Ticks and Tick-borne Diseases 16 (2025) 102546

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Original article

Expression patterns of chemokine mediators and TLR10 in natural bovine babesiosis and anaplasmosis infections

Mayne Barboza Sarti [a], Geovana Menegão de Souza [a], Camila Fagionato Agostinho [b], Gustavo Henrique Carvalho Borges [a], Flávia Fernanda Carneiro Santana [b], Luciana Morita Katiki [b], Anibal Eugênio Vercesi Filho [b], Ana Gonçalves Domingos [c], Rodrigo Giglioti [a,b,*]

[a] Universidade Estadual Paulista Júlio de Mesquita Filho, Jaboticabal, São Paulo, Brazil
[b] Instituto de Zootecnia, Nova Odessa, São Paulo, Brazil
[c] Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal

ARTICLE INFO

Keywords:
Chemokines
TLRs
Tick fever
Immune modulation
Gene expression

ABSTRACT

Cattle tick fever, caused by the protozoa *Babesia bovis* and *B. bigemina* and the rickettsial bacterium *Anaplasma marginale*, represents a significant constraint to livestock productivity worldwide. Taurine cattle are considered more susceptible to these hemoparasites than zebuine breeds. Chemokines and Toll-like Receptors (TLRs) play key roles in immune processes such as chemotaxis and inflammation, primarily through interactions with respective receptors. This study assessed the relationship between the expression of four chemokine-related genes (*ccr3, cxcl12, cxcl8,* and *cxcr1*) and one TLR (*tlr10*) with the infection levels of *B. bovis, B. bigemina*, and *A. marginale* in 24 naturally infected calves from two genetic groups (Angus [$n = 13$; 100% taurine] and Ultrablack [$n = 11$; 82% Angus, 18% zebuine]). Blood samples were collected every 30 days over six time points (Nov 2021–Apr 2022). DNA was used to quantify hemoparasite loads by qPCR (log DNA copy number, CNlog), while RNA was used for RT-qPCR-based gene expression analysis. A mixed model was used to assess associations between gene expression and infection levels, controlling for evaluation, sex, genetic group, and their interactions. All genes, except *cxcl12*, showed significant associations with at least one hemoparasite. Higher *B. bovis* and *B. bigemina* CNlog values were linked to reduced expression of most genes, whereas *A. marginale* infection was associated with increased gene expression. These findings highlight distinct immune modulation strategies by hemoparasites and underscore the need for species-specific interventions. Further studies are required to explore the mechanisms by which these mediators influence susceptibility or resistance in cattle.

1. Introduction

Cattle tick fever is a disease complex involving infections by the protozoa *Babesia bovis* and/or *B. bigemina*, as well as the rickettsial bacterium *Anaplasma marginale* (Guglielmone and Robbins, 2023). In Brazil, the tick *Rhipicephalus microplus* is the primary vector of bovine babesiosis and anaplasmosis. Tick fever is considered one of the most significant parasitic diseases affecting cattle, causing substantial economic losses in the Brazilian livestock industry. According to Grisi et al. (2014), parasitic infestations in cattle result in annual economic losses of approximately US$13.96 billion in Brazil, with *R. microplus* alone responsible for about US$3.24 billion.

These infections may occur asynchronously or manifest simultaneously in the same animal. Babesiosis parasitizes erythrocytes, causing intravascular hemolytic anemia, which is the primary clinical sign (Bock et al., 2004). In contrast, anaplasmosis leads to anemia and extensive hemolysis (Kocan et al., 2010; Tucker et al., 2016). The economic impacts of babesiosis and bovine anaplasmosis extend beyond mortality and include morbidity, abortions, reduced meat and milk production, increased veterinary costs, and restrictions on international trade of cattle products (Kocan et al., 2003; Bock et al., 2004). Both diseases have high morbidity and mortality rates, particularly in areas of enzootic instability (McCosker, 1981).

Zebu breeds are widely used in tropical regions due to their greater

* Corresponding author.
E-mail address: rodrigo.giglioti@sp.gov.br (R. Giglioti).

https://doi.org/10.1016/j.ttbdis.2025.102546
Received 15 April 2025; Received in revised form 21 August 2025; Accepted 4 September 2025
Available online 19 September 2025

resistance to ticks and tick-borne diseases. Crossbreeding taurine and zebu breeds is a strategy aimed at increasing productivity and has attracted interest among Brazilian producers seeking to improve herd efficiency (Ibelli et al., 2012). However, productivity gains from the introduction of taurine breeds and their crosses into beef cattle systems are often limited by losses caused by hemoparasite infections and tick infestations (Oliveira et al., 2013). It has been observed that Zebu cattle (*Bos taurus indicus*) show greater resistance to *B. bovis, B. bigemina*, and *A. marginale* infections than European cattle (*Bos taurus*) (Parker et al., 1990; Bock et al., 1997).

Chemokines are small molecules initially described as chemotactic cytokines (Luster, 1998), playing a fundamental role in both innate and adaptive immunity. These proteins regulate the activation and recruitment of leukocytes and other cell types, guiding their migration during inflammation and under basal conditions (Bachelerie et al., 2014; Foxman et al., 1997; Mantovani et al., 2001; Russo et al., 2014). Severe babesiosis is also considered an immune-mediated disease, characterized by an excessive inflammatory response, particularly the overproduction of pro-inflammatory cytokines and chemokines.

Pro-inflammatory cytokines and chemokines — particularly tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1, also known as CCL2), keratinocyte-derived chemokine (KC, or CXCL1), interferon gamma-induced protein 10 (IP-10, or CXCL10), interleukin-6 (IL-6), IL-8 (CXCL8), IL-12, IL-18, granulocyte-macrophage colony-stimulating factor (GM-CSF, or CSF-2), and high-mobility group box 1 protein (HMGB-1) — play roles in the development of these complications (Zygner et al., 2023).

