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Investigation of Selected Biochemical Parameters in Buffaloes (*Bubalus bubalis*) Naturally Infected with *Anaplasma marginale*

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ABSTRACT
This study was conducted to evaluate the serum biochemical changes in water buffaloes naturally infected with *Anaplasma marginale*. The infection was diagnosed by detection of peripheral intraerythrocytic inclusion bodies in Giemsa-stained blood smears and confirmed by a competitive ELISA (cELISA) test. The study included 20 buffaloes that tested positive for *A. marginale* and 15 clinically healthy buffaloes as the control group. Serum samples from both infected and healthy animals were analyzed for the following biochemical parameters: albumin, total protein, total and direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), amylase (AMYL), blood urea nitrogen (BUN), creatinine (CRE), glucose, calcium (Ca), phosphorus (P), sodium (Na), potassium (K), chloride (Cl), creatine kinase (CK), creatine kinase-MB isoenzyme (CK-MB), C-reactive protein (CRP), procalcitonin (PCT), and high-sensitivity cardiac troponin I (hs-cTnI). Clinically, infected buffaloes showed signs such as fever, depression, loss of appetite, and reduced milk production. Compared with the control group, infected animals had significantly lower serum calcium ($p<0.001$) and glucose ($p<0.05$) levels, along with higher hs-cTnI levels ($p<0.05$). No statistically significant differences were observed in the other biochemical parameters between the groups. These findings suggest that although *Anaplasma* infection in buffaloes often presents with mild clinical symptoms, it can still lead to hypocalcemia, hypoglycemia, and myocardial injury.

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Anaplasma marginale ile Doğal Olarak Enfekte Olmuş Mandalarda (*Bubalus bubalis*) Seçilmiş Biyokimyasal Parametrelerin İncelenmesi

ÖZET
Bu çalışma, doğal olarak *Anaplasma marginale* ile enfekte olmuş su mandalarında serum biyokimyasal değişikliklerini değerlendirmek amacıyla yapılmıştır. Enfeksiyon, Giemsa ile boyanmış kan yaymalarında periferik intrasitoplazmik inklüzyon cisimciklerinin saptanmasıyla teşhis edilmiş ve rekabetçi ELISA (cELISA) testi ile doğrulanmıştır. Çalışmaya *A. marginale* pozitif bulunan 20 manda ile kontrol grubu olarak kullanılan klinik olarak sağlıklı 15 manda dahil edilmiştir. Her iki gruptan alınan serum örneklerinde albümin, total protein, total ve direkt bilirubin, alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), alkalen fosfataz (ALP), gama-glutamil transferaz (GGT), amilaz (AMYL), kan üre azotu (BUN), kreatinin (CRE), glukoz, kalsiyum (Ca), fosfor (P), sodyum (Na), potasyum (K), klor (Cl), kreatin kinaz (CK), kreatin kinaz-MB izoenzimi (CK-MB), C-reaktif protein (CRP), prokalsitonin (PCT) ve yüksek duyarlılıklı kardiyak troponin I (hs-cTnI) parametreleri analiz edilmiştir. Klinik olarak enfekte mandalarda ateş, depresyon, iştahsızlık ve süt veriminde azalma gibi belirtiler gözlenmiştir. Kontrol grubuyla karşılaştırıldığında, enfekte hayvanlarda serum kalsiyum ($p<0,001$) ve glukoz ($p<0,05$) düzeyleri düşük, hs-cTnI düzeyleri yüksek ($p<0,05$) bulunmuştur. Diğer biyokimyasal parametreler açısından gruplar arasında istatistiksel

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olarak anlamlı bir farklılık saptanmamıştır. Bu bulgular, su mandalarında *Anaplasma* enfeksiyonunun genellikle hafif klinik semptomlarla seyretmesine rağmen hipokalsemi, hipoglisemi ve miyokardiyal hasara yol açabileceğini göstermektedir.

