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Acute phase protein response in cattle naturally infected with tick-borne haemoparasites in India

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Abstract

Tick-borne haemoparasitic diseases constitute a major constraint to cattle health and productivity in tropical regions. The present study evaluated the acute phase protein (APP) response in cattle naturally infected with major bovine haemoparasites in Tamil Nadu, India. Blood samples ($n = 462$) were collected from cattle across three agro-climatic zones and screened for *Theileria*, *Babesia*, *Anaplasma*, and *Ehrlichia* species using nested PCR targeting 18S and 16S rRNA genes. The acute phase proteins viz., haptoglobin, serum amyloid A, ceruloplasmin, and α_1 -acid glycoprotein, were quantified in PCR-positive animals ($n = 72$) using ELISA. Overall, 84.2% of samples were positive for one or more haemoparasites, with *Anaplasma* spp. being the most prevalent. All APPs showed significant elevation in infected cattle compared to controls, with the highest responses observed in *Theileria annulata* infections. The findings indicate that acute phase proteins reflect the systemic inflammatory response induced by haemoparasites and may serve as valuable early biomarkers for disease detection and monitoring in cattle.

Keywords: Cattle, acute phase proteins, haemoparasites, *Theileria*, *Babesia*, *Anaplasma*

1. Introduction

Tick-borne haemoparasitic diseases represent a major constraint to cattle health and productivity in tropical and subtropical regions, particularly in India, where climatic conditions favour vector proliferation. Diseases caused by *Theileria*, *Babesia*, *Anaplasma*, and *Ehrlichia* species lead to significant economic losses through reduced milk yield, weight loss, reproductive failure, increased treatment costs, and mortality. In addition to overt clinical disease, subclinical and carrier states contribute substantially to unrecognized production losses and perpetuation of infection within herds. These infections reflect complex host-parasite interactions in which parasites not only evade immune mechanisms but also actively modulate host responses, leading to both protection and pathology (Radostits *et al.*, 2007) [1].

Bovine tropical theileriosis, caused by *Theileria annulata*, is among the most important tick-borne protozoan diseases of cattle in India. Soulsby described theileriosis as a disease in which pathology is largely immune-mediated, driven by parasite-induced transformation of host leukocytes and excessive cytokine release.

Early diagnostic methods, such as blood smear examination, lack sensitivity, particularly in carrier animals. Immunodiagnostic techniques, including the fluorescent antibody test and serological assays, were developed to overcome these limitations (Pipano and Cahana, 1969; Kiltz *et al.*, 1986) [2, 3], but these methods cannot reliably distinguish active infection from prior exposure in endemic areas. Molecular diagnostic techniques, particularly PCR-based assays, have improved the detection of haemoparasites and identification of carrier animals. However, molecular detection alone provides limited insight into disease severity or host inflammatory status. Understanding the mechanisms of host-parasite response is essential to interpreting the disease progression. The acute phase response, mediated by cytokines such as IL-1 β , IL-6, TNF- α , and IFN- γ , represents one of the earliest systemic reactions in various disease conditions of human and domestic animals (Paulina and Tadeusz, 2011; Gómez-Laguna *et al.*, 2011; Valente *et al.*, 2012) [4, 5, 6].

In cattle, major positive acute phase proteins include haptoglobin, serum amyloid A, ceruloplasmin, fibrinogen, and α -¹ acid glycoprotein. These proteins function to limit tissue damage, regulate immune responses, and restore homeostasis. Acute phase proteins have been shown to discriminate between acute and chronic inflammatory conditions in cattle (Horadagoda *et al.*, 1999) [7]. Previous studies have demonstrated marked elevation of these proteins in bovine theileriosis and babesiosis, correlating with parasitaemia and disease severity (Nazifi *et al.*, 2009; Mohammadi *et al.*, 2021) [8, 9].

Despite their diagnostic and prognostic potential, limited data are available on acute phase protein responses in naturally infected cattle under field conditions in India. The present study was therefore undertaken to investigate the prevalence of major haemoparasites in cattle across different agro-climatic zones of Tamil Nadu and to evaluate the associated acute phase protein response, with a view to exploring their utility as early biomarkers of bovine haemoparasitosis.