Galán et al. (2018) evaluated immune response mediators in dogs infected with *Babesia canis* and found negative correlations between IL-8 and packed cell volume, suggesting that increased IL-8 concentrations occur in parallel with hemolysis. In a five-year follow-up of a human patient infected with *Babesia venatorum*, Zhao et al. (2020) observed that serum cytokine and chemokine concentrations correlated with symptom severity. Additionally, transcriptome analysis and extensive protein profiling revealed previously unreported cytokines involved in the course of babesiosis, including IL-13, IL-15, IL-17A, CCL2, CCL5, CCL20, CXCL1, CXCL8, CXCL2, CXCL3, and GM-CSF.

Genes encoding Toll-like receptors (TLRs) play a crucial role in the innate immune response, recognizing pathogen-associated molecular patterns (PAMPs) and activate downstream immune signaling (Takeda et al., 2003). TLRs are found in a wide range of organisms (Akira et al., 2006) and function as cell surface signaling molecules essential for initiating defense against infections. TLR10 is expressed in organs such as the lymph nodes, spleen, thymus, and lungs, and in immune cells including macrophages and neutrophils (Chuang and Ulevitch, 2001). The mRNA of *tlr10* is detectable during early B cell development, with translation occurring mainly during differentiation (Bourke et al., 2003).

Therefore, investigating the expression of genes related to immune activation and inflammation, such as chemokines and TLRs, may help identify genetic markers associated with resistance to babesiosis and anaplasmosis in cattle.

2. Materials and methods

2.1 Experimental animals and blood collections

One hundred RNA samples were obtained from 24 calves. The calves originated from the municipality of José Bonifácio (São Paulo, Brazil; coordinates 21°2′23″S, 49°41′28″W) and belonged to two breeds: Angus ($n = 13$; 10 females and 3 males) and Ultrablack ($n = 11$; 5 females and 6 males). At the beginning of the experiment, the average ages of the calves from the Angus and Ultrablack genetic groups were 2.8 ± 1.6 months and 2.1 ± 1.3 months, respectively. For the Angus group, ages ranged from 13 to 150 days, while for the Ultrablack group, ages ranged from 13 to 137 days. According to breeder records, the Ultrablack breed was produced by crossing Brangus cattle (3/8 Zebu and 5/8 Angus), resulting in a zebuine genetic composition of approximately 18%, which corresponds to half the zebuine proportion present in the Brangus parent breed. Six sampling events were conducted at average intervals of 30 days between 10 November 2021 and 19 April 2022 (1st to 6th: 24-Nov-21, 21-Dec-21, 19-Jan-22, 15-Feb-22, 16-Mar-22, 19-Apr-22). This resulted in a total of 100 observations, considering that each animal was present in at least three evaluations. Some evaluations did not allow for the collection of samples suitable for obtaining RNA of acceptable integrity.

Previous studies have shown that the region under study is endemic for *R. microplus* infestations, which also favors the occurrence of babesiosis and anaplasmosis (Giglioti et al., 2016; Frabetti et al., 2023; Azevedo et al., 2024). During the experimental period, the animals were constantly exposed to natural infestation by the tick vector *R. microplus*. At all evaluations, the animals had a tick infestation score ≥ 1 (mean of 1.82 ± 0.19, corresponding to approximately 60 ticks) (Frabetti et al., 2023). The calves were kept in rotated paddocks comprising coast-cross grass (*Cynodon dactylon* (L.) Pers). Tick controls were performed every 21 days by applying of fipronil pour-on (Topline®). Hemoparasite controls were performed when animals showed clinical signs. Throughout the study, all animals were closely monitored and showed no clinical signs of other diseases.

Blood samples from each animal at each evaluation were collected using vacuum tubes with EDTA anticoagulant and submitted for RNA and DNA extraction.

2.2 DNA extraction and qPCR

Blood samples containing EDTA were processed for DNA extraction using the Wizard® Genomic DNA Purification Kit, following the manufacturer's protocol for isolating genomic DNA from 300 μL of whole blood (Promega®, Madison, USA). The purity and concentration of the extracted DNA were assessed using a BioDrop spectrophotometer (BioDrop uLITE, Biochrom Ltd., UK). The DNA was then diluted in TE buffer (Tris-EDTA, pH 7.8) to a concentration of 20 ng/μL and stored at −20 °C until further analysis.

Absolute quantification of copy numbers of *B. bovis* (CN^{bo}_{log}) and *B. bigemina* (CN^{bi}_{log}) was performed by quantitative PCR (qPCR) as described by Okino et al. (2018), using primers and probes targeting the mitochondrial cytochrome b (*mt-cyB*) gene, generating 98 bp amplicons. qPCR assays for quantifying *A. marginale* (CN^{an}_{log}) infection levels were performed according to Giglioti et al. (2019), using primers and probes targeting a 119-nucleotide fragment of the major surface protein 1b (*msp1b*) gene.

The PCR assays were carried out using the CFX™ Real-Time PCR Detection System (Bio-Rad, CA, USA), with a total reaction volume of 10 μL. Each reaction contained 2 μL of 5 × HOT FIREPol Probe Universal qPCR Mix (Solis BioDyne, Tartu, Estonia), 0.5 μL of each primer (10 μM), 0.5 μL of probe (2.5 μM), 4.0 μL of nuclease-free water, and 2.0 μL of DNA template. The thermal cycling protocol included an initial step of 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. All samples were analyzed in duplicate, alongside positive and negative controls. For each hemoparasite, a calibration curve was generated using synthetic gBlocks® gene fragments (IDT, IA, USA). These gBlocks® fragments, representing the target sequences of *B. bovis, B. bigemina* and *A. marginale*, were serially diluted 10-fold to establish the standard curve.

2.3 RNA extraction and cDNA synthesis

RNA was extracted from whole blood samples from each animal following the methodology described by Giglioti et al. (2022). The extraction was performed within 24 h after blood collection. RNA concentration and purity were measured using a BioDrop spectrophotometer (BioDrop). RNA integrity was assessed by electrophoresis on a 1.5%

agarose gel. The RNA samples were then treated with RQ1 RNase-Free DNase (Promega) following the manufacturer's instructions. cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit with RNase inhibitor (Applied Biosystems, CA, USA), following the manufacturer's protocol and using Oligo(dT) primers (IDT). To ensure the reliability of the qPCR results, RNA integrity was confirmed by gel electrophoresis (Supplementary Information 1).

