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Evaluating cytokine levels and selected biochemical parameters at two-time points in crossbred cattle infected with *Anaplasma marginale*

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Abstract

Background: Bovine Anaplasmosis is a destructive disease leading to significant livestock losses. The disease is common in Africa and is caused by *Anaplasma marginale*. Plasma biochemical profiles, including cytokine levels, can be used as helpful indicators in managing and treating the infection. Additionally, these measurements could provide insight into the physiological responses of the animals in a diseased state.

Objective: This study aimed to identify *A. marginale* using PCR and analyse biochemical and cytokine levels related to *A. marginale* in spontaneously infected Holstein-Friesian and Sanga crossbred cattle.

Methods: Forty (40) apparently healthy cattle were randomly selected from the Amrahia Dairy farm in the Greater Accra region between February and March 2021. Blood samples were taken from these animals via jugular venipuncture at two-time points (four-week intervals). Polymerase chain reaction (PCR) was conducted on DNA isolated from blood samples to detect *A. marginale* infection in cattle. Serum samples collected were used to measure levels of cytokines- Interleukin-4 (IL-4), Interleukin-10 (IL-10), and Interferon-alpha (IFN-α), along with selected biochemical parameters to assess the liver (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total Protein, Total Bilirubin, and Direct Bilirubin) and kidney (blood urea nitrogen (BUN), and creatinine(CRE) of the infected animals.

Results: At time point one, 55% of the cattle tested positive, while 70% tested positive for *A. marginale* at time point two. At time point one, there were no significant differences between infected and non-infected cattle for blood concentrations of IL-10, IL-4, IFN-α, ALT, AST, DB, TB, ALP, TP, BUN, and CRE ($p > 0.05$ respectively). However, at time point two, IFN-α ($p = 0.004$), direct bilirubin ($p = 0.02$), and creatinine ($p = 0.004$) differed significantly between the two study groups.

Conclusion: The study identified *A. marginale* by PCR and was diagnostic for Bovine Anaplasmosis. Circulating levels of cytokines and selected biochemical parameters did not alter significantly between infected and non-infected cattle when blood was taken at two-time intervals, suggesting that these cytokines may not directly contribute to the pathogenesis of Bovine Anaplasmosis.

Keywords: *Anaplasma marginale*, cytokines, biochemical, Holstein-Friesian, Crossbreed

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INTRODUCTION

Anaplasma marginale is a rickettsial organism that exclusively lives inside red blood cells and is the primary cause of Bovine Anaplasmosis [1,2]. Within the genus Anaplasma, other significant species infect the red blood cells of ruminants. These include *A. centrale*, which is less harmful and linked to a milder version of the disease, and *A. ovis*, which causes a similar condition in sheep [3,4]. Foreign cattle breeds, such as Holstein Friesian, are more prone to tick-borne haemoparasitic infections caused by

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Anaplasma, Babesia, Theileria, and other significant parasites [5]. This susceptibility is due to their weak natural immunity, resulting in a high rate of illness and death, reaching up to 100%. In contrast, local breeds exhibit more excellent resistance to these pathogens. Furthermore, many Bos taurus breeds, such as Hereford and Brown Swiss, exhibit lower resistance to *A. marginale* infection and are consequently more susceptible to developing severe Anaplasmosis when compared to hybrid cattle, such as Zebu or Creole [6].

The impact of Bovine Anaplasmosis on exotic cattle breeds and their hybrids is significant, leading to the ineffectiveness of livestock production, especially in developing nations. Farmers are concerned about the disease's low recovery rate due to the considerable economic losses incurred from veterinary intervention expenses, medicine usage, and the purchase of acaricide for tick management. In addition, the extended duration of infection in asymptomatic carrier cattle with low levels of Rickettsia enables the disease to spread to immunologically naive animals within the herd. Changes in the levels of particular blood biochemical indices and cytokine profiles are indicators of pathophysiological responses. They provide basic information on animal epidemiology to assess diet, improve healthcare practices, and minimise economic losses. An ongoing topic of discussion is the response of different components of the host immune system during spontaneous Anaplasma infections. Therefore, evaluating inflammatory biomarkers in the interactions between the host and parasite could offer a dependable means of diagnosing and managing this sickness. Anaplasma infection in cattle has been associated with inflammatory and oxidative stress reactions. While research has demonstrated that the disease can lead to abnormalities in specific biochemical measurements, information regarding cytokine dynamics in diseased cattle grown in resource-constrained environments is scarce.