2. Materials and Methods

2.1. Sample collection

Blood samples were collected aseptically from the jugular vein of cattle into vacuum tubes with and without anticoagulant from three agro-climatic zones of Tamil Nadu, India: Northern zone, Southern zone, and High Rainfall zone. A total of 462 samples were collected and transported under cold conditions for laboratory analysis.

2.2. DNA extraction

Genomic DNA was extracted from whole blood using a Qiagen Blood DNA Kit according to the manufacturer's instructions. Briefly, 200 μ L of blood was mixed with proteinase K and lysis buffer, incubated at 56 °C, followed by ethanol precipitation and purification using spin columns. DNA was eluted in elution buffer and stored at -20 °C until further use.

2.3. Nested PCR for haemoparasite detection

Nested PCR assays were performed using primers targeting partial regions of the 18S rRNA gene for *Theileria annulata*, *Theileria orientalis*, *Babesia bovis*, and *Babesia bigemina*, and the 16S rRNA gene for *Anaplasma* spp. and *Ehrlichia* spp. Species-specific primers were synthesized commercially. PCR products were resolved by agarose gel electrophoresis and visualized under UV illumination. Parasite species were identified based on expected amplicon sizes.

2.4. Acute phase protein estimation

Seventy-two cattle positive for haemoparasite infection by nested PCR were selected for acute phase protein analysis. Twelve animals were included for each parasite group. Serum concentrations of haptoglobin, serum amyloid A, ceruloplasmin, and α -¹ acid glycoprotein were quantified using commercially available ELISA kits following the manufacturers' protocols.

2.5. Statistical analysis

Data were expressed as mean $\pm$ SE values. Differences in acute phase protein concentrations between groups were evaluated using appropriate descriptive statistical methods viz., mixed effect logistic regression, principal component analysis and Receiver operating characteristic curve analysis with a significance set at $p < 0.05$.

3. Results

3.1. Molecular prevalence of haemoparasites

Of the 462 blood samples examined, 389 (84.2%) were positive for one or more haemoparasites by nested PCR. *Anaplasma* spp. was the most prevalent parasite across all three agro-climatic zones, followed by *Babesia bovis* and *Babesia bigemina*. *Theileria annulata* and *Theileria orientalis* were detected at lower frequencies, while *Ehrlichia* spp. showed moderate prevalence, particularly in the high rainfall zone.

Table 1: Prevalence of haemoparasites in various agroclimatic zones of Tamil Nadu

Blood parasites	Agro Climatic Zone / Prevalence (%)		
	Northern zone	Southern zone	High Rainfall
<i>Theileria orientalis</i>	3.84	3.63	0
<i>Theileria annulata</i>	5.12	1.36	0
<i>Babesia bovis</i>	16.66	31.81	0
<i>Babesia bigemina</i>	21.47	14.54	0
<i>Anaplasma</i> spp	33.65	41.36	75
<i>Ehrlichia</i> spp	19.23	7.27	25

3.2 Molecular prevalence and risk factor analysis

Of the 462 cattle sampled, 389 were PCR-positive for at least one haemoparasite, yielding an overall molecular prevalence of 84.2% (95% CI: 80.5-87.4). Prevalence differed significantly across agro-climatic zones ($p < 0.001$).

In multivariable mixed-effects logistic regression analysis adjusting for age, breed, and herd-level clustering, cattle from the high rainfall zone had significantly higher odds of infection compared to the northern zone (adjusted OR = 2.87; 95% CI: 1.64-5.02; $p < 0.001$). Age was positively associated with infection risk (OR per year increase = 1.18; 95% CI: 1.05-1.33; $P = 0.006$). Breed was not independently associated after adjustment. Intraclass correlation coefficient (ICC) indicated moderate herd-level clustering (ICC = 0.19), justifying mixed-effects modeling.