2.4 Relative gene expression

Primers for the target genes were designed using PrimerQuest software (IDT). Their specificity and quality were evaluated using NetPrimer, OligoAnalyzer (IDT), NCBI BLAST, and Primer-BLAST. Details of the primer sets for the target genes are provided in Table 1. RT-qPCR reactions were performed using a CFX96 system (Bio-Rad) in a final volume of 10 μL, containing 2 μL of 5 × HOT FIREPol EvaGreen qPCR Mix Plus (Solis Biodyne), 0.3 μL (10 μM) of each primer, 2.4 μL of ultrapure water (Sigma-Aldrich), and 2 μL of cDNA (approximately 50 ng). One initial cycle of enzymatic activation at 95 °C for 12 min was followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 63 °C for 30 s, and extension at 72 °C for 30 s. After amplification, melting curve analysis was performed by increasing the temperature from 60 °C to 95 °C in 0.5 °C increments, with a 5-second hold at each step.

The reference genes used for normalization were those previously described by Giglioti et al. (2022): beta-actin (*actb*), beta-2-microglobulin (*b2m*), glyceraldehyde 3-phosphate dehydrogenase (*gapdh*), peptidylprolyl isomerase A (*ppia*), and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (*ywhaz*). The algorithms used to identify the most stable genes were geNorm (Vandesompele et al., 2002), NormFinder (Andersen et al., 2004), and BestKeeper (Pfaffl et al., 2004). Additionally, a final ranking of the most stable genes was obtained using the RankAggregated tool, which applies a Monte Carlo cross-entropy algorithm (Pihur et al., 2007).

Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The *b2m* and *ppia* genes were identified as the most stable and were used to normalize the quantification cycle (Cq) values (Supplementary Information 2). The Cq values were normalized using the geometric mean of ppia and b2m, and fold changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method. Fold change values for each target gene, along with DNA copy number (CN_{log}), were used as covariates in the mixed model regression. Melting peaks were examined to assess amplification specificity, confirming the presence of a single product for each reaction (Supplementary Information 3).

2.5 Statistical analyses

Statistical analyses were conducted using SAS PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC, USA), employing the Restricted Maximum Likelihood (REML) method to estimate variance components.

Table 1
Sequences of oligonucleotides used in the RT-qPCR assays. Primer sequences (forward [F] and reverse [R]) for amplifying target genes, along with their corresponding GenBank accession IDs, gene symbols, amplicon sizes (base pairs, bp), and 5′→3′ nucleotide sequences.

GenBank ID	Gene	Sequence 5′ - 3′	Size (pb)
GU936961.1	*ccr3*	F: CCAACATCTACCTGCTCAAC R: CCACTCGTTCCACCTAACA	92
NM_001113174.1	*cxcl12*	F: GCCGATTCTTTGAGAGCCA R: GCACACTTGCCTATTGTTGTTC	119
KX013247.1	*cxcl8*	F: GCTGGCTGTTGCTCTCTT R: GGTGGAAAGGTGTGGAATGT	125
JQ410019.1	*cxcr1*	F: GCTGTTCTGCTACGGATTCA R: CGAGCACGACAGCAAAGAT	95
AM086210.1	*tlr10*	F: GCCCAAGGATAGGCGTAAAT R: CAGAACCTCCAAACCCTTCAT	134

Prior to the main analyses, all evaluated variables were pre-corrected for the animal's age effect at each evaluation, using a model that included the fixed effects of genetic group and sex. For each analysis, a dependent variable (fold change values – *ccr3, cxcl12, cxcl8, cxcr1*, or *tlr10* genes) was used in the model. This model included fixed effects for the factors: evaluation, sex, and genetic group, as well as the interactions between sex and genetic group and between genetic group and evaluation. The covariates CN^{bi}_{log}, CN^{bo}_{log}, and CN^{an}_{log} were included in the model with regression coefficients that quantified their influence on the response variable (expression of each target gene). The animal effect was included as a random factor, and repeated measures from the same animal were used. The assumed covariance matrix model was a first-order autoregressive (*AR(1)*) structure. The significance of effects was assessed using the F-test ($\alpha = 0.05$). The model equation is described below:

$$Y_{ijklm} = \mu + A_i + B_j + C_k + B.C_{jk} + C.A_{ki} + \beta_1 D_1 + \beta_2 E_m + G_p + e_{ijklm}$$

where:

Y_{ijklm}	dependent variable (target genes - *ccr3 cxcl12 cxcl8 cxcr1*, or *tlr10*)
μ	mean
A_i	Fixed effect of evaluation
B_j	Fixed effect of sex
C_k	Fixed effect of genetic group
$B.C_{jk}$	Fixed interaction effect between sex and genetic group
$C.A_{ki}$	Interaction between genetic group and evaluation
$\beta_1 D_l$	Effect of the CN^{bi}_{log} covariate
$\beta_2 E_m$	Effect of the CN^{bo}_{log} covariate
$\beta_3 F_n$	Effect of the CN^{an}_{log} covariate
$G_p \sim N(0,\sigma^2_G)$	Random effect of the animal
$e_{ijklm} \sim N(0,\sigma^2)$	Residual error.

3. Results

The mean values of untransformed DNA copy numbers (copies/μL), followed by the frequencies of positives for infections with *B. bovis, B. bigemina*, and *A. marginale*, were 35.0 (log = 1.54; 88.0%, $n = 88$), 791.0 (log = 2.90; 98.0%, $n = 98$), and 20,326 (log = 4.33; 100%, $n = 100$), respectively (Supplementary Information 4).

Data analysis revealed no significant effects ($p > 0.05$) of the studied genes regarding genetic group, sex, or their interactions. Only the evaluation effect was significant ($p < 0.05$). Furthermore, gene expression levels varied across the six evaluations, corresponding to fluctuations in the DNA copy number of hemoparasites (Fig. 1).

Except for *cxcl12*, all genes showed significant associations (positive or negative) with at least two hemoparasite species (Table 2). This association indicates that for each unit increase in CN_{log}, there is a decrease (when negative) or an increase (when positive) in the estimated gene expression value. For example, the estimated value for the relationship between *ccr3* and CN^{big}_{log} shows that, for each unit of CN^{big}_{log}, there is a decrease of 0.43 in *ccr3* gene expression (Table 2). Similarly, the positive estimated value between *tlr10* and CN^{ana}_{log} shows that, for each unit of CN^{ana}_{log}, there is an increase of 0.60 in *tlr10* gene expression (Table 2).