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INTRODUCTION

Anaplasma species are small rickettsial bacteria that infect the erythrocytes of ruminant animals and cause significant economic losses in herds. Transmission occurs primarily through ticks, which are biological vectors; however, the disease can also be spread mechanically through biting flies or iatrogenically through contaminated needles, surgical instruments, or blood products (Nazifi et al., 2012; Özkan et al., 2019; Otranto & Wall, 2024).

Anaplasmosis is widespread worldwide, particularly in tropical and subtropical regions (Nazifi et al., 2012; Aiello et al., 2016). Infected animals can develop a peracute form characterized by sudden fever followed by death within a few hours (Özkan et al., 2019). During the acute and chronic phases, typical clinical signs include fever, anorexia, mucosal pallor, reduced milk yield, and weight loss (Özkan et al., 2019; Namratha & Ramesh, 2020; Salinas Estrella et al., 2022). As the disease progresses, jaundice, severe dyspnea, diarrhea, abortion, and death develop (Özkan et al., 2019). *A. marginale* can infect a broad range of domestic and wild animals. While it is pathogenic in cattle, infections in other species are usually subclinical or mild (Kuttler, 1984). Buffaloes are likewise susceptible to anaplasmosis, although the clinical course is generally less severe than in cattle (Rajput et al., 2005).

There is conflicting information on whether anaplasmosis alters certain biochemical parameters in buffaloes. Jalali et al. (2023) reported no significant changes in ALT, AST, total and direct bilirubin, glucose, total protein, albumin, urea, Ca, P, Mg, Fe, Na, and K levels, whereas Swetha et al. (2023) observed notable changes in total protein, albumin, globulin, AST, bilirubin, BUN, creatinine, Ca, P, Na, K, Cl, glucose, cholesterol, LDH, GGT, and CK-MB levels.

Procalcitonin (PCT) has been increasingly investigated as a biomarker for systemic bacterial and severe parasitic infections, as it closely reflects the intensity of the inflammatory response and disease severity (Lee, 2013; Lum et al., 2022; Tripathy et al., 2023). PCT levels rise markedly in bacterial sepsis and have also been reported to increase in protozoal infections such as malaria and babesiosis (Lee, 2013; Arslan et al., 2018; Tripathy et al., 2023).

Although numerous studies have evaluated the biochemical alterations associated with anaplasmosis in cattle, data regarding buffaloes remain scarce. Moreover, to the best of our knowledge, the effects of Anaplasma marginale infection on novel biomarkers such as high-sensitivity cardiac troponin I (hs-cTnI) and procalcitonin (PCT) have not yet been investigated in buffaloes. Therefore, this study aims to address this knowledge gap by determining levels of selected biochemical parameters, including acute-phase and cardiac biomarkers such as PCT and hs-cTnI, in buffaloes naturally infected with Anaplasma marginale, thereby elucidating the biochemical and potential cardiac alterations associated with anaplasmosis in this species.

MATERIAL and METHOD

Ethics statement

This study was approved by the Local Ethics Committee for Animal Experiments of Tekirdağ Namık Kemal University (Approval No. T2025).

Sampling

This study was conducted on two different farms located in the Çatalca district of Istanbul Province, where buffalo deaths had been reported. A smear prepared from the kidney, spleen, and liver of the buffalo was examined in the laboratory. The samples were stained with Giemsa stain according to the procedure and examined microscopically. Upon detection of intraerythrocytic inclusion bodies resembling *A. marginale* within erythrocytes, the same farms were visited.

A total of 89 buffaloes on two farms were examined, and blood samples were collected from 33 animals exhibiting clinical signs consistent with anaplasmosis, including fever (>39.5 °C), depression, anorexia, reduced milk yield, and/or pale mucous membranes, together with Rhipicephalus spp. tick infestation. The buffaloes were between 2

and 8 years of age. Blood samples were collected from the animals via the jugular vein into both anticoagulant (EDTA) and plain tubes, and peripheral blood samples were also obtained by cutting the tip of the ear.

