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Breed-Dependent IgG Subclass Responses Are Associated with Differential Bm86 Vaccine Efficacy Against Rhipicephalus microplus in Taurine Cattle

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Abstract

The cattle tick Rhipicephalus microplus is a major constraint to livestock production worldwide, and alternatives to chemical acaricides are needed. This study evaluated breed-associated differences in humoral immune responses, with particular emphasis on IgG subclass dynamics, in two tick-susceptible taurine breeds, Aberdeen Angus and Hereford, following vaccination with a Bm86-based commercial vaccine and controlled Rhipicephalus microplus challenge. Tick biological parameters and anti-Bm86 total IgG, IgG1, and IgG2 responses were assessed using an in-house indirect ELISA and a commercial immunochromatographic strip assay. The rBm86-CG ELISA agreed closely with a commercial immunoblot (AUC 0.998; 95% CI 0.87-1.00; $P < 0.0001$). Hereford cattle developed significantly higher total IgG, IgG1, and IgG2 responses early after vaccination (days 30 and 57), whereas Angus cattle exhibited stronger IgG subclass responses after tick challenge (days 57 and 85). Despite the higher early antibody levels in Hereford animals, vaccine efficacy differed markedly between breeds, reaching 65.87% in Angus but only 21.16% in Hereford cattle. The greater protection observed in Angus was associated with elevated IgG2 responses and an IgG1/IgG2 balance skewed toward IgG2 rather than with higher total anti-Bm86 IgG titers. These findings suggest that differences in IgG subclass profiles may be more informative indicators of protection than total antibody levels alone, and support the hypothesis that breed-associated immune background contributes to differential responsiveness to Bm86-based vaccination. The potential contribution of IgG2 allotypic variation was not tested here and warrants direct investigation in future studies.

1. Introduction

Rhipicephalus microplus is a hard tick belonging to the class Acari and family Ixodidae and represents one of the most economically important ectoparasites affecting cattle worldwide. It is the predominant cattle tick species and a major vector of pathogenic microorganisms, particularly in tropical and subtropical regions, where environmental conditions favor its establishment and dissemination (Weaver et al. 2022). Infestation by R. microplus causes substantial direct and indirect economic losses to the livestock industry through blood loss, skin damage, reduced weight gain and milk production, hide depreciation, and transmission of tick-borne pathogens, resulting in annual losses estimated in the billions of dollars globally (Jonsson et al. 2001; Jongejan and Uilenberg 2004; Strydom et al. 2023). In Brazil, which harbors one of the largest commercial cattle populations worldwide, the combined annual economic impact of endo- and ectoparasites has been estimated at no less than USD 13.96 billion (Grisi et al. 2014; Rodriguez-Vivas et al. 2018). Brazilian cattle production generated approximately USD 100 billion in revenue in 2021, so losses of this magnitude have a direct impact on the sector.

Despite decades of research, a reliable and sustainable strategy for controlling R. microplus infestations remains unavailable. Although chemical acaricides continue to be the primary method for tick control in many countries, their effectiveness is increasingly compromised by the emergence of acaricide resistance, environmental contamination, and concerns regarding chemical residues in animal products. Vaccination has emerged as a promising complementary strategy to reduce tick infestations in cattle

and decrease dependence on chemical control by lowering the frequency and intensity of acaricide application. Commercial anti-tick vaccines based on the concealed antigen Bm86, a glycoprotein predominantly expressed on the membrane of digestive cells in the tick gut, have demonstrated efficacy as an integrated control approach, offering a cost-effective and environmentally sustainable alternative to conventional acaricides (de la Fuente et al. 2007). The efficacy of commercially available vaccines ranges from 51% to 91%, depending on tick populations, bovine genetics, nutritional status, and herd composition (Cunha et al. 2013; Ndawula 2021; Pereira et al. 2022). One of the most effective vaccines commercially available is GAVAC®, formulated with the recombinant glycoprotein Bm86 from R. microplus (rBm86) (de la Fuente et al. 1999; Blecha et al. 2018). In Brazil, GAVAC reduced the infestation in vaccinated bovines by 47% (Andreotti 2006).

A major limitation to the universal efficacy of anti-tick vaccines is the genetic variability of target antigens among geographically distinct tick populations. Recent genomic analyses of 167 R. microplus isolates from the Americas and Pakistan demonstrated that the Bm86 glycoprotein is among the least conserved vaccine targets, exhibiting a protein similarity index of only 0.889 relative to the original Australian TickGARD reference strain (Busch et al. 2025). The high frequency of amino acid substitutions, many of which occur within predicted B-cell epitopes, highlights the challenge of achieving broad cross-protection using a single reference antigen across genetically diverse tick populations. Consequently, in addition to antigenic variability, host-related factors, particularly immune genetic background, may represent critical determinants of vaccine responsiveness and overall efficacy. Bovine hosts present contrasting and heritable phenotypes for tick infestations (Ndawula 2021; Pereira et al. 2022). Breeds of Bos indicus (indicine) cattle are more resistant to R. microplus than those of B. taurus (taurine), possibly because the former were domesticated in Asia (Blecha et al. 2018; de la Fuente et al. 1999), which is also believed to be the place of origin of the parasite (Andreotti 2006). Even after repeated infestations, taurine breeds remain susceptible to tick loads that are unacceptable in terms of animal productivity and health (Kashino et al. 2005; Carvalho et al. 2011; Gong et al. 2014; Tabor et al. 2017; Vlasova and Saif 2021).