MATERIALS AND METHODS

Blood sample collection and biochemical analysis
This cross-sectional study utilised a total of forty (40) Holstein-Friesian and Sanga crossbred cattle that were over six months old. The cattle were sourced from the Amrahia Dairy farm in the Greater Accra region between February and March 2021. The eligible cattle were selected using a random sample technique, which involved consulting the farm records and conducting physical examinations on cattle in good health. Aseptically, five millilitres of blood was obtained from the jugular vein. Out of these, 2 millilitres were transferred into a vacutainer tube containing EDTA as an anticoagulant to extract genomic DNA. The remaining 3 millilitres were put into empty tubes without anticoagulant and left undisturbed for 10-20 minutes to allow coagulation. Afterwards, the tubes were centrifugated at 3,000 revolutions per minute for 10 minutes, all at room temperature. The obtained sera were placed into 0.5 ml Eppendorf tubes and kept at -80°C until needed for cytokines and other specific biochemical studies. The sera were utilised for evaluating hepatic function, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), total proteins, and albumin. An automated laboratory chemistry analyser was used to measure these biochemical parameters (URIT-8021 AVet, China).

Measurement of IL-4, IL-10, and IFN- α Cytokines The Sandwich enzyme-linked immunosorbent assay (ELISA) technique was used to measure Interferon-Alpha (IFN-α), Interleukin 4 (IL-4), and 10 (IL-10) levels in serum samples. This analysis used a micro-ELISA strip-plate analyser manufactured by SUNLONG BIOTECH Company Limited, China. The analyser was pre-coated with an antibody that specifically binds to these tests. The standard tests for each cytokine were prepared and diluted serially. Ten wells were formed by adding 50 µl of each of the five standards in duplicate to specific microplate wells. Two microplate wells were kept empty as a control. 40 µl of sample dilution buffer and 10 µl of samples were pipetted into the bottom of each microplate well without direct contact. It was agitated by shaking gently. The assay plates were incubated at 37°C for 30 minutes while covered with a closure plate membrane. After carefully removing the closure plate membrane, the wells were aspirated and washed with a wash solution. After a 30-second break, the wash solution was removed, and the washing process was repeated five times, concluding with flipping and wiping the microplates on a fresh paper towel. Each well of the microplate was filled with 50 µl of Chromogen solution A and 50 µl of Chromogen solution B, then gently mixed. The sample was then placed in an incubator at 37°C for 15 minutes, shielded from direct light. 50 µl of stop solution was applied to each well to stop the reaction, causing a colour change from blue to yellow. The absorbance (OD) at 450 nm was measured using a microtiter plate reader (Varioskan lux, Thermo Fisher Scientific, USA), with the blank control's OD value and the reference point set to zero.

DNA extraction from whole blood samples
By making a minor modification, DNA was isolated from whole blood stored in EDTA tubes according to the blood kit instructions provided by the manufacturer (QIAGEN Inc. Valencia, USA). 0.5 ml of whole blood was combined with 1.5 ml of RBC lysis solution and left to incubate at room temperature for ten minutes. To ensure complete destruction of red blood cells, the tube was repeatedly turned upside down during incubation. Subsequently, the samples were centrifuged at 8,000 revolutions per minute for 10 minutes. Following centrifugation, the liquid portion above the sedimented cells, known as the supernatant, was discarded, leaving the cell pellet immersed in the remaining liquid behind. To reconstitute the cells, the solid mass at the bottom of the tube was vigorously mixed with the remaining liquid portion. A volume of 0.4 ml of Cell Lysis solution was carefully transferred into the resuspended cells to disrupt the cellular membranes. The samples were