Microscopic and serological Examination

Smears were prepared from the anticoagulated and peripheral blood samples, air-dried, and fixed with methanol for 5 minutes. The smears were then stained with Giemsa stain for 45 minutes and examined microscopically for the presence of *A. marginale*-like inclusion bodies within erythrocytes. Samples found to be A. marginale-positive under the microscope were tested using a competitive enzyme-linked immunosorbent assay (cELISA) kit (VMRD, Pullman, USA) to confirm the diagnosis serologically. The test was performed according to the manufacturer's protocol, and the evaluation was conducted according to Gattan et al. (2025). In evaluating the cELISA test results, the manufacturer's recommended inhibition percentage was used as the reference. The percentage of inhibition (%I) was calculated using the formula %I = 100 × [1 − (sample OD / negative control OD)]. Test validity was determined in accordance with the manufacturer's instructions; accordingly, the assay was considered valid when the mean optical density (OD) of the negative controls was greater than 0.40 and less than or equal to 2.10, and when the mean inhibition value of the positive controls was ≥30%. Only results obtained under valid test conditions were included in the analysis, and samples with an inhibition rate ≥30% were considered positive.

A total of 20 buffaloes that tested positive by both microscopic examination and cELISA were defined as the *Anaplasma*-infected group. In contrast, 15 animals from the same region that appeared clinically healthy, had no intraerythrocytic inclusion bodies in their blood smears, and tested negative by cELISA were considered the control group.

Biochemical Analyses

Blood samples collected in non-anticoagulant tubes were centrifuged at 3000 rpm for 10 minutes, and the resulting sera were separated and stored at –20°C in a deep freezer. In the serum samples, levels of albumin, total protein, total and direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), amylase (AMYL), blood urea nitrogen (BUN), creatinine (CRE), glucose, calcium (Ca), phosphorus (P), sodium (Na), potassium (K), and chloride (Cl), as well as the enzyme activities of creatine kinase (CK), creatine kinase-MB (CK-MB), and C-reactive protein (CRP), were measured using an autoanalyzer (COBAS 8000, Roche, Switzerland) with appropriate commercial kits. Serum levels of procalcitonin (PCT) and high-sensitivity cardiac troponin I (hs-cTnI) were measured using the Maglumi 2000 (Snibe Diagnostic, China) device.

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics 24 software. The Shapiro-Wilk test was used to assess whether the data followed a normal distribution. For between-group comparisons, variables that were not normally distributed were analyzed using the non-parametric Mann–Whitney U test (TP, ALP, GGT, D.BIL, T.BIL, CRE, hs-cTnI, PCT), whereas variables with a normal distribution were analyzed using the independent-samples t-test (Glucose, ALB, ALT, AST, AMYL, BUN, CK, CK-MB, Cl, K, Na, P, Ca, CRP). For parametric analyses, results are presented as mean ± standard error (X ± SE), while non-parametric data are presented as the median with minimum–maximum values (Xmin–Xmax). A p-value of <0.05 was considered statistically significant.

RESULTS and DISCUSSION

During the clinical examination of the animals, clinical signs such as fever, depression, anorexia, decreased milk yield, and pale mucous membranes were observed. Ticks belonging to the genus *Rhipicephalus* were detected on the animals. No statistically significant differences were found in the levels of biochemical parameters including TP, ALB, ALP, ALT, AST, GGT, D.BIL, T.BIL, BUN, CRE, CK, CK-MB, CRP, PCT, Na, K, Cl, P, and AMYL (Table 1). In infected buffaloes, decreased Ca ($p<0.001$) and glucose ($p<0.05$) levels and increased hs-cTnI levels ($p<0.05$) were observed (Table 1, Figure 1).

In this study, changes in certain biochemical parameters in naturally infected buffaloes with anaplasmosis were evaluated, and significant differences were observed compared with the healthy control group. Infected buffaloes showed a significant decrease in calcium and glucose levels, and a significant increase in hs-cTnI levels. No notable differences were observed in other biochemical parameters. Although buffaloes are susceptible to the anaplasmosis agent similarly to cattle, it has been emphasized that they generally carry the infection at a subclinical carrier level and therefore play an important reservoir role for *A. marginale* in nature (Namratha & Ramesh, 2020; Jalali et al., 2023).