Table 2: Multiple Mixed effect Logistic Regression for haemoparasite infection

Variable	Adjusted Odds Ratio	95% CI	p-value
High rainfall zone	2.87	1.64-5.02	< 0.001
Southern zone	1.42	0.91-2.23	0.12
Age (per year)	1.18	1.05-1.33	0.006
Crossbred vs indigenous	1.09	0.74-1.62	0.65
Random effect (herd variance)	0.38	—	—

3.3. Acute phase protein response

All four acute phase proteins evaluated were markedly elevated in haemoparasite-infected cattle compared to control animals. APP concentrations demonstrated right-skewed distributions and were log-transformed prior to modeling. Linear mixed-effects models adjusted for zone, age, breed, and herd revealed significant differences in APP concentrations across parasite species.

The magnitude of elevation varied among parasite species. Cattle infected with *Theileria annulata* exhibited the highest concentrations of haptoglobin, serum amyloid A, ceruloplasmin, and α -¹ acid glycoprotein. Elevated APP levels were also observed in infections caused by *Babesia* spp., *Anaplasma* spp., and *Ehrlichia* spp., indicating a robust systemic inflammatory response associated with haemoparasitism.

3.3.1. Alpha¹ Acid Glycoprotein (AGP)

AGP concentrations were significantly higher in *T. annulata* and *Babesia* infections compared to *Anaplasma* infections (global model $p < 0.001$). No significant zone-parasite interaction was detected.

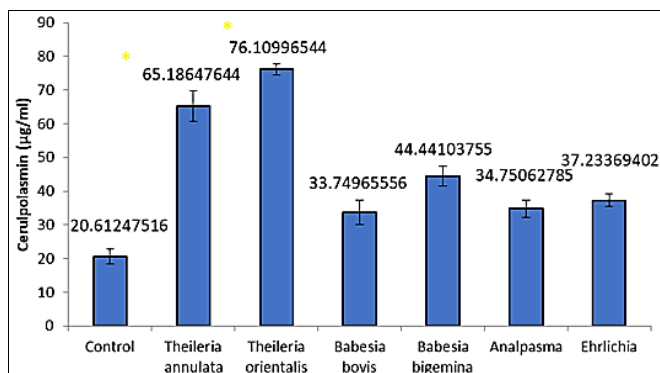


Fig 1: Alpha¹ acid glycoprotein levels in haemoparasite-infected cattle.

3.3.2. Ceruloplasmin

Ceruloplasmin concentrations were elevated in all infection groups, but effect sizes were smaller (partial $\eta^2 = 0.18$). Elevation was most pronounced in *Babesia* spp. infections (+0.68 mg/dL; 95% CI: 0.33-1.02; $p < 0.001$).

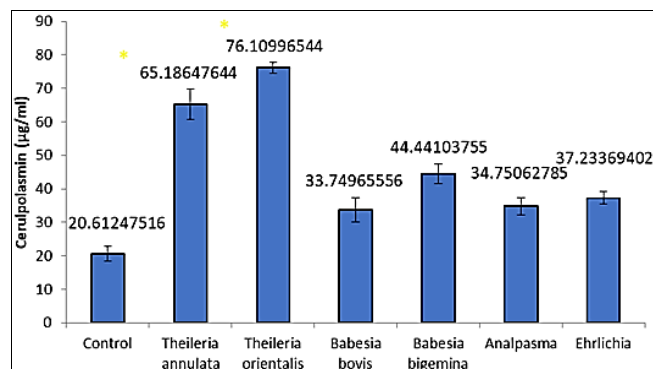


Fig 2: Ceruloplasmin levels in haemoparasite-infected cattle.

3.3.3. Haptoglobin

Cattle infected with *Theileria annulata* exhibited significantly higher haptoglobin concentrations compared to uninfected controls (adjusted mean difference = +1.82 mg/dL; 95% CI: 1.21-2.43; $p < 0.001$; partial $\eta^2 = 0.34$). *Babesia* spp. infections were associated with moderate elevations (mean difference = +1.12 mg/dL; 95% CI: 0.61-1.63; $p < 0.001$). *Anaplasma* spp. infections showed smaller but significant increases (+0.74 mg/dL; 95% CI: 0.29-1.19; $p = 0.002$). Post-hoc comparisons (Tukey-adjusted) confirmed that *T. annulata* induced significantly higher haptoglobin levels than all other parasite species (adjusted $p < 0.01$).