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allowed to cool at room temperature of 25 ± 2 ºC before adding 0.3 ml of protein precipitation solution to the cell lysate. The tube was vigorously vortexed for 20 seconds at a high rotational speed to thoroughly blend the protein precipitation solution with the lysate. Subsequently, it was centrifuged for 10 minutes at 8,000 revolutions per minute. This procedure resulted in the protein precipitating into a conspicuous dark brown pellet. The DNA supernatant was poured off and put into a 1.5 mL Eppendorf tube with 1 ml of isopropanol. The presence of DNA was observable as a compact, white mass when the tube was delicately rotated 50 times. The visible DNA was meticulously extracted using a pipette and transferred to a 1.5 ml tube. It was then allowed to dry in the air for 10 minutes and reconstituted in 200 µl of DNA hydration solution. Before storing at -20 °C, the DNA pellets from different samples were incubated in a water bath at 65°C for 1 hour to facilitate complete dissolution in the hydration buffer.

Detection of *A. marginale* using PCR

Individual cattle DNA fragments of 250 base pair (bp) were amplified using *Anaplasma marginale*-specific PCR primers. The primers used to amplify the 250 bp segment of the msp1b gene were as follows: The given sequence is 5′-GCTCTAGCAGGTTATGCGTC-3′, and its complementary sequence is 5′-CTGCTTGGGAGAATGCACCT-3′. A total reaction volume of 25 µl was used for the PCR test, consisting of 0.5 µl of 10 µM forward and reverse primers, 12.5 µl of 1x Quick-Load One-Taq MasterMix with Standard Buffer (New England Biolabs, Inc.), 4.0 µl DNA template, and 7.5 µl of Nuclease-free PCR water. The amplification reactions consisted of an initial denaturation phase at a temperature of 95ºC for 5 minutes. This was followed by 35 cycles of denaturation at 94ºC for 45 seconds, annealing at 55ºC for 60 seconds, and extension at 72ºC for 2 minutes. The PCR reaction included both positive and negative controls. Ultimately, the products underwent an extension process at a temperature of 72°C for 7 minutes, followed by storage at a temperature of 4°C. The PCR products were separated and run on a 1.2% agarose gel stained with Ethidium bromide at an electric potential of 80 volts for 1 hour and 30 minutes. The results were visualised using a high-performance ultraviolet transilluminator (Cole-Palmer, France).

Data analysis

We structured the study's data in Microsoft Excel and examined it using IBM's Statistical Package for the Social Sciences (IBM-SPSS v 26.0). The software Adamsel (RemaqueR) was utilised to transform the OD values acquired from the ELISA cytokine measurement into concentrations and subsequently analysed using SPSS version 26. Descriptive statistics were generated to maximise the collected data's usefulness, and the relationships between different biochemical and cytokine parameters were examined using the paired-sample t-test. We established the statistical significance level at $p < 0.05$.

RESULTS

PCR results for testing of crossbred cattle for *A. marginale*

The PCR products obtained when run and separated on a 1.2% agarose gel stained with Ethidium bromide showed amplified products of approximately 250bp size, which are shown in Figures 1 and 2, respectively. At time point one, 55% (n = 22) of the 40 cattle tested positive for A. *marginale*, while 28 (70%) tested positive at time point two (Figures 1 and 2). In Figure 3, the results show that, out of the 18 animals that tested negative at time-point one, 11 further retested positive for A. *marginale*, while seven remained negative at time-point two.

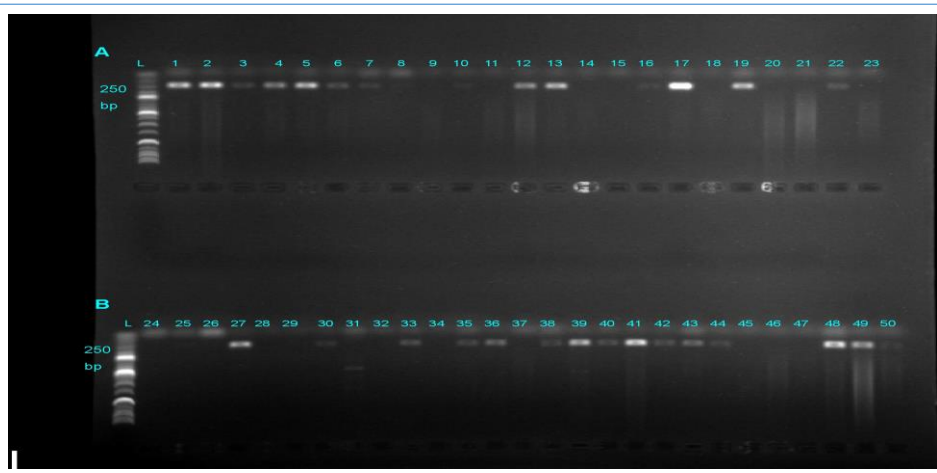

Figure 1. Detection of *A. marginale* by PCR.