Table 1. Serum biochemical parameters in buffaloes naturally infected with *A. marginale* and healthy control buffaloes

Çizelge 1. Doğal olarak A. marginale ile enfekte mandalarda ve sağlıklı kontrol mandalarında serum biyokimyasal parametreler.

Parameters	*Infected buffaloes (n=20)*	*Healthy controls (n=15)*	*P value*
Glukoz (mg dL^{-1})	51.39 ± 1.92	60.87 ± 3.62	p=0.019(t-test)
TP (g dL^{-1})	8.01 (5.96-8.42)	7.95 (3,37-8.56)	p= 0.531(Mann-Whitney U)
ALB (g dL^{-1})	3.11 ± 0.09	3.21 ± 0.07	p=0.440(t-test)
ALP (U L^{-1})	59.00 (21.00-267.00)	85.50 (38.00-212.00)	p=0.416(Mann-Whitney U)
ALT (U L^{-1})	15.38 ± 2.08	14.42 ± 1.63	p=0.738(t-test)
GGT (U L^{-1})	13.00 (12.00-43.00)	24.00 (17.00-39.00)	p=0.489(Mann-Whitney U)
AST (U L^{-1})	81.44 ± 7.57	78.36 ± 5.76	p=0.766(t-test)
D.BIL (mg dL^{-1})	0.03 (0.01-0.09)	0.025 (0.01-0.55)	p=0.456(Mann-Whitney U)
T.BIL (mg dL^{-1})	0.16 (0.04-0.76)	0.12 (0.04-1.21)	p=0.856(Mann-Whitney U)
BUN (mg dL^{-1})	19.22 ± 2.70	22.17 ± 2.90	p=0.472(t-test)
CRE (mg dL^{-1})	1.69 (0.70-3.96)	1.58 (1.18-1.71)	p=0.541(Mann-Whitney U)
CK (U L^{-1})	14.00 ± 3.74	24.00 ± 2.00	p=0.176(t-test)
CK-MB (U L^{-1})	14.87 ± 3.02	7.90 ± 0.60	p=0.174(t-test)
hs-cTnI (ng mL^{-1})	0.028 (0.01-0.30)	0.02 (0.01-0.05)	p=0.045(Mann-Whitney U)
AMYL (U L^{-1})	9.14 ± 1.18	16.38 ± 6.58	p=0.330(t-test)
Cl (mmol L^{-1})	93.13 ± 0.61	94.08 ± 0.83	p=0.350(t-test)
K (mmol L^{-1})	7.20 ± 0.31	7.62 ± 0.52	p=0.483(t-test)
Na (mmol L^{-1})	139.75 ± 0.76	139.50 ± 1.26	p=0.859(t-test)
P (mg dL^{-1})	5.50 ± 0.30	5.75 ± 0.44	p=0.634(t-test)
Ca (mg dL^{-1})	5.78 ± 0.75	9.70 ± 0.20	p=0.000(t-test)
CRP (mg L^{-1})	0.160 ± 0.029	0.099 ± 0.030	p=0.168(t-test)
PCT (ng mL^{-1})	0.017 (0.01-0.057)	0.010 (0.01-0.059)	p=0.857(Mann-Whitney U)

Values are presented as mean ± SE for parametric data and as median (min–max) for non-parametric data.Statistical significance was considered at $p<0.05$.