All estimated values for each target gene associated with the CN_{log} of each hemoparasite species are shown in Fig. 1. It was observed that for all significant CN_{log} values of *B. bovis* and *B. bigemina*, gene expression decreased, whereas for *A. marginale*, the relationship was inverse (Fig. 1).

4. Discussion

The results of this study demonstrate distinct patterns of gene expression in response to different hemoparasite infections in cattle. The observed downregulation of chemokine genes (*ccr3, cxcl8*, and *cxcr1*) during *Babesia bovis* and *B. bigemina* infections suggests a potential mechanism of immune modulation or suppression by these protozoan

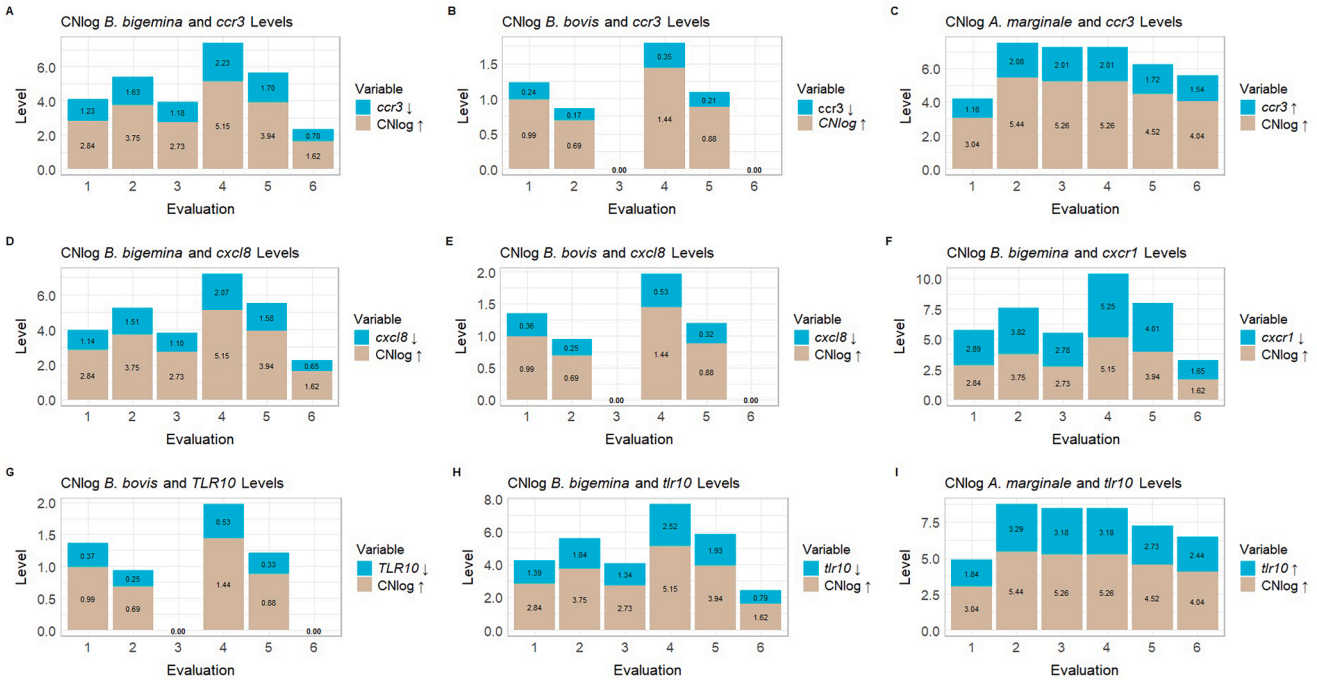

Fig. 1. Relationship between DNA copy number (CNlog, copies/μL) and estimated gene expression (fold change) levels. Bars represent the regression estimates (solution for fixed effects) for the genes *ccr3, cxcl8, cxcr1*, and *tlr10* across six evaluations. The genes *ccr3, cxcl8*, and *cxcr1* are chemokines, while *tlr10* is a Toll-like receptor. Panels A, D, F, and H show CNlog for *B. bigemina*; panels B, E, and G show CNlog for *B. bovis*; and panels C and I show CNlog for *A. marginale*. Arrows indicate the relationship between CNlog and gene expression: arrows in opposite directions (cyan and beige bars) suggest an inverse relationship (i.e., increased CNlog associated with decreased gene expression), while arrows in the same direction (beige bars) indicate a positive relationship (i.e., both CNlog and gene expression increase). Evaluations: 1st to 6th (24-Nov-21, 21-Dec-21, 19-Jan-22, 15-Feb-22, 16-Mar-22, 19-Apr-22).

Table 2
Estimated effects of the association between DNA copy number (CN_{log}) and gene expression levels. Negative effect values indicate an inverse relationship, where increased CN_{log} is associated with decreased gene expression. Positive values indicate a direct relationship, where both CN_{log} and gene expression increase. CN_{log}^{big} = CN_{log} *B. bigemina;* $CN_{log}^{bov} = CN_{log}$ *B. bovis;* $CN_{log}^{ana} = CN_{log}$ *A. marginale.*

DNA_{log} copy number	Expression gene levels									
	ccr3		*cxcl8*		*cxcl12*		*cxcr1*		*tlr10*	
	effect	P value	effect	P value	effect	P value	effect	P value	effect	P value
CN_{log}^{big}	−0.43	0.006**	−0.40	0.046**	.	ns	−0.88	0.009**	−0.49	0.015
CN_{log}^{bov}	−0.24	0.037**	−0.35	0.073*	.	ns	.	ns	−0.37	0.056*
CN_{log}^{ana}	0.38	0.003**	.	ns	.	ns	–	ns	0.60	0.004

*p-value ≤ 0.10; **p-value ≤ 0.05; ns: not significant.

parasites. This finding aligns with previous reports that *Babesia* species interfere with host immune responses to establish persistent infections. Goff et al. (1998) provided evidence that *B. bovis* infection induces the production of interleukin-10 (IL-10), which can downregulate the expression of pro-inflammatory cytokines and nitric oxide, thereby aiding parasite persistence. According to Brown et al. (2006), *B. bovis* typically induces severe, potentially lethal infections in mature cattle. Following recovery, animals develop persistent infections without clinical signs. These persistently infected cattle acquire resistance to subsequent infections by similar parasite strains, a phenomenon known as concomitant immunity.