Lane A: 1 - 23 represents samples taken at time-point one with 14 samples testing positive for *A. marginale*. Lane B: 24 - 40 represents samples taken at time-point 1 with 8 testing positive, while 41 - 50 are samples taken at time point two with 7 testing positive. L- 100bp DNA Ladder.

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Table 1. Comparison between biochemical parameters and PCR results at two-time points

Biochemical parameters	Time	Result	Mean	SEM	t-test	p-value
Direct Bilirubin (µmol/L)						
	Time 1	Negative	1.31	0.13	1.01	0.32
		Positive	1.86	0.50		
	Time 2	Negative	7.33	3.61	3.17	0.02*
		Positive	2.08	0.32		
Alanine aminotransferase (U/L)						
	Time 1	Negative	37.44	37.44	0.12	0.91
		Positive	39.00	39.00		
	Time 2	Negative	20.58	20.58	-1.01	0.32
		Positive	27.54	27.54		
Aspartate aminotransferase (U/L)						
	Time 1	Negative	61.33	3.88	1.11	0.27
		Positive	60.36	6.82		
	Time 2	Negative	90.17	19.8	1.05	0.30
		Positive	77.79	7.27		
Total Bilirubin (µmol/L)						
	Time 1	Negative	6.00	0.50	0.14	0.89
		Positive	5.43	0.90		
	Time 2	Negative	7.75	2.83	1.32	0.19
		Positive	5.73	0.80		
Blood Urea Nitrogen (mmol/L)						
	Time 1	Negative	8.07	0.70	1.63	0.11
		Positive	11.40	2.16		
	Time 2	Negative	15.52	6.71	0.55	0.59
		Positive	12.95	3.37		
Alkaline Phosphatase (U/L)						
	Time 1	Negative	98.17	6.82	0.38	0.71
		Positive	133.40	17.64		
	Time 2	Negative	98.33	20.87	0.34	0.37
		Positive	92.21	14.51		
Total Protein (g/l)						
	Time 1	Negative	59.14	1.82	0.92	0.37
		Positive	55.80	2.87		
	Time 2	Negative	63.54	3.43	1.03	0.31
		Positive	60.73	2.10		
Creatinine (µmol/L)						
	Time 1	Negative	69.39	5.13	0.17	0.17
		Positive	82.95	7.92		
	Time 2	Negative	85.00	11.10	-2.99	-2.99
		Positive	113.11	7.42		
Interferon-Alpha (pg/ml)						
	Time 1	Negative	189.50	27.62	1.37	0.18
		Positive	189.07	15.01		
	Time 2	Negative	213.15	37.88	2.59	0.01*
		Positive	152.76	14.81		
Interleukin-4 (pg/ml)						
	Time 1	Negative	188.41	48.97	1.99	0.53
		Positive	204.25	28.24		
	Time 2	Negative	178.91	41.64	0.33	0.75
		Positive	170.00	18.54		
Interleukin-10 (pg/ml)						
	Time 1	Negative	200.78	15.67	1.38	0.18
		Positive	216.16	28.95		
	Time 2	Negative	208.32	19.04	0.57	0.57
		Positive	219.90	17.10		

Positive = infected with *A. marginale*, Negative = not infected with *A. marginale*, *p < 0.05 indicates significance, SEM= Standard Error of Mean.