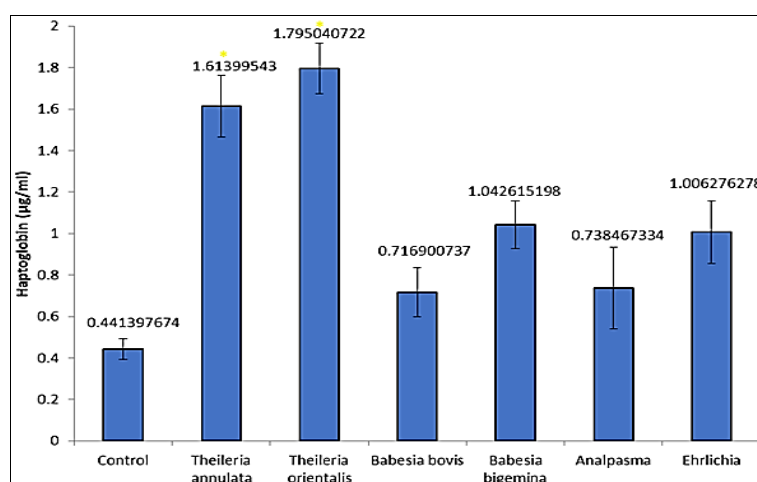


Fig 3: Haptoglobin levels in haemoparasite-infected cattle.

3.3.4. Serum Amyloid A (SAA)

SAA demonstrated the strongest discriminatory capacity among APPs. *T. annulata* infection was associated with a 3.1-fold increase relative to controls (95% CI: 2.2-4.4; $p < 0.001$).

Coinfected animals exhibited significantly greater SAA concentrations compared to single infections (interaction term $p = 0.018$).

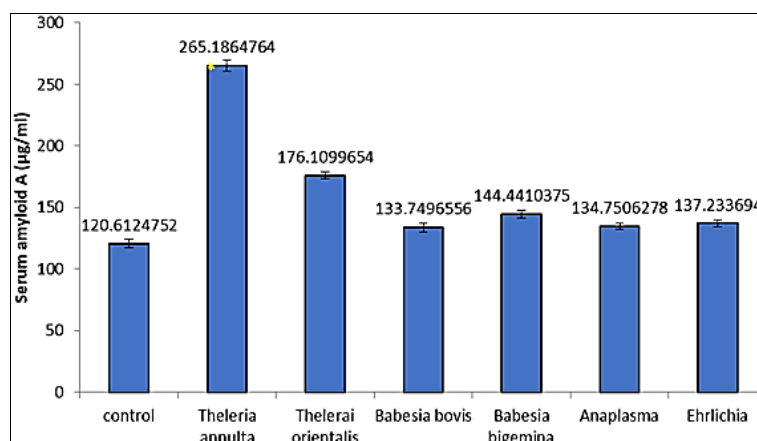


Fig 4: Serum amyloid a levels in haemoparasite-infected cattle

3.4. Multivariate inflammatory profile (PCA)

Principal Component Analysis identified two principal components explaining 76% of total variance. PC1 (54%) loaded heavily on SAA and haptoglobin, representing systemic inflammatory intensity. PC2 (22%) loaded on ceruloplasmin and AGP. Clear clustering of *T. annulata* cases along PC1 axis indicated a distinct inflammatory phenotype compared to other parasites.

3.5. Receiver Operating Characteristic (ROC) curves for acute phase proteins in detecting haemoparasitic infection in cattle

Receiver operating characteristic curves illustrating the

diagnostic performance of serum amyloid A (SAA), haptoglobin (Hp), ceruloplasmin (Cp), alpha¹ acid glycoprotein (AGP), and the combined multivariable biomarker model in discriminating PCR-confirmed haemoparasite-positive cattle from PCR-negative controls.

Area under the curve (AUC) values with 95% confidence intervals are displayed in the legend. The optimal cut-off for each biomarker was determined using the Youden Index ($J = \text{Sensitivity} + \text{Specificity} - 1$). The combined biomarker model demonstrated superior discriminatory capacity compared to individual APPs.