This immunosuppressive profile is further supported by the functional roles of the downregulated chemokines. The receptor CXCR1 (also known as IL-8RA, IL-8R-I, or IL-8R) interacts with chemokines such as CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8, and is expressed in polymorphonuclear granulocytes, monocytes, astrocytes, endothelial cells, and mast cells. Meanwhile, the CCR3 receptor (also known as CKR3, CC CKR3, EotR, or CMKBR3) binds to CCL5, CCL7, CCL8, CCL11, CCL13, CCL14, CCL15, CCL24, and CCL26, acting on eosinophils, basophils, T cells (Th2 > Th1), dendritic cells, platelets, and mast cells. Additionally, CXCL8 (IL-8/NAP-1), an inflammatory chemokine, primarily induces chemotaxis in neutrophils, but also in T cells, basophils, and endothelial cells (Le et al., 2004; Palomino and Marti, 2015). The suppression of these pathways during *Babesia* infection may therefore reflect a parasite strategy to dampen inflammatory and adaptive immune responses, facilitating chronicity.

The negative correlation between parasite load and chemokine expression suggests an evolutionary adaptation of parasites to evade host defenses through the suppression of inflammatory responses. In contrast, the positive correlation between *A. marginale* infection levels and the upregulation of *ccr3* and *tlr10* suggests a distinct immune strategy against this rickettsial pathogen. Elevated expression of *ccr3,* which, as previously mentioned, interacts with chemokines and play crucial roles in immune signaling, could enhance chemokine production and the recruitment of immune cells. This response may be directly influenced by the increasing parasitic burden of *A. marginale*. The

upregulation of these genes could indicate an active inflammatory response, as *tlr10* has been implicated in recognizing bacterial components and initiating innate immune defenses (Hasan et al., 2005). Extensive studies on *tlr2* have demonstrated that its association with *tlr1* and *tlr6* is essential for efficient ligand binding, enabling the discrimination between triacylated and diacylated bacterial lipopeptides (Takeda et al., 2002). Thus, according to Hasan et al. (2005), *tlr10* may potentially act as a coreceptor for these TLRs and therefore recognize similar ligands. This differential response between protozoan and bacterial infections highlights the complexity of host–pathogen interactions in co-infection scenarios.

The lack of significant differences in chemokine and *tlr10* gene expression between Angus (100% taurine) and Ultrablack (82% taurine/18% zebuine) cattle aligns with Azevedo et al. (2024), who studied the same herd and found that the low percentage of zebu ancestry in Ultrablack animals did not confer measurable resistance to *Babesia* spp. According to these authors, due to Mendelian segregation and crossing-over processes, the estimated proportion of zebu genetics resulting from Ultrablack mating may differ from the actual proportion (~18%).

Temporal variations in gene expression across the six sampling periods underscore the dynamic nature of host-parasite interactions. The significant effect of evaluation time on gene expression levels, independent of genetic group or sex, suggests that environmental factors or infection progression may play a substantial role in modulating immune responses. This variability may reflect natural fluctuations in parasite load or the cyclical activation of the immune system during persistent infections (Bock et al., 2004), emphasizing the importance of longitudinal studies for understanding the immunology of hemoparasitic diseases. As noted by Brown et al. (2001, 2006), evolutionary pressure favors parasites that establish persistent infections through immune modulation rather than causing host mortality, with parasitemia often cycling unnoticed in infected but otherwise clinically healthy animals. This typically involves the induction of host responses that limit pathogen proliferation without achieving complete clearance (Brown et al., 2006). Therefore, the use of repeated measures and mixed models in this study effectively captured these temporal dynamics, providing a robust analysis of the complex relationship between parasite load and gene expression.

The contrasting relationships between gene expression and different parasite species have important implications for understanding disease pathogenesis and developing control strategies. The negative associations between *Babesia* infections and chemokine expression suggest that these parasites may actively suppress host immune responses, potentially contributing to their ability to establish chronic infections. This finding is consistent with studies showing that *Babesia* can modulate host cytokine production (Zygner et al., 2023). According to Zygner et al. (2023), the immune response to the infection by *B. canis* is driven by pro-inflammatory cytokines and chemokines, especially IFN-γ, TNF-α, IL-6, and IL-8. Their findings further indicate that disease pathogenesis, including the development of anemia, results from a disrupted equilibrium between pro-inflammatory and anti-inflammatory cytokine networks.

In contrast, the positive association between *A. marginale* infection and immune gene expression suggests that the host mounts a more pronounced inflammatory response to this bacterial pathogen. This immune profile differs from that observed in babesiosis and may help explain variations in clinical manifestations and disease outcomes between the two infections. Under the conditions of the present study, these findings indicate that therapeutic or preventive strategies may need to be tailored accordingly. Currently, there are no specific studies directly investigate the *ccr3* and *tlr10* genes in cattle infected with *A. marginale*. Ahlawat et al. (2023) demonstrated that *A. marginale* infection significantly alters the expression of multiple cytokines and chemokines, including CXCL2 and CXCL8, which signal through CXCR1. However, in our study, a significant association between *cxcr1* gene expression and *A. marginale* infection was not observed. Müller et al. (2021) investigated the immune response of murine neutrophils to *Anaplasma phagocytophilum* infection and noted that TLR7, TLR9, and the TRIF pathway play significant roles in the production of pro-inflammatory cytokines such as MIP-1α, TNF, and IL-6. Although TLR10 was not directly studied, these findings highlight the importance of Toll-like receptors in the immune response to *Anaplasma* infections. Our results reinforce the hypothesis of an active inflammatory response during *A. marginale* infection and provide insight into the mechanisms by which this pathogen interacts with the bovine immune system. Consequently, immunomodulatory strategies that harness or regulate this response could enhance disease control.