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Table 2. Comparison between biochemical parameters and gender at time point 2

Biochemical parameters	Gender	Mean	SEM	t-test	p-value
Direct Bilirubin (µmol/L)					
	Female	1.9	0.2	2.50	0.01*
	Male	5.3	2,5		
Alanine aminotransferase (U/L)					
	Female	31.9	5.3	0.01	0.10
	Male	31.8	8.7		
Aspartate aminotransferase (U/L)					
	Female	73.8	5.6	1.10	0.23
	Male	62.1	5.4		
Total Bilirubin (µmol/L)					
	Female	5.7	0.5	1.05	0.30
	Male	7.1	1.9		
Blood Urea Nitrogen (mmol/L)					
	Female	9.8	0.8	2.34	0.02*
	Male	18.8	6.7		
Alkaline Phosphatase (U/L)					
	Female	110.4	9.9	1.08	0.28
	Male	89.9	8.6		
Total Protein (g/l)					
	Female	59.7	1.5	0.48	0.64
	Male	58.3	2.7		
Creatinine (µmol/L)					
	Female	90.3	5.2	0.20	0.84
	Male	92.4	7.6		
Interferon-Alpha (pg/ml)					
	Female	180.30	11.30	0.03	0.97
	Male	179.40	28.50		
Interleukin-10 (pg/ml)					
	Female	179.40	13.10	0.21	0.84
	Male	208.60	16.80		
Interleukin-4 (pg/ml)					
	Female	178.90	14.80	0.70	0.50
	Male	205.40	50.10		

*p < 0.05 indicates significance, SEM= Standard Error of Mean.

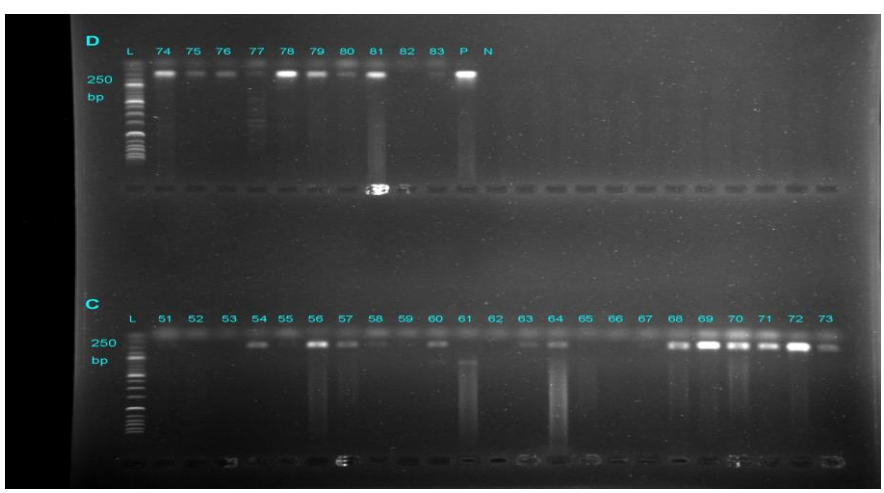

Figure 2. Detection of *A. marginale* by PCR.
Lane C: 51- 73 samples were taken at time point two, with 14 testing positive. Lane D: 74-80 represent samples taken at time-point two, with all 7 testing positive for *A. marginale*, P- positive control for *A. marginale*, and N-negative control.

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Also, 22 positive animals at time point one had five showing negative PCR tests after four weeks, with 17 remaining positive at the end of the study. Figure 4 shows the pattern of infection among the genders at four-week intervals. 61% of females and 33% of males tested positive at time point one. In comparison, 58% of females retested positive, and 67% of males retested positive out of the negative animals for A. *marginale* at time point 2. Table 1 shows the biochemical and PCR results at the two-time points. At time-point one, there was no significant difference between infected and non-infected cattle for DB, ALT, AST, TB, BUN, ALP, TP, and CRE ($p > 0.05$). However, there was a significant difference in the levels of DB ($p = 0.02$) and CRE ($p = 0.004$) among the infected and non-infected cattle at time point two. Among the cytokines determined at both time points, IFN-α was the only cytokine non-infected cattle at time point two. There was a statistically significant difference in the levels of DB (p =

Table 3: Comparison between biochemical parameters and age at the two-time points