One potential heritable determinant of resistance to tick infestation is variation in the constant region of the IgG2 heavy chain. Cattle possess three IgG subclasses (IgG1, IgG2, and IgG3), and during blood feeding, ticks ingest substantial amounts of host immunoglobulins, creating a biologically relevant interface between parasite survival and host humoral immunity (Vlasova and Saif 2021). Following repeated exposure to R. microplus, anti-tick salivary IgG2 antibody levels remain stable in indicine cattle but decline in taurine breeds, despite the greater intake of potentially antigenic tick saliva by the latter (Kashino et al. 2005). These findings suggest that breed-associated differences in IgG2-mediated responses may contribute to variability in resistance to tick infestation.

Polymorphisms in bovine IgG2 allotypes have been associated with differential immune responses to infectious agents (Tabor et al. 2017). The IgG2a and IgG2b allotypes differ in amino acid sequence across the constant region of the heavy chain, encompassing the CH1–CH3 immunoglobulin domains, with functional evidence indicating that IgG2b more effectively activates the bovine complement

cascade. In contrast, cattle carrying the IgG2a allotype appear to exhibit increased susceptibility to extracellular pyogenic infections (Tabor et al. 2017). At the host–parasite interface, Carvalho et al. (2011) proposed that specific IgG2 allotypes may impair the activity of tick immunoglobulin-binding proteins (IGBPs), potentially limit parasite immune evasion, and reinforce the role of IgG2-mediated mechanisms in resistance to tick parasitism.

In addition, host neonatal Fc receptors (FcRn), which protect endocytosed IgG from intracellular degradation and extend its circulatory half-life in cattle, exhibit differential binding affinities toward distinct IgG allotypes (Cervenak and Kacskovics 2009; West and Bjorkman 2000). Furthermore, bovine breeds display quantitative differences in antibody responses against tick salivary antigens, suggesting that host genetic background may influence the magnitude and quality of anti-tick immunity (Kashino et al. 2005).

The present study aimed to characterize breed-associated differences in humoral immune responses following Bm86-based vaccination and controlled R. microplus challenge in two tick-susceptible taurine breeds, Aberdeen Angus and Hereford. Specifically, we evaluated total anti-Bm86 IgG, IgG1 and IgG2 subclass dynamics in relation to tick biological parameters and vaccine efficacy. We expected that differences in the magnitude and subclass composition of the humoral response would follow the breed-dependent variation in protection against R. microplus challenge. No genotyping of IgG2 allotypes was performed; any reference to allotypic variation is therefore presented as a hypothesis rather than as a finding of this study.

2. Materials and Methods

2.1. Animal ethics

All protocols and procedures were reviewed and approved by the Ethics Committee on Animal Experimentation (CEEA No. 2129) of the Universidade Federal de Pelotas (UFPel). The CEEA of UFPel is accredited by the Brazilian National Council for the Control of Animal Experimentation (CONCEA). Cattle were maintained and handled under veterinarian supervision throughout the experiment.

2.2. Expression of recombinant Bm86-CG

The rBm86 gene from the Campo Grande (MS, Brazil) tick strain (rBm86-CG) was cloned into the Komagataella phaffii (Pichia pastoris) expression system, strain GS115, and the rBm86-CG protein was expressed, concentrated, and purified as previously described (Cunha et al. 2011).

2.3. Study area and experimental design

Animals were provided by a local farm located in southern Brazil (33° 31' 8" S, 53° 22' 4" W). This zone is recognized as a "tick- and tick fever-free zone" due to its location below parallel 32°S, since the low temperatures observed during June hinder the survival of the parasite during the free-living stage (Brum et al. 1985).

Twelve healthy female Bos taurus calves (6 Angus and 6 Hereford), aged 9 months and with an average weight of 220 kg, were used. The animals were divided into groups: 4 Angus and 4 Hereford were vaccinated with the commercial vaccine (Gavac®, Herber Biotec S.A, La Habana, Cuba) on days 0, 30, and 57, according to the manufacturer's instructions. The control group was composed of 2 Angus and 2 Hereford heifers receiving placebo injections on days 0, 30, and 57, remaining as unvaccinated controls. All animals had been previously vaccinated with Sintoxan® Polivalente T (Boehringer Ingelheim, São Paulo, SP, Brazil), a commercial C. chauvoei vaccine. During the experiment, animals were treated with intramuscular Imidocarb dipropionate (1.2 mg kg^{-1}) every 15 days to prevent interference from hemoparasites. Blood was harvested from the caudal vein on days 0, 30, 57, and 87 of the experiment.

2.4. Stall test

R. microplus larvae were obtained at the Laboratory of Parasitic Diseases (LADOPAR) of the Faculty of Veterinary Medicine, Federal University of Pelotas, Brazil. The isolates used in this study were obtained from parasitic infestations in beef and dairy herds, from properties with a history of chemical resistance to several active ingredients commonly used to control tick infestations. Engorged adult female R. microplus ticks were incubated in a biological oxygen demand (BOD) chamber at 27 ± 1°C and relative humidity (RH) ≥ 80% to allow oviposition. Eggs were harvested on the 14th day of incubation, stored in empty test tubes, sealed, and incubated under the same conditions until hatching.

The same twelve heifers were housed in individual stalls with slats and a daily tick collection system in the isolation unit of Fazenda Três Figueiras, in Cascata, municipality of Pelotas, RS. The diet was based on hay, commercial ration concentrated at 16% crude protein, mineral salt, and water available ad libitum. Each heifer was artificially infested with 2,000 R. microplus larvae directly on the back (day 75), with alternate infestations on days 77 and 79, totaling 6,000 larvae. Heifers were monitored daily for tick development (day 93 to day 113). Each stall was washed individually every day to recover detached ticks, which were then sorted, dried, and sent to the laboratory for incubation.

