρ = 0.0163 (p > 0. 05)	ρ = 0.1556 (p > 0. 05)	ρ = −0.0967 (p > 0. 05)	ρ = −0.0076 (p > 0. 05)	ρ = 0.0163 (p > 0. 05)

Bold letters highlight the significant correlation coefficients ($p < 0.05$).

CRediT authorship contribution statement

Cintia Hiromi Okino: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Simone Cristina Méo Niciura:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization. **Alessandro Pelegrine Minho:** Writing – review & editing, Methodology, Conceptualization. **Sérgio Novita Esteves:** Methodology. **Gláucia Roberta Melito:** Validation, Methodology, Investigation. **Hélio José Montassier:** Writing – review & editing, Formal analysis. **Ana Carolina de Souza Chagas:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dci.2024.105216.

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