While our study did not find statistically significant associations between hemoparasite species and *cxcl12* gene expression, CXCL12 plays a crucial and multifaceted biological role in immune regulation, cellular trafficking, and even general cell proliferation. Known as SDF-1α, CXCL12 and its receptor CXCR4 are fundamental for leukocyte trafficking and the retention and mobilization of hematopoietic cells, including neutrophils, within the bone marrow (Pello et al., 2006; Siberski-Cooper et al., 2024). Beyond immunity, CXCL12 also influences broader cellular dynamics, including cell growth and proliferation (Zhang et al., 2019). Given its role in general cell proliferation (Zhang et al., 2019) and the dynamic host-pathogen interactions in persistent infections, the lack of statistical significance here does not preclude CXCL12 from playing a crucial, albeit indirect, role in the host response or parasite persistence mechanisms. In the context of persistent infections, which are hallmarks of *A. marginale* that evade clearance through mechanisms such as antigenic variation despite antimicrobial treatment (Curtis et al., 2021; Mauri Pablo et al., 2025), our findings of *ccr3* and *tlr10* upregulation suggest a sustained inflammatory response, contrasting with the immunosuppression observed in *Babesia*.

These findings provide an additional foundation for future research aimed at clarifying the specific mechanisms through which hemoparasites influence host gene expression. Further studies will be essential to advance our understanding of host-pathogen interactions and to support the development of targeted control strategies.

5. Conclusion

Our study elucidates the roles of chemokines and Toll-like receptors in bovine immune responses to infections with *Babesia bovis, B. bigemina*, and *Anaplasma marginale*. The observed downregulation of *ccr3, cxcl8*, and *cxcr1* in response to *Babesia* spp. suggests a parasite-driven immune evasion strategy that may contribute to chronic infection. Conversely, the upregulation of *ccr3* and *tlr10* during *A. marginale* infection indicates a more pronounced pro-inflammatory response, which may contribute to the elimination or reduction of the infection. The absence of significant differences between Angus and Ultrablack cattle underscores the complexity of genetic resistance, suggesting that a higher proportion of zebuine ancestry may be necessary to produce detectable phenotypic effects. Our findings contribute to a better understanding of host-pathogen dynamics in tick-borne diseases and emphasize the importance of species-specific immunomodulatory approaches.

Ethical approval

This experiment adhered to the ethical principles of animal experimentation of the Instituto de Zootecnia Ethics Committee on Animal Experimentation (Protocol Nr. 328–2021).

Funding

This project was supported by grants from the São Paulo State Research Support Foundation (FAPESP) (Grant #2019/22675–6, #2022/16262–3) and the Institute of Animal Science (APTA-SAA).

CRediT authorship contribution statement

Mayne Barboza Sarti: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Geovana Menegão de Souza:** Writing – original draft, Validation, Resources, Methodology, Investigation. **Camila Fagionato Agostinho:** Validation, Methodology, Investigation. **Gustavo Henrique Carvalho Borges:** Visualization, Methodology, Investigation. **Flávia Fernanda Carneiro Santana:** Validation, Methodology, Investigation. **Luciana Morita Katiki:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Conceptualization. **Anibal Eugênio Vercesi Filho:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Conceptualization. **Ana Gonçalves Domingos:** Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Conceptualization. **Rodrigo Giglioti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2025.102546.

Data availability

Data will be made available on request.

References

Ahlawat, S., Bharti, P.K., Arora, R., Kishore, A., 2023. Unraveling the genetic mechanisms governing the host response to bovine anaplasmosis. Gene 877, 147532. https://doi.org/10.1016/j.gene.2023.147532.

Akira, S., Uematsu, S., Takeuchi, O., 2006. Pathogen recognition and innate immunity. Cell 124 (4), 783–801. https://doi.org/10.1016/j.cell.2006.02.015.

Andersen, C.L., Jensen, J.L., Ørntoft, T.F., 2004. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res. 64 (15), 5245–5250. https://doi.org/10.1158/0008-5472.CAN-04-0496.

Azevedo, B.T., Oliveira, H.N., Katiki, L.M., Vercesi Filho, A.E., Domingos, A.G., Antunes, S., Okino, C.H., Oliveira, M.C.S., Ibelli, A.M.G., Giglioti, R., 2024. A small proportion of Zebu genetic background in crossbred calves may not be enough to improve resistance against natural bovine *Babesia* spp. Infections. Vet. Parasitol. 328, 110165. https://doi.org/10.1016/j.vetpar.2024.110165.

Bachelerie, F., Ben-Baruch, A., Burkhardt, A.M., Combadière, C., Farber, J.M., Graham, G.J., Zlotnik, A., 2014. International Union of Basic and Clinical Pharmacology. [corrected]. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. Pharmacol. Rev. 66 (1), 1–79. https://doi.org/10.1124/pr.113.007724.

Bock, R.E., De Vos, A.J., Kingston, T.G., McLellan, D.J., 1997. Effect of breed of cattle on innate resistance to infection with *Babesia bovis, B. bigemina* and *Anaplasma marginale*. Aust. Vet. J. 75 (5), 337–340. https://doi.org/10.1111/j.1751-0813.1997.tb15712.x.

Bock, R., Jackson, L., De Vos, A., Jorgensen, W., 2004. Babesiosis of cattle. Parasitology, 129 (S1), S247–S269. https://doi.org/10.1017/S0031182004005190.

Bourke, E., Bosisio, D., Golay, J., Polentarutti, N., Mantovani, A., 2003. The toll-like receptor repertoire of human B lymphocytes: inducible and selective expression of TLR9 and TLR10 in normal and transformed cells. Blood 102 (3), 956–963. https://doi.org/10.1182/blood-2003-01-0264.

Brown, W.C., 2001. Molecular approaches to elucidating innate and acquired immune responses to *babesia bovis*, a protozoan parasite that causes persistent infection. Vet. Parasitol. 101 (3–4), 233–248. https://doi.org/10.1016/S0304-4017(01)00562-9.

