ORIGINAL ARTICLE

Haematological Alterations and Molecular Detection of *Anaplasma marginale* in Cattle, Western Iraq

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ABSTRACT

Background: *Bovine anaplasmosis is a communicable disease transmitted via ticks, which carries significant veterinary and economic implications. Data from western Iraq remains scarce despite high cattle exposure to vector-borne infections.* ***Objective:*** *To determine the incidence of* Anaplasma marginale *in cattle in Anbar Province, compare the diagnostic performance of microscopy, ELISA, and PCR, and assess associated haematological changes.* ***Methodology:*** *Between January and December 2024, three hundred blood samples were drawn from cattle (1–10 years, both sexes) across 20 farms in Haditha, Heet, Al-Qaim, and Rutba districts; Giemsa-stained smears, a commercial ELISA, and PCR targeting the msp5 gene were used for diagnosis. Haematological parameters (RBC, Hb, PCV, WBC) were measured using an automated analyser. Prevalence was determined by sex, age, and season, while haematological data were compared by t-tests (α = 0.05).* ***Results:*** *Prevalence was 28.0% by microscopy, 36.3% by ELISA, and 41.3% by PCR, with PCR being most sensitive. Infection rate was higher among females (46.9%) compared to males (33.6%), and in older cattle than in younger ones; Peak rates occurred in the summer (62.6%), with the lowest rates in winter (17.3%). Infected cattle had reduced Hb, PCV, RBC, and elevated WBC counts.* ***Conclusion:*** *This study provides the first detailed epidemiological report on bovine anaplasmosis in western Iraq. The results confirm that PCR is the most sensitive tool, underlining host and seasonal influences on infection and revealing distinct haematological alterations. These findings provide a surveillance baseline and support control strategies against A. marginale in endemic regions.*

INTRODUCTION

Bovine anaplasmosis is a communicable disease, a primary veterinary and economic concern worldwide. The causative agent, *Anaplasma marginale*, is a Gram-negative obligate intraerythrocytic bacterium that invades red blood cells, leading to haemolytic anaemia, reduced productivity, abortion, and in severe cases, death[1]. Transmission occurs primarily through hard ticks of the genera *Rhipicephalus, Dermacentor*, and *Boophilus*; mechanical spread also occurs via blood-feeding flies or contaminated veterinary instruments. Clinically affected cattle often present with fever, jaundice, lethargy and decreased milk, or meat yield, rendering the disease a significant constraint on livestock production [2].

In Iraq, several tick-borne haemoparasites have been reported in ruminants, yet systematic investigations of bovine anaplasmosis remain scarce, especially in the western desert areas such as Anbar Province. Previous studies have confirmed the presence of *A. ovis* in goats from central Anbar [3] and *A. phagocytophilum* in cattle from Mosul City [4]. Anaplasmosis has also been detected in buffaloes from Wasit Province by serological testing[5]. Moreover, infestations with multiple tick species, notably *Hyalomma* spp., have been documented in various regions of Iraq [6]. These findings indicate that ecological conditions are favourable for the circulation of tick-borne pathogens; however, specific epidemiological data on *A. marginale* in cattle from Anbar are still lacking.

Accurate diagnosis is critical for effective control of bovine anaplasmosis. Conventional methods- such as Giemsa-stained blood smears are inexpensive and widely used, yet their sensitivity is limited, particularly for detecting carrier animals. Serological assays, such as ELISA, have improved surveillance by identifying antibodies across different stages of infection; however, they cannot reliably distinguish between past and current exposure. Molecular approaches, particularly

Polymerase Chain Reaction (PCR), are considered the most reliable tools because of their high sensitivity, specificity, and ability to detect low-level infections in asymptomatic carriers [7, 8].

Given the paucity of data on bovine anaplasmosis in western Iraq, this research aimed to assess the incidence of *A. marginale* in cattle from Anbar Province, compare the diagnostic accuracy of microscopy, ELISA, and PCR, and examine the associated haematological changes, these findings provide a baseline for the region's epidemiological data and will guide the development of effective surveillance and control strategies.