Biochemical parameters	Age	Mean	SEM	t-test	p-value
Direct Bilirubin (µmol/L)					
Time 1	>2.5	1.33	0.11	0.93	0.36
	<2.5	1.85	0.5		
Time 2	>2.5	5.67	2.47	1.64	0.11
	<2.5	2	0.24		
Alanine aminotransferase (U/L)					
Time 1	>2.5	25.67	6.67	1.5	0.14
	<2.5	48.64	12.67		
Time 2	>2.5	29.67	9.74	0.85	0.4
	<2.5	22	1.93		
Aspartate aminotransferase (U/L)				1.1	0.28
Time 1	>2.5	65.78	6.52		
	<2.5	56.72	5.16	2.49	0.02*
Time 2	>2.5	101.44	15		
	<2.5	65.18	4.91	0.67	0.51
Total Bilirubin (µmol/L)					
Time 1	>2.5	6.09	0.58	1.77	0.09
	<2.5	5.35	0.87		
Time 2	>2.5	8.24	2.03	1.5	0.14
	<2.5	4.77	0.65		
Blood Urea Nitrogen (mmol/L)					
Time 1	>2.5	7.88	0.59	1.48	0.15
	<2.5	11.54	0.87		
Time 2	>2.5	10.33	0.52	1	0.32
	<2.5	16.49	5.53		
Total Protein (g/l)					
Time 1	>2.5	59.06	2.51	0.89	0.38
	<2.5	55.87	2.51		
Time 2	>2.5	66.77	2.06	2.88	0.01*
	<2.5	57.31	2.45		
Creatinine (µmol/L)					
Time 1	>2.5	66.28	7.92	1.99	0.05
	<2.5	85.5	5.88		
Time 2	>2.5	108.56	8.45	0.54	0.59
	<2.5	101.5	9.56		
Interferon-Alpha (pg/ml)					
Time 1	>2.5	188.73	12.65	0.03	0.98
	<2.5	189.66	25		
Time 2	>2.5	150.31	11.99	1.19	0.24
	<2.5	187.71	26.65		
Interleukin-10 (pg/ml)					
Time 1	>2.5	194.94	13.48	0.75	0.46
	<2.5	220.93	29.51		
Time 2	>2.5	199.54	10.64	1.17	0.25
	<2.5	230.23	22.11		
Interleukin-4 (pg/ml)					
Time 1	>2.5	175.55	19.28	0.73	0.47
	<2.5	214.77	45.96		
Time 2	>2.5	149.44	142.25	1.19	0.24
	<2.5	191.68	29.78		

*$p < 0.05$ indicates significance, SEM is standard Error of Mean.

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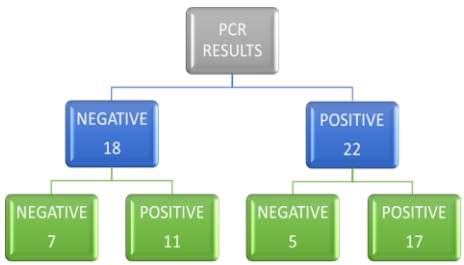

Figure 3. Overall infection status of cattle at two time-points.

Blue boxes represent time-point one and green boxes represent time-point 2. The time-point is 4-week intervals.

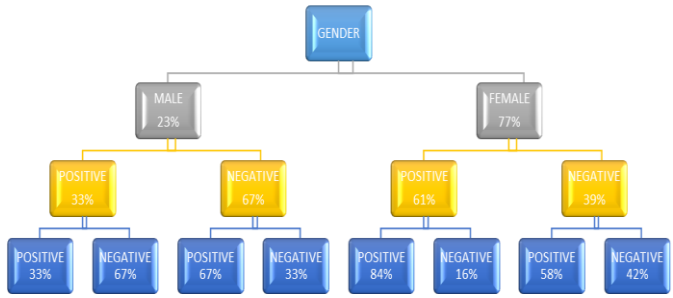

Figure 4. Distribution of infection among the gender at four-week intervals.

Yellow boxes represent time-point one, blue boxes represent time point 2

0.01) and BUN (p = 0.02) between male and female cattle, with a significant difference (p = 0.01) between infected and at time-point 2 (Table 2). There were, however, no significant differences in ALT, AST, TB, ALP, TP, CRE, IL-10, IL-4, and IFN-α (p > 0.05) between the genders at time-point 2. When comparing biochemical parameters and age, a significant difference was observed in AST (p = 0.02) and TP (p = 0.01) at time-point 2 (Table 3).