Brown, W.C., Norimine, J., Goff, W.L., Suarez, C.E., McElwain, T.F., 2006. Prospects for recombinant vaccines against *Babesia bovis* and related parasites. Parasite Immunol. 28 (7), 315–327. https://doi.org/10.1111/j.1365-3024.2006.00849.x.

Chuang, T., Ulevitch, R.J., 2001. Identification of hTLR10: a novel human toll-like receptor preferentially expressed in immune cells. Biochim. Biophys. Acta (BBA) - Gene Struct. Expr. 1518 (1–2), 157–161. https://doi.org/10.1016/S0167-4781(00)00289-X.

Curtis, A.K., Kleinhenz, M.D., Anantatat, T., Martin, M.S., Magnin, G.C., Coetzee, J.F., Reif, K.E., 2021. Failure to eliminate persistent *anaplasma marginale* infection from cattle using labeled doses of chlortetracycline and oxytetracycline antimicrobials. Vet. Sci. 8 (11), 283. https://doi.org/10.3390/vetsci8110283.

Foxman, E.F., Campbell, J.J., Butcher, E.C., 1997. Multistep navigation and the combinatorial control of leukocyte chemotaxis. J. Cell Biol. 139 (5), 1349–1360. https://doi.org/10.1083/jcb.139.5.1349.

Frabetti, A.F., Katiki, L.M., Caetano, L., Sarti, M.B., Falasca, T.M., Polli, H., Verissimo, C. J., Vercesi Filho, A.E., Oliveira, H.N., Oliveira, M.C.S., Giglioti, R., 2023. Natural levels of *rhipicephalus microplus* infestation and *anaplasma marginale* infection in Angus and Ultrablack calves. Exp. Appl. Acarol. 89 (1–2), 131–140. https://doi.org/10.1007/s10493-023-00778-3.

Galán, A., Mayer, I., Rafaj, R.B., Bendelja, K., Sušić, V., Cerón, J.J., Mrljak, V., 2018. MCP-1, KC-like and IL-8 as critical mediators of pathogenesis caused by *Babesia canis*. Plos One 13 (1), e0190474. https://doi.org/10.1371/journal.pone.0190474.

Giglioti, R., Azevedo, B.T., Oliveira, H.N., Katiki, L.M., Vercesi Filho, A.E., Oliveira, M.C. S., Okino, C., 2022. How long does the mRNA remains stable in untreated whole bovine blood? Mol. Biol. Rep. 49 (1), 789–795. https://doi.org/10.1007/s11033-021-06808-w.

Giglioti, R., Bassetto, C.C., Okino, C.H., de Oliveira, H.N., de Sena Oliveira, M.C, 2019. Development of a loop-mediated isothermal amplification (LAMP) assay for the detection of *anaplasma marginale*. Exp. Appl. Acarol. 77 (1), 65–72. https://doi.org/10.1007/s10493-018-0327-y.

Giglioti, R., Oliveira, H.N., Santana, C.H., Ibelli, A.M.G., Néo, T.A., Bilhassi, T.B., Rabelo, M.D., Machado, R.Z., Brito, L.G., Oliveira, M.C.S., 2016. *Babesia bovis* and *Babesia bigemina* infection levels estimated by qPCR in Angus cattle from an endemic area of São Paulo state, Brazil. Ticks Tick-Borne Dis. 7, 657–662. https://doi.org/10.1016/j.ttbdis.2016.02.011.

Goff, W.L., Johnson, W.C., Cluff, C.W., 1998. *Babesia bovis* immunity: in vitro and in vivo evidence for IL-10 regulation of IFN-γ and iNOS. Ann. N. Y. Acad. Sci. 849, 161–180. https://doi.org/10.1111/j.1749-6632.1998.tb11046.x.

Grisi, L., Leite, R.C., Martins, J.R.S., Barros, A.T.M., Andreotti, R., Cançado, P.H.D., Villela, H.S., 2014. Reassessment of the potential economic impact of cattle parasites in Brazil. Rev. Bras. Parasitol. Vet. 23 (2), 150–156. https://doi.org/10.1590/S1984-29612014042.

Guglielmone, A.A., Robbins, R.G., 2023. Hard ticks (Acari: ixodida: ixodidae) parasitizing domestic animals and humans in Africa, Latin America, and the Caribbean. Exp. Appl. Acarol. 89 (1–2), 1–45. https://doi.org/10.1007/s10493-022-00770-3.

Hasan, U., Chaffois, C., Gaillard, C., Saulnier, V., Merck, E., Tancredi, S., Bates, E.E., 2005. Human TLR10 is a functional receptor, expressed by B cells and plasmacytoid dendritic cells, which activates gene transcription through MyD88. J. Immunol. 174 (5), 2942–2950. https://doi.org/10.4049/jimmunol.174.5.2942.

Ibelli, A.M.G., Ribeiro, A.R.B., Giglioti, R., Regitano, L.C.A., Alencar, M.M., Chagas, A.C. S., Paço, A.L., Oliveira, H.N., Duarte, J.M.S., Oliveira, M.C.S., 2012. Resistance of cattle of various genetic groups to the tick *rhipicephalus microplus* and the relationship with coat traits. Vet. Parasitol. 186 (1–2), 425–430. https://doi.org/10.1016/j.vetpar.2011.11.019.

Kocan, K.M., de la Fuente, J., Blouin, E.F., Coetzee, J.F., Ewing, S.A., 2010. The natural history of *Anaplasma marginale*. Vet. Parasitol., 167 (2–4), 95–107. https://doi.org/10.1016/j.vetpar.2009.09.032.

Kocan, K.M., de la Fuente, J., Guglielmone, A.A., Meléndez, R.D., 2003. Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. Clin. Microbiol. Rev. 16 (4), 698–712. https://doi.org/10.1128/CMR.16.4.698-712.2003.

Le, Y., Zhou, Y., Iribarren, P., Wang, J.M., 2004. Chemokines and chemokine receptors: their manifold roles in homeostasis and disease. Cell. Mol. Immunol. 1 (2), 95–118. https://doi.org/10.1038/cmi.2004.1.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods 25 (4), 402–408. https://doi.org/10.1006/meth.2001.1262.