2.5. Tick biological parameters and calculation of vaccine efficacy

For each animal, detached and engorged females (≥ 4.5 mm) were collected and divided into three subsets of 10 ticks, weighed and measured to establish uniformity among the different experimental groups, fixed, and identified in disposable Petri dishes inside an incubator (27 ± 1°C and RH ≥ 80%) for oviposition. After 18 days, the laid eggs were weighed and transferred to adapted transparent plastic syringes (10 ml), identified, and sealed with cotton. They were then placed back in the incubator under the same conditions for egg hatching (Drummond et al. 1973). After 15 days, the hatching rate was evaluated from the syringes. Readings were performed visually by a single trained technician.

The mean weight of the eggs laid was used to express the reproductive capacity of ticks, and fertility was expressed as the percentage of viable larvae (Andreotti 2006). These parameters were used to

calculate the vaccine efficacy as a combination of the reduction in the number of engorged ticks, in their egg-laying capacity, and in the hatchability of the eggs, according to Cunha et al. (2013):

Efficacy (%) = 100 × [1 − (CRT × CRO × CRF)]

where CRT, CRO, and CRF are, respectively, the ratio of the number of engorged ticks, the ratio of egg mass weight, and the ratio of hatchability in the vaccinated group, as compared with the control group. CRT and CRO were computed from the data presented in Table 1, and CRF from the data presented in Table 2.

2.6. Commercial immunochromatographic strip (ICS) test

An indirect lateral flow immunochromatographic system for the rapid detection of anti-Bm86 antibodies in vaccinated cattle was used (Vargas-Hernández et al. 2021). Briefly, each strip was introduced into bovine serum previously diluted 1:8 (60 µl in 440 µl of phosphate-buffered saline – PBS), dissolving the Bm86 conjugate labeled with colloidal gold.

2.7. In-house indirect ELISA

An in-house indirect rBm86-CG ELISA test was performed according to Cunha et al. (2011), and an in-house indirect C. chauvoei ELISA was performed according to Crichton et al. (1990) and Santos et al. (2021). Briefly, the assay conditions were as follows: 96-well microtiter plates (Nunc-Immuno MicroWell PolySorp, Nunc) were coated overnight with 200 ng/well of respective antigen, rBm86-CG and C. chauvoei antigen diluted in CBB at 4°C. Plates were washed 3× with PBS-T, blocked for 1 h at 37°C with PBS-T plus 0.5% skim milk powder and 0.3% casein. After washing, serum samples diluted in PBS-T (1:800 for rBm86-CG and 1:100 for C. chauvoei) were added individually in duplicate to the wells, and the plates were incubated for 1 h at 37°C. After three additional washes, horseradish peroxidase (HRP)-conjugated goat anti-bovine IgG (Sigma-Aldrich) was diluted 1:10,000 in PBS-T and added to the plates, followed by incubation at 37°C for 1.5 h. Subsequently, plates were washed five times with PBS-T, and 100 µl/well of peroxidase substrate o-phenylenediamine dihydrochloride (OPD; Sigma-Aldrich) diluted in 0.07 mM citrate-phosphate buffer (pH 4.2), with 0.03% (v/v) H_2O_2, was added. Plates were incubated for 15 min in the dark, and the reaction was stopped with 2 N H_2SO_4. Optical densities were read using an ELISA TP-Reader plate spectrophotometer (ThermoPlate) at 492 nm, expressing the total specific IgG against rBm86-CG or against total C. chauvoei antigens.

2.8. IgG isotyping against rBm86-CG and C. chauvoei

IgG1 and IgG2 isotype levels against rBm86-CG and total C. chauvoei antigens were evaluated by in-house indirect ELISA using individual sera. The plates were washed 3× and incubated for 1 h at 37°C with serum samples diluted 1:800 and 1:100 in PBS-T, respectively, for rBm86-CG and C. chauvoei antigens. Then, anti-isotype antibodies were diluted 1:5,000, and the ELISA was performed according to the instructions of the Abcam isotyping kit for IgG1 and IgG2 detection.

2.9. Statistical analysis

Vaccination effects on tick biology and efficacy were determined as described by García-García et al. (2000) and Andreotti (2006). Briefly, reduction rates associated with the anti-tick vaccine immunization, relative to the unvaccinated group, were determined for the number of adult female ticks (%DT), weight of adult female ticks (%DW), egg-laying capacity (%DO), and fertility (%DF). Vaccine efficacy was calculated as 100 × [1 – (CRT × CRO × CRF)], according to Cunha et al. (2013). Tick biological parameters measured at the level of the individual animal (number, weight and egg mass of engorged females, and hatching rate) were compared between groups using the Mann-Whitney non-parametric test. Mean antibody levels were determined for each group and compared using ANOVA, as described previously (Cunha et al. 2013; Andreotti 2006). Vaccine efficacy was computed at the group level as a composite ratio and is therefore reported as a point estimate without an associated significance test, since the calculation does not operate on individual experimental units. Given the limited number of control animals per breed (n = 2), efficacy estimates should be interpreted as descriptive and carry substantial uncertainty. Receiver-operating characteristics (ROC) curves, the area under the curve (AUC), the determination of the optimal cut-off point, and the inter-rater agreement (κ coefficient) were obtained using IBM SPSS Statistics, version 20. The κ coefficient was calculated to determine the magnitude of the statistical agreement between two tests.