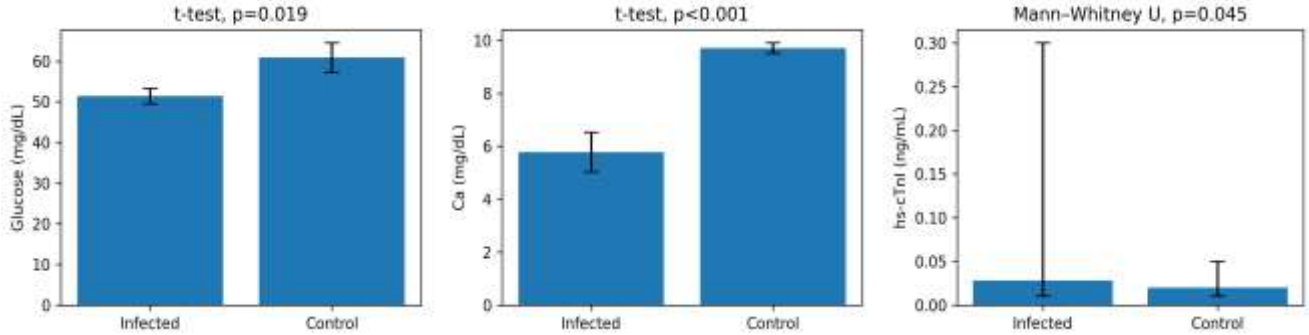

Figure 1. Bar graphs showing serum glucose, calcium, and hs-cTnI values in Anaplasma-infected buffaloes and healthy controls.

Şekil 1. Anaplasma ile enfekte mandalar ve sağlıklı kontrol grubunda serum glukoz, kalsiyum ve hs-cTnI düzeylerini gösteren sütun grafikler.

In an experimental study conducted by Lima et al. (2019), buffaloes inoculated with A. marginale were clinically more resistant than cattle and did not develop significant symptoms, whereas splenectomized cattle infected with the same strain developed severe anemia and died (Lima et al., 2019). In this study, the farms were investigated following reports of buffalo deaths; however, these fatalities were more likely attributable to predisposing factors—such as heavy tick infestation, advanced age, high infection pressure, and possible concurrent infections—than to

the typical course of *A. marginale* infection. During farm visits, only a limited number of buffaloes exhibited pronounced clinical signs, whereas the majority were mildly affected or remained subclinical. This observation is consistent with previous reports describing buffaloes as relatively resistant carriers of anaplasmosis. (Lima et al., 2019; Namratha & Ramesh, 2020; Jalali et al., 2023) Notably, only a subset of animals showed marked clinical signs, whereas most buffaloes on the farms appeared mildly affected or subclinical, which aligns with the literature describing buffaloes as relatively resistant carriers. However, the marked hypocalcemia and hypoglycemia observed in infected buffaloes suggest that the infection may also have clinical effects in these animals. Indeed, in a study conducted on naturally infected buffaloes in India, significantly lower levels of total protein, albumin, glucose, calcium, phosphorus, sodium, potassium, and chloride were detected in infected animals compared to healthy controls, while increases were reported in globulin, AST, bilirubin, BUN, creatinine, GGT, and CK-MB levels (Swetha et al., 2023). In the same study, infected buffaloes exhibited prominent clinical signs, including anorexia, ruminal stasis, marked decrease in milk production, fever, depression, weight loss, jaundice, lymphadenopathy, dyspnea, and ocular-nasal discharge (Swetha et al., 2023). These findings indicate that severe infections in buffaloes can lead to metabolic and biochemical imbalances.