METHODOLOGY

Study Area and Animals

This study was conducted in Anbar Province, western Iraq, throughout 2024, covering all four seasons to examine potential seasonal differences in infection; the province is characterised by a desert environment with long, scorching summers, mild winters, and minimal rain each year. 300 cattle, aged 1–10 years and of both sexes, were selected from 20 farms in Haditha, Heet, Al-Qaim, and Rutba districts. Animals underwent thorough clinical examination, and data regarding age, sex, and management practices were recorded; sampling included both clinically suspected cases showing signs consistent with anaplasmosis and apparently healthy controls, the latter confirmed by the absence of clinical signs and negative results in preliminary Giemsa-stained smears.

Collecting Blood Samples

About 10 milliliters of blood were collected aseptically from each animal's jugular vein using sterile disposable syringes, and the samples were split into two aliquots:

1- Blood with ethylenediaminetetraacetic acid (EDTA), an anticoagulant used for microscopic, molecular, and haematological analyses.
2- Blood without an anticoagulant used for serum separation and serological testing. Serum samples were separated by centrifugation at 3000 rpm for 10 minutes and stored at –20°C until tested. All samples were transported in ice-cooled containers to the College of Science, University of Anbar's laboratories, and processed within six hours of collection.

Microscopic Examination

For every sample, two thin blood smears were made, stained using a 10% Giemsa solution, and fixed with absolute methanol; the stained smears were then examined under a light microscope at 1000× magnification to identify the existence of *A. marginale* (Figure 2) within erythrocytes [9].

Molecular Diagnosis (PCR of the msp5 Gene)

A commercial kit (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany; Catalogue No. 51104), was used to extract genomic DNA from EDTA blood samples. Using specific primers, PCR amplification targeted the *A. marginale msp5* gene following the manufacturer's protocol:

Forward: 5'-ATG AAA AAC ACG GTG GAG AAT G-3'

Reverse: 5'-CTA CTC GCA CTT GCT TCT TAA G-3'

The anticipated size of the amplicon was 407 bp. PCR reactions were conducted in a 25 µL total volume with 12.5 µL of 2× Master Mix (Promega, Madison, WI, USA), 1 µL of each primer (10 pmol), 2 µL of DNA template, and nuclease-free water. The polymerase chain reaction (PCR) was performed using a 25-cycle heat regimen. The first step was to heat the reaction mixture for five minutes to 95°C. The DNA strands were fully split, allowing the primers to join. Subsequently, the reactions underwent several heat cycles. Following the thermal decomposition stage, the temperature was lowered to 58°C for 30 seconds to allow the primers to bind to the sequence to be amplified. The temperature was then raised to 72°C for one minute to enable the polymerase enzyme to lengthen the chain and synthesise new DNA copies. After the 25 cycles were completed, the temperature was maintained at 72°C for an additional seven minutes to ensure that all transcription steps were fully completed. The reaction products were then transferred onto a 1.5% agarose gel stained with ethidium bromide, and the resulting bands were observed under UV light (Figure 1A). Each run included both positive and negative controls [10].

Serological Diagnosis (ELISA)

Sera were tested for antibodies against *A. marginale* using a commercial ELISA kit (ID Screen® *Anaplasma* Indirect ELISA, IDvet, Grabels, France; Cat. No. ANAPLASMA-2P). The assay was performed according to the manufacturer's instructions, and optical density (OD) values were determined with a microplate reader (BioTek ELx808, Winooski, VT, USA) at 450 nm (Figure 1B), and samples exceeding the cut-off value were classified as positive[11].