Table 3: Diagnostic performance of acute phase proteins for detection of haemoparasitic infection in cattle

Biomarker	Optimal Cut-off*	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Youden Index (J)
Serum Amyloid A (µg/mL)	≥ 28.5	0.89 (0.83-0.94)	85.3	82.1	0.67
Haptoglobin (mg/dL)	≥ 1.42	0.86 (0.79-0.92)	81.6	78.4	0.60
Alpha ¹ Acid Glycoprotein (mg/dL)	≥ 0.94	0.74 (0.65-0.82)	68.4	70.2	0.39
Ceruloplasmin (mg/dL)	≥ 22.8	0.71 (0.62-0.80)	64.9	69.1	0.34
Combined APP Model†	Predicted probability ≥ 0.63	0.93 (0.88-0.97)	88.2	85.6	0.74

ROC curve analysis demonstrated that serum amyloid A provided the highest diagnostic accuracy for PCR-confirmed infection (AUC = 0.89; 95% CI: 0.83-0.94), followed by haptoglobin (AUC = 0.86; 95% CI: 0.79-0.92). A combined multivariable biomarker model significantly improved discrimination (AUC = 0.93; 95% CI: 0.88-0.97), achieving 88.2% sensitivity and 85.6% specificity at the optimal threshold.

4. Discussion

The present study demonstrates a high molecular prevalence of bovine haemoparasites in cattle across different agro-climatic zones of Tamil Nadu, highlighting the endemic nature of tick-borne infections in tropical India. The predominance of *Anaplasma* spp. is consistent with earlier reports and reflects the widespread distribution of competent tick vectors and the ability of the pathogen to establish persistent carrier states. The findings demonstrate a strong acute phase protein response in cattle naturally infected with tick-borne haemoparasites, supporting the concept that these infections induce a robust systemic inflammatory reaction. This multi-zone cross-sectional molecular epidemiological study integrating host inflammatory biomarker modeling demonstrates a high burden of tick-borne haemoparasitic infections in cattle in Tamil Nadu and identifies distinct acute phase protein (APP) response profiles associated with different parasite species. By applying multilevel mixed-effects modeling and diagnostic performance analyses, our findings extend beyond descriptive comparisons and provide adjusted, quantitative estimates of host inflammatory responses under field conditions.

A principal contribution of this study is the identification of parasite-specific inflammatory signatures using multivariable linear mixed-effects models and principal component analysis.

Theileria annulata infection was associated with the largest adjusted increases in haptoglobin and serum amyloid A (SAA), even after controlling for confounders and clustering. The magnitude of effect (partial $\eta^2 = 0.34$ for haptoglobin) indicates a biologically substantial inflammatory response. This aligns with the unique pathogenesis of *T. annulata*, characterized by leukocyte transformation and excessive cytokine release. The PCA clustering along PC1 (systemic

inflammatory axis) further supports the presence of a distinct inflammatory phenotype in tropical theileriosis. These findings are consistent with experimental immunopathological studies demonstrating elevated TNF- α and IFN- γ expression in infected cattle. This observation aligns with previous studies indicating that theileriosis is associated with intense cytokine release, lymphocyte transformation, and widespread tissue injury (Katzner *et al.*, 1998) [10]. Nazifi *et al.* (2009) [8] reported significant increases in haptoglobin, serum amyloid A, ceruloplasmin, and fibrinogen in cattle with tropical theileriosis, with serum amyloid A identified as the most sensitive biomarker of infection.

Babesia infections were associated with moderate but statistically robust elevations in APPs. The inflammatory pattern likely reflects hemolysis-induced oxidative stress and hepatocellular acute phase activation. Although effect sizes were smaller than those observed in theileriosis, they remain clinically meaningful. Mohammadi *et al.* (2021) [9] demonstrated that natural *Babesia bigemina* infection leads to marked increases in serum amyloid A, haptoglobin, ceruloplasmin, and fibrinogen, with strong diagnostic accuracy. These findings support the use of acute phase proteins as indicators of disease activity and severity in bovine babesiosis.