3. Results

3.1. Tick counting, biological parameters, and vaccine efficacy

After the experimental infestation period, the mean number of engorged females collected (%DT) from the Angus vaccinated group (103 ± 18; n = 4) was 69.36% lower than that obtained from the Angus control group (337 ± 18; n = 2). In contrast, for the Hereford vaccinated group (293 ± 46; n = 4), no reduction was observed when compared to the Hereford control group (284 ± 21; n = 2); the mean was in fact 3.08% higher, although this difference was not statistically significant (Table 1).

Table 1 *Rhipicephalus microplus* engorged females recovered from each animal: number, average weight and average egg mass. Means are given as mean ± standard deviation, calculated from the individual values of each group

Group-Breed	Animal	Number of engorged females	Engorged female average weight (g)	Average egg mass (g)
Control Angus	615	324	0.317	0.157
	619	350	0.351	0.168
	Mean	337 ± 18	0.334 ± 0.024	0.163 ± 0.008
Control Hereford	679	269	0.243	0.089
	680	299	0.242	0.153
	Mean	284 ± 21	0.243 ± 0.001	0.121 ± 0.045
Gavac Angus	624	113	0.307	0.151
	640	79	0.328	0.172
	643	101	0.319	0.167
	644	120	0.353	0.190
	Mean	103 ± 18	0.327 ± 0.020	0.170 ± 0.016
Gavac Hereford	670	313	0.236	0.087
	674	346	0.216	0.098
	684	269	0.284	0.107
	685	243	0.265	0.116
	Mean	293 ± 46	0.250 ± 0.030	0.102 ± 0.012

The weight of engorged females (%DW) did not differ statistically between breeds or groups ($P > 0.05$). Ticks fed on Hereford animals (vaccinated and control) presented a lower average egg mass (0.102 ± 0.012 and 0.121 ± 0.045 g, respectively) than those fed on Angus animals (0.170 ± 0.016 and 0.163 ± 0.008 g, respectively). The mean hatching rates obtained from individual animal counts (Table 2) were 77.72 ± 2.97% (Angus vaccinated), 73.00 ± 1.13% (Angus control), 73.67 ± 6.94% (Hereford vaccinated), and 81.20 ± 5.52% (Hereford control).

Table 2 Egg hatching rate per animal and vaccine efficacy per group. Vaccine efficacy was calculated at the group level as 100 × [1 − (CRT × CRO × CRF)] and is therefore reported as a single value per vaccinated group

Group-Breed	Animal	Hatching rate (%)	Vaccine efficacy (%), group level
Control Angus	615	72.2	–
	619	73.8	
	Mean	73.00 ± 1.13	
Control Hereford	679	77.3	–
	680	85.1	
	Mean	81.20 ± 5.52	
Gavac Angus	624	77.8	65.87
	640	73.5	
	643	80.0	
	644	79.6	
	Mean	77.72 ± 2.97	
Gavac Hereford	670	64.2	21.16
	674	80.9	
	684	74.6	
	685	75.0	
	Mean	73.67 ± 6.94	

Applying the formula proposed by Cunha et al. (2013) to all three components (CRT × CRO × CRF), vaccine efficacy reached 65.87% in the Angus group and 21.16% in the Hereford group (Table 2). The decomposition by parameter shows that the protective effect in the Angus group is essentially driven by the reduction in the number of engorged females (%DT = 69.36%), whereas %DO and %DF were close to zero or marginally negative (–4.62% and –6.47%, respectively). In the Hereford group, the modest overall efficacy results from a contribution of %DO (15.70%) and %DF (9.27%), against an absence of effect on the number of engorged females (%DT = –3.08%). The protective phenotypes observed in the two breeds therefore differ in magnitude and in the biological parameter affected.

3.2. Immunochromatographic strip (ICS) test

The HeberFast® Line Gavac (Heber Biotec S.A., Havana, Cuba) for Bm86 did not show any positive response on day 0 in any group. By day 57, three out of six animals in each vaccinated group were positive. On day 85, four out of six were positive in the Hereford group and three out of six in the Angus group. Reactivity was observed only in vaccinated animals, although one vaccinated Angus animal did not present reactivity (Table 3).

Table 3 Results of the commercial immunochromatographic strip (ICS) test for anti-Bm86 antibodies in each animal on days 0, 57 and 85. Animals 615, 619, 679 and 680 were unvaccinated controls

Breed	Animal	Day 0	Day 57	Day 85
Hereford	670	Negative	Positive	Positive
	674	Negative	Negative	Positive
	679	Negative	Negative	Negative
	680	Negative	Negative	Negative
	684	Negative	Positive	Positive
	685	Negative	Positive	Positive
Angus	615	Negative	Negative	Negative
	619	Negative	Negative	Negative
	624	Negative	Positive	Positive
	640	Negative	Positive	Positive
	643	Negative	Negative	Negative
	644	Negative	Positive	Positive

3.3. Development of the rBm86-CG ELISA and ROC analysis

The optimal concentration of rBm86-CG for plate coating was established at 200 ng/well, with sera diluted at 1:800. When compared to the commercial Immunoblotting test, the rBm86-CG ELISA developed here showed an area under the ROC curve (AUC) of 0.998 (Fig. 2a). The 95% confidence interval (CI) of the AUC ranged from 0.87 to 1.00, with a significance level (against Area = 0.5) of $P < 0.0001$. Based on the confusion matrix, the overall proportion of correctly classified samples was 97.2% (35/36).