DISCUSSION

This study investigated the detection of *A. marginale* by PCR. Further, it evaluated the biochemical markers of liver and kidney function and inflammatory cytokines in Holstein-Friesian and Sanga crossbred cattle infected with *A. marginale* at two different time points. The findings suggest that PCR is a precise and sensitive technique for diagnosing *A. marginale* infection in cattle, capable of detecting low levels of rickettsemia. Previous studies in different areas have shown similar findings in accurately diagnosing *A. marginale* infections by PCR methods, identifying Rickettsaemia levels of approximately 0.03% [7,8]. This is also consistent with the study by Noaman et al. [9], whereby 50% of 150 blood samples in Iran were positive for *A. marginale*, highlighting the importance of molecular identification of A. *marginale* in carrier cattle in detecting persistently infected calves.

There was variation in infection levels among individual cattle from time point one to time point two, with some cattle retaining their infection status while others eliminated the pathogen. The elevated rate of reinfection in cattle could be attributed to factors like the immune system's reaction to the pathogen, different life cycle phases of the organism, and the disease's enzootic stability in the host [10,11]. This study did not include the various levels of rickettsemia in individual cattle, which could have influenced the infection dynamics and resulted in a high infection rate. The disease's progression is greatly impacted by the pathogen's life stage inside the host. Sahni et al. [12] suggested that the cyclical presence of Rickettsia in the host during acute and chronic infection results from the initial immune response regulating the cycle. During *A. marginale* infection, this process creates antigenic variants, which initiates a new cycle of invasion and replication. Thus, the animals that tested positive may be in the first phase of infection and exhibit a subdued immunological reaction to the pathogen. Antigenic diversity allows intracellular infections to continue within the host despite a robust immune response. A declining immunity may have led to the resurgence of the original antigenic type, resulting in an increased reinfection rate among the cattle in this study.

Several significant bovine pathogens have developed defence mechanisms that enable them to compromise the immune system and cause persistent infections [11 13]. Animals that survive the initial infection might become continuously infected or "carriers," exhibiting a cyclical low level of Rickettsaemia within the herd. These carriers possess extended immunity, remain asymptomatic, and likely played a role in the 70% reinfection rate observed in cattle after the study. Palmer et al. [14] linked the continuous infection in cattle to the antigenic shift in the immunodominant major surface protein (MSP2) and related MSP3 of *A. marginale*. Thus, these chronically infected carrier cattle serve as a biological or mechanical transmission source among the herd [15,16]. The study demonstrated that cattle are susceptible to the disease due to multiple variables, irrespective of age or sex, with reinfection and prevalence rates exceeding 50% at both time points. Bovine Anaplasmosis is common worldwide, especially in tropical regions, with a prevalence of up to 100% in Africa [1,2,17].

The study revealed a prevalence exceeding 50% at both time points, lower than the 63% prevalence reported by Beckley et al. [18] in their cross-sectional investigation on

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A. marginale in cattle throughout three vegetative zones in Ghana. Beckley et al. studied 397 indigenous cattle breeds with strong resistance to tick-borne diseases over two years, while the current study examined exotic crossbred cattle at four-week intervals. Discrepancies in illness rates can be attributed to seasonal changes, variations in the duration of the vector species' transmission cycle due to weather conditions, the abundance of various pathogenic strains, and the presence and susceptibility of the host to infection [19-21].

Ticks have a crucial role in spreading the illness, whereas the enzootic stability within the herd significantly impacts the development and prevalence of the disease [22]. As stated by Coskun et al., the elevated prevalence of the disease in the area under investigation can be linked to factors including natural immunological resistance and humid weather conditions that aid in transmitting ticks [23]. Female animals showed a higher susceptibility to infection than males, with 61% testing positive for *A. marginale* at the first time point. Subsequently, 84% of the female animals tested positive again, indicating a high infection rate among females of all ages. Coşkun et al. [23] studied forty dairy cows diagnosed with Anaplasmosis and found a high incidence of infection in the cows. Jassem and Agaar [24] confirmed similar findings, indicating a higher infection rate in females than males. The prolonged illness was attributed to compromised immune systems associated with the postpartum state of cows who were pregnant or nursing. The cows in the current study were healthy, not lactating, and of different ages.