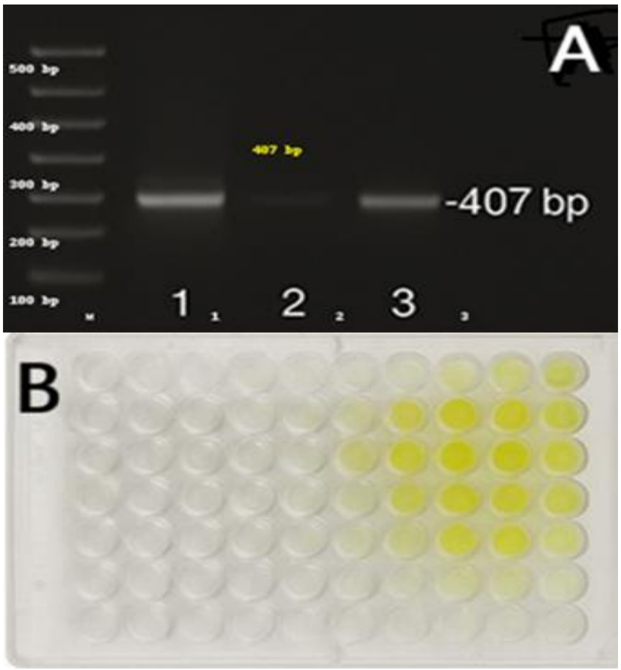

Fig. 1. Diagnostic panels: (A) Agarose gel electrophoresis showing the msp5 PCR amplicon of *A. marginale* (407 bp); (B) The ELISA microplate showing positive and negative wells for detecting *A. marginale* antibodies.

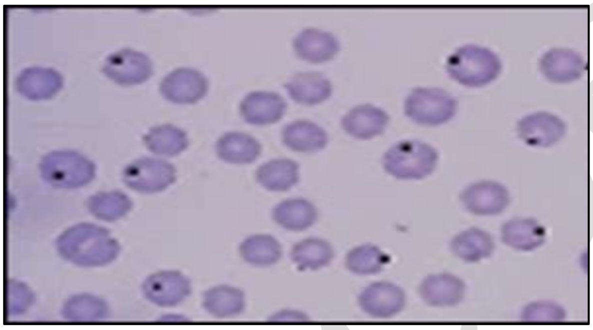

Figure 2. Microscopic findings: Giemsa-stained erythrocytes showing *A. marginale* inclusions (1000X).

Haematological Analysis

Blood samples collected in EDTA tubes were processed within two hours to ensure accuracy, haematological parameters measured included haemoglobin concentration (Hb), Red blood cell (RBC) and white blood cell (WBC) counts, volumes of packed cells (PCV), measurements were performed using an automated veterinary haematology analyser (Mindray BC-2800Vet, Mindray, China) that applies impedance-based technology for cell counts and colorimetric methods for haemoglobin determination[12].

Statistical Analysis

The latest version of SPSS, 26.0 (IBM Corp., Armonk, NY, USA), was used to analyse the data. The test of chi-square (χ^2) evaluated associations between infection rates and categorical variables (sex, age, season). Independent-samples t-tests were used to compare the haematological parameters of infected and non-infected cattle. The parameters were presented as mean ± standard deviation (SD). Statistical significance was defined as a p-value < 0.05 [13].

RESULTS

Overall Prevalence

Out of 300 examined cattle, *A. marginale* was detected in 84 animals (28.0%) by microscopy, 109 animals (36.3%) by ELISA, and 124 animals (41.3%) by PCR. Among the three diagnostic methods, PCR consistently yielded the highest detection rate, followed by ELISA; in contrast, microscopy demonstrated a lower prevalence (Table 1; Figure 3A).

Prevalence by gender

The infection rate differed significantly between sexes (Table 2). By PCR, females showed a prevalence of 46.9% compared with 33.6% in males ($p < 0.001$). ELISA and microscopy also detected higher positivity in females (40.0% and 32.0%, respectively) than in males (31.2% and 22.4%, respectively).

Prevalence by age group

Prevalence varied significantly with age (Table 2). Animals older than 3 years had the highest prevalence (45.2%), followed by those aged 1–3 years (34.2%), while calves under 1 year had the lowest prevalence (32.4%). The statistical significance of the age group differences was ($p < 0.05$).

Seasonal prevalence

Marked seasonal variation was observed (Table 3). The highest prevalence occurred in summer (62.6%), followed by spring (45.3%), autumn (40.0%), and the lowest in winter (17.3%). These seasonal fluctuations were statistically significant ($p < 0.001$).

Haematological Alterations

Significant differences were detected between infected and control cattle in all haematological parameters (Table 4; Figure 3B). infected cattle exhibited reduced mean haemoglobin concentration (8.35 ± 0.71 g/dL) compared with controls (11.2 ± 0.70 g/dL). Similarly, mean PCV values (23.4 ± 3.2% vs. 31.6 ± 2.1%) and RBC counts (4.02 ± 0.52 $\times10^6/\mu L$ vs. 7.30 ± 0.50 $\times10^6/\mu L$) were significantly decreased in infected cattle. Conversely, mean WBC counts were significantly higher in infected animals (13.8 ± 2.3 $\times10^3/\mu L$) compared with controls (8.6 ± 1.4 $\times10^3/\mu L$).