The ROC analysis showed that the optimal cut-off point was the optical density at 492 nm of 0.271, corresponding to a sensitivity of 100% and a specificity of 95.2%. All 15 Immunoblotting-positive results were considered true positives, since the corresponding ELISA readings were above the optimized cut-off. Among the 21 Immunoblotting-negative results, 1 sample (4.76%) was considered a false positive, with the ELISA reading above the cut-off, while 20 samples (95.24%) showed ELISA readings below the cut-off and were considered true negatives (Fig. 2b). A κ coefficient of 0.952 was calculated between the commercial Immunoblotting test and the rBm86-CG ELISA.

3.4. Dynamics of specific IgG and isotypes against rBm86-CG and C. chauvoei

All groups responded to vaccination. The Angus breed showed a highly significant ($P < 0.0001$) increase in total anti-rBm86-CG IgG compared to its control group on day 57. After the third dose (day 57), both Hereford and Angus presented a significant ($P < 0.0095$ and $P < 0.0048$, respectively) increase in anti-rBm86-CG IgG by day 85 (Fig. 3). The rBm86-CG indirect ELISA showed differences in IgG1 dynamics on day 30 between vaccinated Hereford and Hereford control ($P < 0.005$), and between vaccinated Hereford and Angus control ($P < 0.001$) (Fig. 3). On days 57 and 87, both vaccinated groups presented significant statistical differences compared to their control groups ($P < 0.001$).

The Angus group did not show statistically significant differences in IgG2 on day 30, although the overall dynamics paralleled that of IgG1: both vaccinated groups presented statistical significance ($P < 0.001$) on days 57 and 87 when compared to their respective controls (Fig. 3).

The Angus group exhibited progressively increasing anti-*C. chauvoei* IgG levels following each vaccination, whereas the Hereford group displayed a different pattern, with high baseline IgG levels already present on day 0 (Fig. 4).

Statistically significant differences ($P < 0.001$) were observed for *C. chauvoei* IgG1 when comparing Angus and Hereford breeds on days 0, 30, and 57; only on day 87 did the Hereford group show an increased IgG1 response. The *C. chauvoei* IgG2 response was significantly lower in the Hereford group on days 0 ($P < 0.009$), 30 ($P < 0.001$), 57 ($P < 0.001$), and 87 ($P < 0.001$) (Fig. 4).

4. Discussion

In this study a stronger antibody response did not result in greater protection. Hereford cattle mounted the higher and earlier anti-Bm86 IgG response yet showed the lower vaccine efficacy, whereas Angus cattle, with lower early titres, were better protected. This dissociation between antibody levels and parasitological outcome is the main result reported here, and indicates that total antibody levels alone are poor correlates of protection. Instead, vaccine efficacy was associated with the quality of the humoral response, as animals with higher IgG2 levels and an IgG1/IgG2 ratio skewed toward IgG2 exhibited the lowest tick burdens. These findings agree with previous reports linking IgG2-dominant responses to enhanced protection against Rhipicephalus microplus (Scoles et al. 2022) and support the concept that IgG subclass polarization, reflecting Th1/Th2 balance, is a more informative predictor of vaccine efficacy than total IgG levels (Estes and Brown 2002).

Few studies have examined breed-associated IgG responses to anti-tick vaccines. Here, we compared two tick-susceptible, R. microplus-naïve taurine breeds (Bos taurus), Aberdeen Angus and Hereford, immunized with a commercial Bm86-based vaccine. The in-house rBm86-CG ELISA showed high concordance with the commercial assay while allowing characterization of IgG1 and IgG2 responses. Vaccine efficacy differed markedly between breeds, reaching 65.87% in Angus and 21.16% in Hereford cattle. In Angus, protection was primarily associated with reduced numbers of engorged females, whereas surviving ticks showed little impairment in reproductive parameters, suggesting that antibodies mainly affected tick feeding. Conversely, Hereford cattle showed little reduction in tick burden but a

tendency toward reduced egg mass and hatching, indicating partial effects on reproduction. These contrasting patterns indicate that the components contributing to the composite efficacy estimate differed between breeds. Whether this reflects a genuinely distinct biological mechanism of protection cannot be established from the present data and requires confirmation in larger studies.

Protection induced by Bm86 vaccines depends on antibodies that cross the tick gut, reach the hemolymph, and bind internal tissues, impairing parasite survival and reproduction (Ackerman et al. 1981; Fujisaki et al. 1984; Wang and Nuttall 1999). However, tick immunoglobulin-binding proteins (IGBPs) can interfere with antibody function, emphasizing that antibody quality, rather than quantity alone, determines vaccine efficacy (Gong et al. 2014).

Breed-associated differences in IgG subclass responses have been reported previously, although results remain inconsistent (Kashino et al. 2005; Piper et al. 2009, 2017; Garcia et al. 2017). In the present study, Angus cattle developed stronger IgG1 responses than Hereford cattle, whereas animals with predominant IgG2 responses exhibited the greatest protection. Likewise, Cunha et al. (2021) reported that booster vaccination preferentially increased IgG2 over IgG1, consistent with a Th1-biased response. Because IgG2 more efficiently promotes opsonization, phagocytosis, and complement activation (McGuire et al. 1979; Bastida-Corcuera et al. 1999), these observations support a functional role for IgG2 in protective anti-tick immunity.

One possible mechanism is bovine IgG2 allotypic variation. Previous work has described functional differences between IgG2 allotypes, including differences in complement activation and in recognition by commonly used anti-bovine IgG2 reagents (Kooyman et al. 2025). Because IgG2 allotypes were not genotyped in the present study, this mechanism remains speculative and cannot be linked directly to the breed-associated differences observed here. Moreover, widely used monoclonal antibodies, including clone MCA626, preferentially detect IgG2a and poorly recognize IgG2b (Noble et al. 2024; Kooyman et al. 2025). Consequently, animals with strong IgG2b responses may be underestimated in conventional serological assays, potentially explaining discrepancies among studies evaluating breed-associated immunity. Differential IGBP binding to IgG allotypes may further contribute to variation in host resistance (Carvalho et al. 2011).