Luster, A.D., 1998. Chemokines–chemotactic cytokines that mediate inflammation. N. Engl. J. Med. 338 (7), 436–445. https://doi.org/10.1056/NEJM199802123380706.

Mantovani, A., Locati, M., Vecchi, A., Sozzani, S., Allavena, P., 2001. Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. Trends Immunol. 22 (6), 328–336. https://doi.org/10.1016/S1471-4906(01)01941-X.

Mauri Pablo, J.D., Del Solar, J.J.C., Hinojosa Enciso, E.T., Polveiro, R.C., Vieira, D.d.S., Ramos Sanchez, E.M., Bardales Escalante, W., Maicelo Quintana, J.L., Lopez Lapa, R. M., 2025. Anaplasmosis in the Amazon: diagnostic challenges, persistence, and control of *anaplasma marginale* and *anaplasma phagocytophilum*. Front. Vet. Sci. 12, 1571694. https://doi.org/10.3389/fvets.2025.1571694.

McCosker, P.J., 1981. The global importance of babesiosis. In: Ristic, M., Kreier, J.P. (Eds.), Babesiosis. Academic Press, pp. 1–24.

Müller, B.J., Westheider, A., Birkner, K., Seelig, B., Kirschnek, S., Bogdan, C., von Loewenich, F.D., 2021. *Anaplasma phagocytophilum* induces TLR- and MyD88-dependent signaling in in vitro generated murine neutrophils. Front. Cell. Infect. Microbiol. 11, 627630. https://doi.org/10.3389/fcimb.2021.627630.

Okino, C.H., Giglioti, R., Silva, P.C., Oliveira, H.N., Oliveira, M.C.S., 2018. Comparative evaluation of DNA extraction kit, matrix sample and qPCR assays for bovine babesiosis monitoring. Mol. Biol. Rep. 45 (6), 2671–2680. https://doi.org/10.1007/s11033-018-4330-5.

Oliveira, M.C.S., Alencar, M.M., Giglioti, R., Beraldo, M.C.D., Aníbal, F.F., Correia, R.O., Boschini, L., Chagas, A.C.S., Bilhassi, T.B., Oliveira, H.N., 2013. Resistance of beef cattle of two genetic groups to ectoparasites and gastrointestinal nematodes in the state of São Paulo, Brazil. Vet. Parasitol. 197 (1–2), 168–175. https://doi.org/10.1016/j.vetpar.2013.04.031.

Palomino, D.C.T., Marti, L.C., 2015. Chemokines and immunity. Einstein (São Paulo), 13 (3), 469–473. https://doi.org/10.1590/S1679-45082015RB3438.

Parker, C.M., Groh, V., Band, H., Porcelli, S.A., Morita, C., Fabbi, M., Brenner, M.B., 1990. Evidence for extrathymic changes in the T cell receptor gamma/delta repertoire. J. Exp. Med. 171 (5), 1597–1612. https://doi.org/10.1084/jem.171.5.1597.

Pello, O.M., Moreno-Ortiz, M.D.C., Rodríguez-Frade, J.M., Martínez-Muñoz, L., Lucas, D., Gómez, L., Lucas, P., Samper, E., Aracil, M., Martínez-A, C., Bernad, A., Mellado, M., 2006. SOCS up-regulation mobilizes autologous stem cells through CXCR4 blockade. Blood, 108 (12), 3928–3937.

Pfaffl, M.W., Tichopad, A., Prgomet, C., Neuvians, T.P., 2004. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: bestKeeper–Excel-based tool using pair-wise correlations. Biotechnol. Lett. 26 (6), 509–515. https://doi.org/10.1023/B:BILE.0000019559.84305.47.

Pihur, V., Datta, S., Datta, S., 2007. Weighted rank aggregation of cluster validation measures: a Monte Carlo cross-entropy approach. Bioinformatics 23 (13), 1607–1615. https://doi.org/10.1093/bioinformatics/btm158.

Russo, R.C., Garcia, C.C., Teixeira, M.M., Amaral, F.A., 2014. The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases. Expert Rev. Clin. Immunol. 10 (5), 593–619. https://doi.org/10.1586/1744666X.2014.894886.

Siberski-Cooper, C.J., Mayes, M.S., Gorden, P.J., Kramer, L., Bhatia, V., Koltes, J.E., 2024. The genetic architecture of complete blood counts in lactating Holstein dairy cows. Front. Genet. 15, 1360295. https://doi.org/10.3389/fgene.2024.1360295.

Takeda, K., Kaisho, T., Akira, S., 2003. Toll-like receptors. Annu. Rev. Immunol. 21, 335–376. https://doi.org/10.1146/annurev.immunol.21.120601.141126.

Takeda, K., Takeuchi, O., Akira, S., 2002. Recognition of lipopeptides by toll-like receptors. J. Endotoxin Res. 8 (6), 459–463. https://doi.org/10.1179/096805102125001073.

Tucker, T.R., Aly, S.S., Maas, J., Davy, J.S., Foley, J.E., 2016. Investigation of *anaplasma marginale* seroprevalence in a traditionally managed large California beef herd. Vet. Med. Int. 2016, 1768624. https://doi.org/10.1155/2016/1768624.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3 (7). https://doi.org/10.1186/gb-2002-3-7-research0034. RESEARCH0034.

Zhang, M., Cao, T.T., Wei, Z.G., Zhang, Y.Q., 2019. Silk sericin hydrolysate is a potential candidate as a serum-substitute in the culture of Chinese hamster ovary and Henrietta lacks cells. J. Insect Sci. 19 (1), 10. https://doi.org/10.1093/jisesa/iey137, 1–12.

Zhao, L., Jiang, R., Jia, N., Ning, N., Zheng, Y., Huo, Q., Cao, W., 2020. Human case infected with *Babesia venatorum*: a 5-year follow-up study. Open Forum Infect. Dis. 7 (3), ofaa062. https://doi.org/10.1093/ofid/ofaa062.

Zygner, W., Gójska-Zygner, O., Norbury, L.J., 2023. Pathogenesis of anemia in canine babesiosis: possible contribution of pro-inflammatory cytokines and chemokines - a review. Pathogens 12 (2), 166. https://doi.org/10.3390/pathogens12020166.