As also reported by Jalali et al. (2023), no differences were observed in many parameters such as ALT, ALP, total protein, and albumin. However, consistent with the findings of Swetha et al. (2023), a decrease in calcium and glucose levels was observed. Jalali et al. (2023) emphasized that buffaloes may carry the infection subclinically and may not develop organ dysfunction at the laboratory level. In contrast, it was demonstrated that in buffaloes experiencing clinical anaplasmosis, certain biochemical alterations may occur as a result of anorexia and metabolic stress. The hypoglycemia detected in infected buffaloes is thought to be related to reduced energy intake due to appetite loss and the negative energy balance caused by the infection. In fact, complete anorexia and a sharp decline in milk production are common findings in buffaloes with anaplasmosis (Swetha et al., 2023). As a result of energy deficiency, hepatic gluconeogenesis capacity may decrease, and peripheral utilization may increase. Clinically, hypoglycemia may lead to a reduction in milk yield by limiting the metabolic energy required for lactation, and the resulting negative energy balance may create a predisposing condition for the development of ketosis. Similarly, decreases in total protein and glucose have been reported in a cow with *Anaplasma* infection (Jayalakshmi et al., 2020). Hypocalcemia, on the other hand, may be associated with reduced dietary calcium intake due to anorexia (Ramadevi et. al., 2017), impaired intestinal absorption, and infection-related alterations in calcium metabolism (Schafer & Shoback, 2016; Goyal et al., 2023). From a clinical perspective, hypocalcemia may impair neuromuscular function and rumen motility, thereby exacerbating inappetence, reducing milk synthesis, and predisposing to the development of clinical hypocalcemia (milk fever). Consistent with previous reports, Swetha et al. (2023) in buffaloes, and Ganguly et al. (2018) and Al-Shammari et al. (2024) in cattle also reported hypocalcemia in cases of anaplasmosis.

Marked increases in BUN and creatinine levels have been reported in some studies (Swetha et al., 2023). However, these findings are inconsistent across studies in the literature. In a study of asymptomatic anaplasmosis cases, no significant change in blood urea nitrogen (BUN) levels was reported (Jalali et al., 2023), and in symptomatic anaplasmosis-infected buffaloes, BUN and creatinine levels were also found to be statistically unchanged (Ganguly et al., 2022). Similarly, no significant difference was observed between the infected and control groups of buffaloes in BUN and creatinine levels. This suggests that *Anaplasma* infection may not cause significant kidney damage in buffaloes, or that the infection, at this stage, does not significantly affect renal function. These results are consistent with the findings reported by Ganguly et al. (2022) and Jalali et al. (2023), but differ from those reported by Swetha et al. (2023).

One of the most striking observations was the increase in hs-cTnI levels in infected buffaloes. Cardiac troponins are important biomarkers for identifying myocardial injury. There is no significant difference between cTnI and cTnT; both are widely used in clinical settings. However, hs-cTn tests can detect even minimal increases in troponin levels, making them useful for earlier and more reliable diagnosis of myocardial damage (Loughrey & Young, 2014). To date, no previous data exist in the literature regarding cardiac troponin levels during anaplasmosis in buffaloes. In this regard, the current results demonstrate potential cardiac involvement due to anaplasmosis in buffaloes. The findings suggest that the infection may lead to myocardial injury in these animals. The mild but statistically significant increase in hs-cTnI suggests that the infection may induce subclinical myocardial stress, which may pose a risk of long-term cardiac dysfunction, particularly in high-producing buffaloes. Yogeshpriya et al. (2022) reported a marked increase in cTnI levels in dairy cows naturally infected with *Anaplasma*. They emphasized that although other cardiac or muscle enzymes such as CK-MB and LDH were elevated in infected animals, troponin was more reliable for identifying myocardial damage (Yogeshpriya et al., 2023). Similarly, in a case report by Jayalakshmi et al. (2020), a cow with co-infection of anaplasmosis and fasciolosis exhibited a significant elevation in cTnI, along with reduced total protein and glucose levels, which were linked to cardiac injury. *Anaplasma* infections have also been shown to affect the heart in small ruminants. In a study conducted