Although Bm86 sequence variation may also influence vaccine efficacy, the Campo Grande antigen shares approximately 96.5% amino acid identity with the recombinant antigen used in commercial vaccines (Andreotti et al. 2008). Therefore, the pronounced breed-dependent differences observed here likely reflect host immune variation in addition to antigenic diversity. The detectable anti-Clostridium chauvoei antibody responses in both breeds suggest that the lower Bm86 vaccine efficacy observed in Hereford cattle cannot be readily explained by a generalized inability to mount humoral responses. However, differences in baseline levels and subclass dynamics against C. chauvoei indicate that breed-associated variation may extend beyond the Bm86 antigen. These results should therefore be read as complementary evidence of immune responsiveness and not as proof of Bm86-specific immune regulation.

This study has some limitations. The small number of animals, inherent to controlled stall-based experiments, may limit the generalizability of the findings. In addition, IgG2 allotypes were not directly genotyped; thus, the proposed role of IgG2a/IgG2b variation is inferred from subclass dynamics rather than confirmed genetically. Larger studies incorporating IgG2 allotyping will be important to validate the relationship between IgG subclass polarization and protection against R. microplus.

5. Conclusion

This study has several limitations that should be considered when interpreting the findings. First, the number of animals was small and the design was unbalanced, with only two control animals per breed. This was dictated by the constraints of controlled stall-test experiments and by the ethical principle of using the minimum number of animals, but it substantially reduces statistical power and means that the vaccine efficacy estimates, which are ratios computed from these group means, carry considerable uncertainty that is not captured by a confidence interval. Second, distinct IgG subclass profiles were observed together with differences in vaccine efficacy, but the study was not designed to establish a causal relationship between IgG2 responses and protection. Third, IgG2 allotypes were not genotyped, so any contribution of IgG2a/IgG2b variation remains hypothetical. Finally, the results were obtained under controlled stall-test conditions and require validation in larger and independent populations under field conditions.

The rBm86-CG indirect ELISA developed in this study is a reliable alternative to commercial immunoblotting assays for detecting anti-Bm86-specific IgG while enabling characterization of IgG1 and IgG2 subclass responses. Our findings demonstrate marked breed-dependent differences in the efficacy of a commercial Bm86-based vaccine between two tick-susceptible taurine breeds, with protection reaching 65.87% in Aberdeen Angus and 21.16% in Hereford cattle. Importantly, vaccine efficacy was more closely associated with IgG subclass polarization than with total anti-Bm86 IgG titers, with an IgG1/IgG2 balance skewed toward IgG2 corresponding to greater protection. These results support breed-associated immune background as a potential contributor to differential responsiveness to Bm86-based vaccination. The distinct IgG subclass profiles and the contrasting efficacy estimates indicate that the IgG1/IgG2 balance is a potential correlate of protection. However, these are preliminary data and this needs to be further evaluated in future studies, combining functional antibody assays with direct IgG2 allotyping.

Declarations

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Competing interests

The authors declare that they have no conflicts of interest.

Ethics approval

Cattle were maintained and handled under veterinarian supervision. All protocols and procedures were reviewed and approved by the Ethics Committee on Animal Experimentation (CEEA No. 2129) of the Universidade Federal de Pelotas (UFPel). The CEEA of UFPel is accredited by the Brazilian National Council for the Control of Animal Experimentation (CONCEA).

Consent to participate

Not applicable. This study did not involve human participants.

Consent for publication

Not applicable.

Author contributions

Araujo IL contributed to the experiment design, performed most of the experiments, and wrote most of the manuscript. Martins KR contributed to the stall test and to the tick collections and counts. Andreotti R provided the Bm86-CG antigen and contributed to the analyses of results. Piraine REA contributed to the production of the recombinant antigen and to the standardisation of the ELISA. Conrad NL contributed to the ELISA and to the IgG isotyping assays. Albuquerque PMM contributed to the serological assays and to the analyses of results. Cunha RC contributed to the experiment design and to the analyses of results, and Leite FPL designed the study, analysed the results, and wrote the manuscript. All authors of this paper have approved the submission of this manuscript.

Data availability

All data generated or analysed during this study are included in this published article.

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Figures

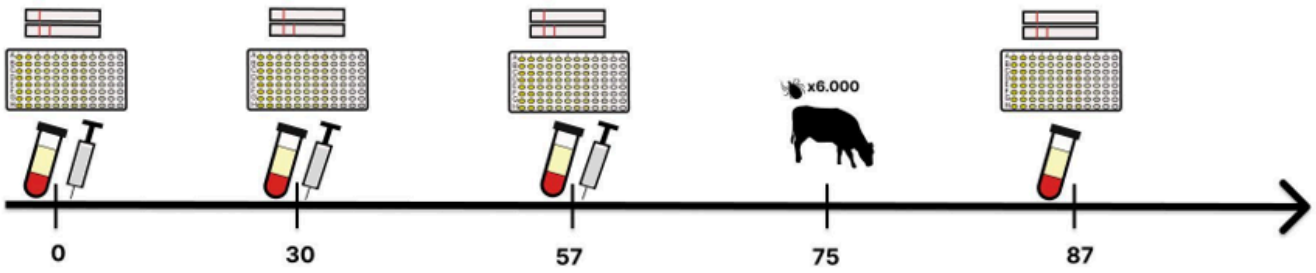


Figure 1

Schematic presentation of the study design. Twelve healthy female calves aged nine months received vaccinations on days 0, 30 and 57, and blood samples were collected on days 0, 30, 57 and 87. Animals were artificially infested with *Rhipicephalus microplus* larvae from day 75, and detached engorged females were recovered daily