When comparing the biochemical and cytokine parameters at two distinct time periods, there was no statistically significant difference between the infected and non-infected cattle at the first time point. This contradicts Coskun et al. [23], who observed increased AST, ALP, CRE, BIL, TP, and BUN levels. Our results are however consistent with a recent study [25], which reported no significant variations in the blood's TP, TB, DB, and ALT concentrations. At time point two, there was an increase in DB and CRE blood levels. Jaseem and Agaar [24] discovered that crossbred cattle infected with *A. marginale* showed notably higher ALT, AST, TBIL, and indirect bilirubin levels than healthy cattle. Mazzullo et al. [26] observed significant increases in TP, TB, and ALT levels in dairy cattle across different age groups, which are consistent with the findings of this study. Prior studies have shown that several factors, such as illness, breed, age, sex, time of year, muscle use, diet, pregnancy, heat, and stress, can influence the levels of specific biochemical parameters in animals [27,28].

Cytokines are vital in the inflammatory response and function as mediators to fight against infections. IL-10 and IL-4 are anti-inflammatory cytokines that suppress inflammatory responses and T-cell proliferation. In contrast, pro-inflammatory cytokines, including Interferon-gamma, Tumor necrosis factor, and IL-12, promote inflammation. At time-point one, there was no statistically significant difference in the IFN-α, IL-10, and IL-4 levels. IFN-α mean values considerably increased at time point two. IFN-α's significance in bovine Anaplasmosis has not been documented despite its importance in experimental studies involving knocked-out mice [29]. Therefore, the immunological pathway responsible for this action has to be studied further. Nonetheless, IFN- α is known to induce an antiviral state in cells, decreasing viral replication within cells to inhibit infection.

This study examined the correlation between gender and various biochemical indicators. At time point one no significant alterations were observed in any biochemical measurements at time point two for cattle over 2.5 years. At time point two, there were significant gender variations in DB and BUN levels (Table 2). Ruminants show fluctuations in overall protein levels influenced by factors like age, climate, and animal genotype, including genetic and non-genetic factors [30,31]. Moreover, ageing animals with reduced resistance and weakening immunity to *A. marginale* may change some biochemical indicators. In contrast to previous studies [1,25], cattle can maintain a persistent infection with a disease irrespective of age, with susceptibility rising as the animal ages. The current study had limitations. Replicating this work to include a more extensive sample size is recommended. Future research should also concentrate on additional cytokines, both pro-inflammatory and anti-inflammatory, that were not evaluated in the study to gain a deeper understanding of the dynamics of the cytokine response.

Conclusion

In summary, the PCR method identified A. *marginale* making it useful for diagnosing bovine Anaplasmosis at two specific time points. When blood was drawn at two-time intervals, circulating cytokine levels and selected biochemical markers did not differ substantially between infected and uninfected cattle, suggesting that these cytokines may not have a direct role in the development of Bovine Anaplasmosis.

DECLARATIONS

Ethical consideration

Ethical approval was obtained from the Ethical and Protocol Review Committee (EPRC) of the College of Health Sciences, University of Ghana (CHS-Et/M.3-5.6/2020-2021). Verbal and written consent was sought from cattle owners before their cattle were recruited as study participants. The owners were briefed on the research objectives, the technique for collecting specimens, and the potential benefits or drawbacks of the study.

Consent to publish

All authors agreed on the content of the final paper.

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Competing Interest
The authors declare that there is no conflict of interest regarding the publication of this article.

Author contributions
FKD, EKO and HRO were involved in the conceptualisation. FKD was involved in funding acquisition. EKO, SDA, HAA and HRO participated in the supervision, writing, editing and review of the manuscript. FKD, LAD, WD, BKS and AB participated in the methodology. ESY participated in the Statistical analysis.

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Availability of data
Data is available upon request to the corresponding author.

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