on sheep infected with *Anaplasma ovis*, cTnI levels in the infected group were found to be significantly higher than those in the healthy group, providing evidence that the parasite can impair cardiac function (Uslu et al., 2023). All these findings support the notion that *Anaplasma* infections can cause cardiac damage in ruminants. Histopathological examination of the heart in cattle with anaplasmosis has revealed irregularities in myocardial fibers, mild to moderate multifocal myocardial degeneration, and coagulative necrosis (Jaswal et al., 2015). Although the exact mechanism of cardiac damage in anaplasmosis remains unclear, several potential pathogenic pathways have been proposed. The most significant factor is tissue hypoxia caused by severe anemia. In hemoparasitic infections, the destruction of erythrocytes reduces oxygen-carrying capacity, limiting oxygen delivery to vital organs—including the heart—and potentially leading to ischemic injury (Yogeshpriya et al., 2022). Another possible mechanism is that during prolonged tachycardia, the increased myocardial oxygen consumption coincides with reduced oxygen delivery due to the shortened diastolic period, thereby exacerbating myocardial hypoxia (Fartashvand et al., 2013). Metabolic disturbances such as hypocalcemia and hypoglycemia observed in infected buffaloes also create an unfavorable environment for cardiac muscle. Calcium is a key ion for cardiac contraction; calcium deficiency can weaken myocardial contractility and increase susceptibility to arrhythmias. Glucose is the primary energy source for the myocardium; in severe hypoglycemia, the heart muscle may not be able to produce sufficient ATP, leading to functional impairment. The concurrent evaluation of decreased calcium and glucose levels with elevated troponin levels in infected buffaloes may explain how the overall metabolic stress induced by the infection is reflected in cardiac function. Another important cardiac biomarker is CK-MB (Loughrey & Young, 2014). Although increased levels were observed in the infected group, the difference was not statistically significant. Notably, cardiac troponins T (cTnT) and I (cTnI) can reliably detect myocardial micro-necrosis even when CK-MB levels are not elevated (Loughrey & Young, 2014).

No significant increase in CRP and procalcitonin (PCT) levels was observed in the infected group. These findings are consistent with those of Uslu et al. (2023), who also did not detect a significant increase in CRP levels in sheep with anaplasmosis. CRP is considered a moderate acute-phase protein in sheep and cattle (Cray et al., 2009). The absence of significant changes in CRP and PCT suggests that A. marginale infection in buffaloes may not elicit a pronounced systemic inflammatory response, or that the acute phase response remains limited under the field conditions examined.

There is no evidence indicating a significant change in amylase levels in buffaloes with anaplasmosis. In a case of human granulocytic anaplasmosis, amylase levels were within the normal range (Malik et al., 2005). Since amylase measurement in buffaloes or cattle in the context of anaplasmosis is not common, there is no evidence suggesting that the infection has a significant effect on amylase levels. In the present study, no significant change in amylase levels was detected.

In this research, 20 infected and 15 healthy buffaloes were evaluated. The relatively small sample size may have limited the statistical power and restricted the generalizability of the findings. Further studies with larger populations and data obtained from different geographical regions are needed to validate and strengthen these results. Future studies are also recommended to include larger cohorts stratified by infection stage and to incorporate functional cardiac assessments, such as echocardiography, to better characterize potential subclinical myocardial involvement and its clinical relevance.

CONCLUSION

This study demonstrated that buffaloes naturally infected with *A. marginale* exhibited specific biochemical alterations, including decreased serum calcium and glucose levels and elevated high-sensitivity troponin I (hs-cTnI) concentrations. These findings contribute to a better understanding of the disease's pathophysiology and emphasize the importance of regularly monitoring these parameters in infected animals. In practical terms, these results suggest that routine monitoring of serum calcium, glucose, and hs-cTnI levels in buffaloes with suspected A. marginale infection may be useful for the early detection of subclinical metabolic and cardiac complications. In particular, the increase in hs-cTnI levels suggests that anaplasmosis may be associated with cardiac damage in buffaloes, underscoring the need for further research in this area. The current results add to the limited literature on biochemical alterations associated with anaplasmosis in buffaloes and provide new perspectives for the diagnosis, monitoring, and treatment of the disease. The data obtained serve as a guide for veterinarians in the early recognition of anaplasmosis-related complications and in developing supportive treatment and monitoring strategies.

Contribution Rate Statement Summary of Researchers

Sezai Arslan: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Supervision. Taraneh Öncel: Investigation, Methodology, Validation, Writing – review & editing. Mesut Şenel: Investigation, Methodology, Data curation, Resources.

Conflict of Interest

The authors declare that they have no conflict of interest

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