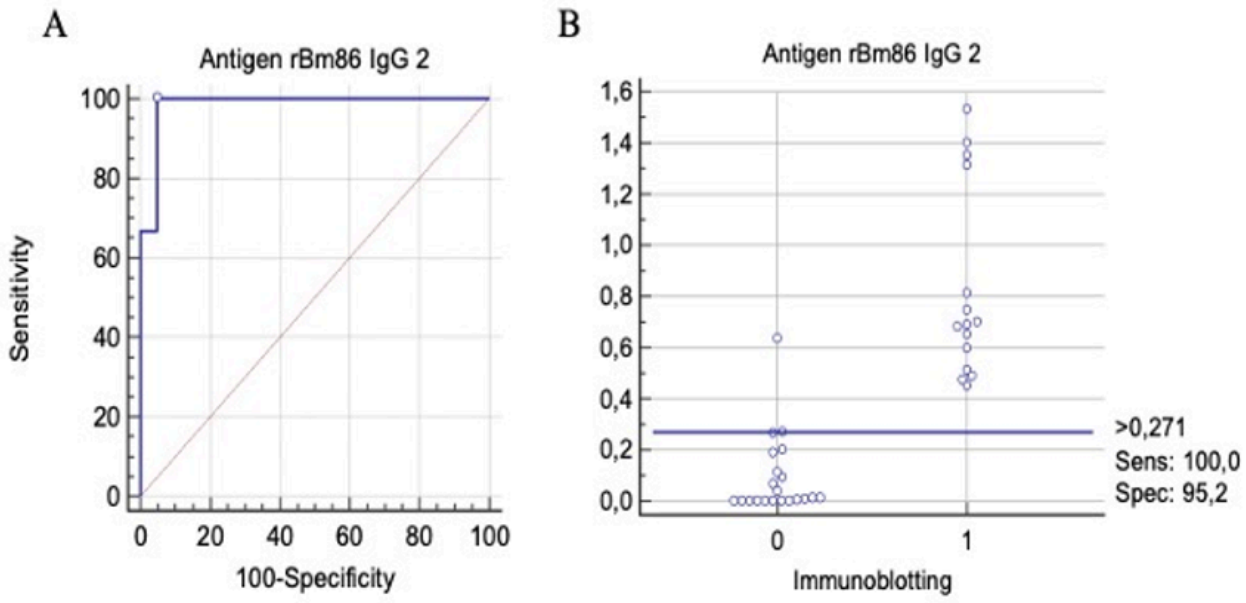


Figure 2

Receiver operating characteristic (ROC) analysis of the in-house rBm86-CG indirect ELISA validated against the commercial immunoblotting assay. a ROC curve, area under the curve 0.998 (95% CI 0.87-1.00). b Distribution of optical density values at 492 nm relative to the optimised cut-off of 0.271