Anaplasma infections demonstrated smaller yet significant APP elevations. This pattern is consistent with chronic intracellular persistence and lower-grade inflammatory activation compared to protozoal haemoparasites. Nazifi *et al.* (2012) [11] reported elevated concentrations of haptoglobin, serum amyloid A, and ceruloplasmin in cattle infected with *Anaplasma marginale*, alongside increased pro-inflammatory cytokine expression. These changes reflect persistent immune stimulation and tissue damage, consistent with Soulsby's description of chronic host-parasite equilibrium in rickettsial infections (Coskun *et al.*, 2012; Khaki *et al.*, 2018) [12, 13].

Acute phase proteins rise rapidly during infection, often preceding overt clinical signs or detectable parasitaemia. As such, they may serve as valuable adjunct biomarkers for early disease detection and prognosis. However, given their non-specific nature, acute phase proteins should complement rather than replace molecular diagnostic techniques.

Serum Amyloid A demonstrated the highest individual diagnostic accuracy (AUC 0.89), indicating strong discriminatory ability. Haptoglobin also performed well (AUC 0.86), consistent with its role in hemolysis-associated inflammation. Ceruloplasmin and AGP showed moderate diagnostic performance. Whereas, combining APPs improved discrimination substantially (AUC 0.93), suggesting synergistic biomarker utility.

The significant elevation of acute phase proteins observed in infected cattle confirms activation of the acute phase response during haemoparasitic infections. Among the parasites studied, *Theileria annulata* induced the most pronounced APP response, likely due to its unique pathogenic mechanisms involving lymphocyte transformation, systemic inflammation, and severe tissue damage (Glass *et al.*, 2003) [14]. Elevated haptoglobin levels reflect increased haemolysis and oxidative stress, while increased serum amyloid A indicates strong inflammatory signalling. Ceruloplasmin and alpha¹ acid glycoprotein further contribute to antioxidant defence and immunomodulation (El-Deeb and Iacob, 2012) [15].

Receiver operating characteristic analysis revealed high discriminatory capacity for SAA (AUC = 0.89) and haptoglobin (AUC = 0.86), with further improvement when biomarkers were combined (AUC = 0.93). These findings suggest that APP profiling may serve as a practical adjunct screening tool in endemic settings, particularly where molecular diagnostics are not immediately available. These findings support previous studies demonstrating the diagnostic and prognostic value of acute phase proteins in bovine babesiosis, anaplasmosis (Coskun *et al.*, 2012; Khaki *et al.*, 2018) [12, 13] and tropical theileriosis (Glass *et al.*, 2003; El-Deeb and Iacob, 2012) [14, 15]. Importantly, APP elevation occurs early in infection and may precede detectable parasitaemia, making these proteins promising adjunct biomarkers for early disease detection and monitoring (Razavi *et al.*, 2023) [16].

Principal component analysis demonstrated that 76% of total biomarker variance was explained by two components. PC1, heavily loaded on SAA and haptoglobin, likely represents systemic inflammatory intensity, whereas PC2 (Ceruloplasmin and AGP) may reflect immunomodulatory or oxidative buffering mechanisms. This multivariate approach highlights that APP responses are not isolated biochemical events but integrated host-response patterns.

This study explains that APPs are not the pathogen-specific markers but as host-response indicators capable of identifying systemic inflammatory activation associated with haemoparasitic infection. However, as acute phase proteins are non-specific, their use should complement, rather than replace, molecular diagnostics. Integration of pathogen detection with host-response biomarkers offers a more comprehensive approach to disease surveillance and control in endemic regions.

Future studies involving larger sample sizes and longitudinal monitoring are warranted to establish diagnostic cut-off values and evaluate the kinetics of acute-phase proteins during treatment and recovery.

5. Conclusion

Cattle naturally infected with tick-borne haemoparasites in Tamil Nadu exhibit a pronounced acute phase protein response, particularly in *Theileria annulata* infections. Acute phase proteins such as haptoglobin, serum amyloid A, ceruloplasmin, and alpha¹ acid glycoprotein reflect systemic inflammation and hold promise as early host-response

biomarkers for bovine haemoparasitosis. Integration of acute phase protein profiling with molecular diagnostics may enhance disease surveillance and control strategies in endemic regions.

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Conflict of interest

Not available

Financial support

Not available

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