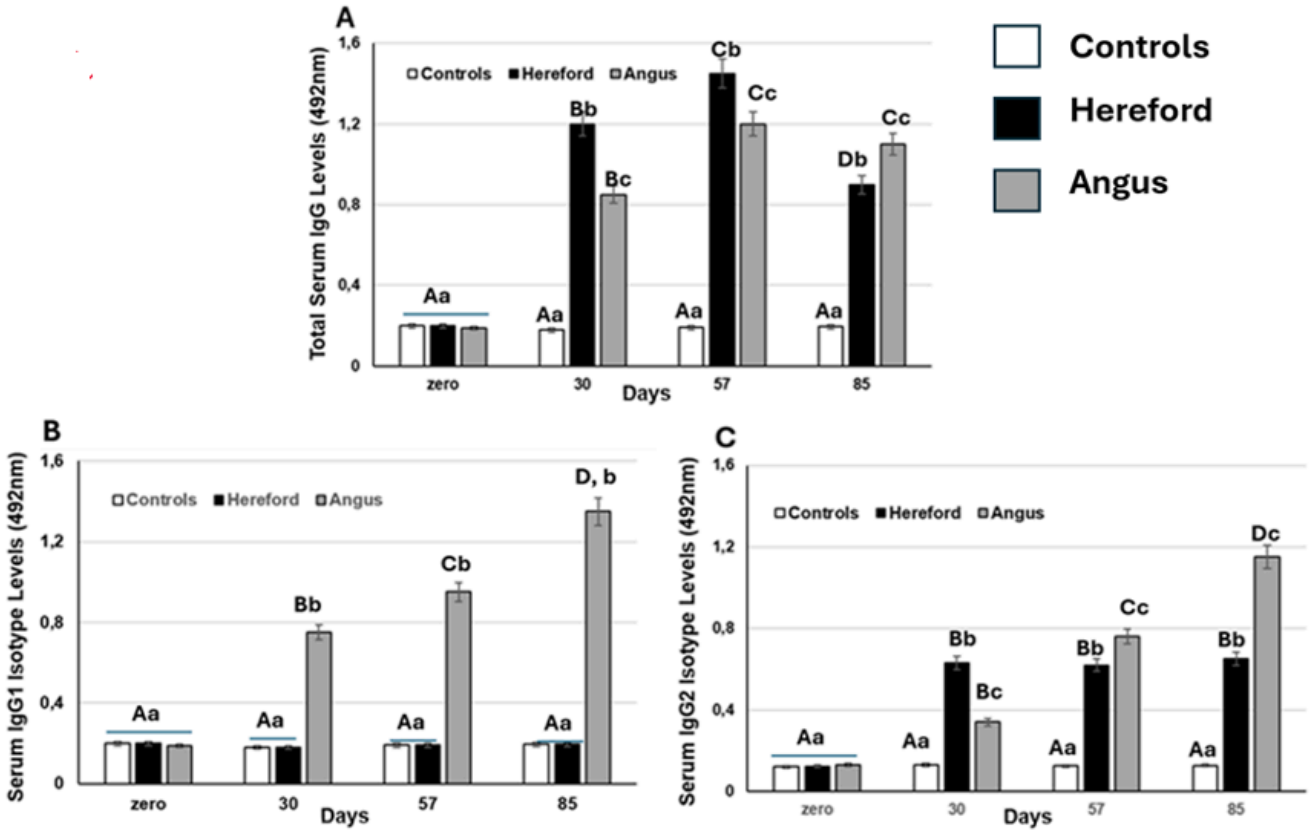


Figure 3

Dynamics of total IgG, IgG1 and IgG2 against rBm86-CG in vaccinated and control Aberdeen Angus and Hereford cattle on days 0, 30, 57 and 87. Asterisks indicate statistical differences ($P < 0.05$) relative to the corresponding control group

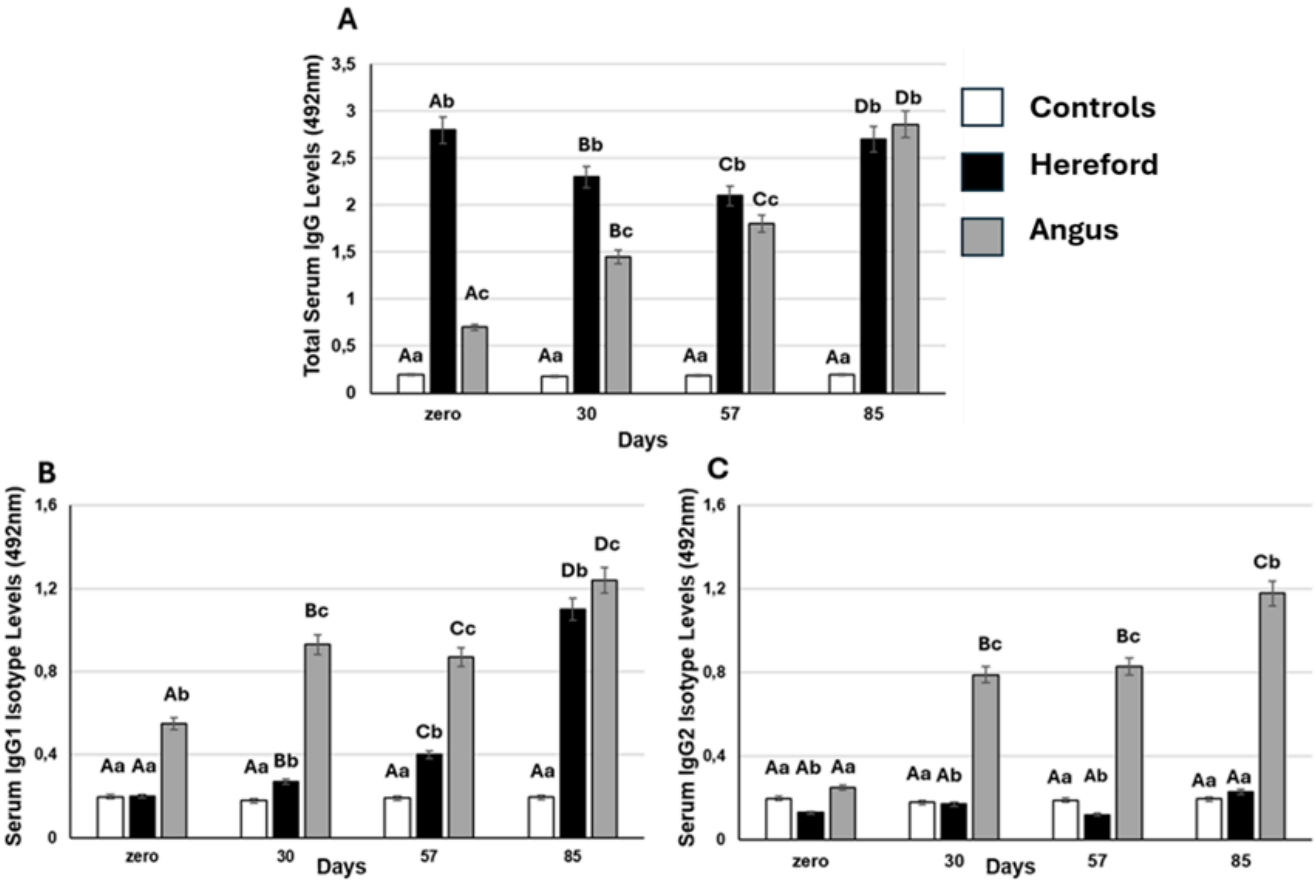


Figure 4

Dynamics of total IgG, IgG1 and IgG2 against *Clostridium chauvoei* in vaccinated and control Aberdeen Angus and Hereford cattle on days 0, 30, 57 and 87. Asterisks indicate statistical differences ($P < 0.05$) between breeds