Table 1. Overall prevalence of *A. marginale* in cattle by different diagnostic techniques.

Diagnostic method	Positive cases (n = 300)	Prevalence (%)
Microscopy	84	28.0
ELISA	109	36.3
PCR	124	41.3

The chi-square test showed a significant difference among diagnostic methods ($p < 0.05$), with PCR giving the highest prevalence.

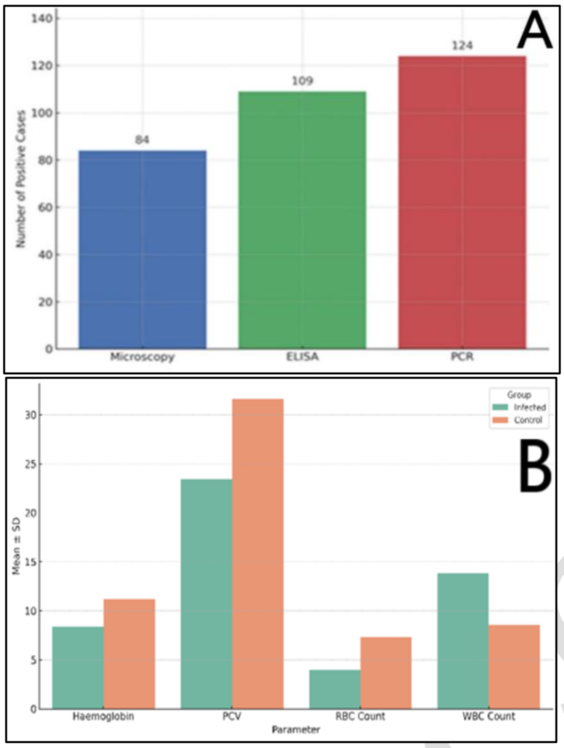

Fig. 3*:* Detection rates and haematological changes: (A) Detection rates of A. marginale by microscopy, ELISA, and PCR; (B) Comparison of haematological parameters between infected and control cattle.

Table 2. Gender and age distribution of *A. marginale* infection in cattle by PCR.

Category	Group	No. of animals	PCR positive (n)	Prevalence (%)
Gender	Male	125	42	33.6
	Female	175	82	46.9
	Total	300	124	41.3
Age	< 1 year	74	24	32.4
	1–3 years	111	38	34.2
	> 3 years	115	62	53.9
	Total	300	124	41.3

Values represent the number of PCR-positive animals with percentages in parentheses.
Chi-square test showed a significant difference by gender ($p < 0.001$) and by age ($p < 0.05$).

Table 3: Seasonal distribution of *A. marginale* infection in cattle by PCR.

Season	No. of samples	PCR-positive (n)	Prevalence (%)
Winter	75	13	17.3
Spring	75	34	45.3
Summer	75	47	62.6
Autumn	75	30	40.0
Total	300	124	41.3

Values show PCR-positive animals with prevalence (%); the Chi-square test across seasons was significant ($p < 0.001$).

Table 4: Haematological parameters in infected versus control cattle.

Parameter	Infected (Mean±SD)	Control (Mean±SD)	p-value
Haemoglobin (g/dL)	8.35 ± 0.71	11.2 ± 0.70	< 0.01
PCV (%)	23.4 ± 3.2	31.6 ± 2.1	< 0.01
RBC ($\times 10^6/\mu L$)	4.02 ± 0.52	7.30 ± 0.50	< 0.01
WBC ($\times 10^3/\mu L$)	13.8 ± 2.3	8.6 ± 1.4	< 0.01

Values are mean ± SD; the independent t-test showed significant differences for all parameters ($p < 0.01$).

DISCUSSION

The current investigation found that cattle from western Iraq had a comparatively high incidence of *A. marginale* infection, with an overall rate of 41.3% by PCR. This finding is higher than reports from Babylon Province (5.17%)[14] but comparable to that reported in Nigeria (41.3%)[15], lower rates were documented in Egypt (12.5%)[16] and Thailand (12.7%) [17], reflecting differences in ecological conditions, management practices, and diagnostic methods employed; the superior performance of PCR over microscopy and ELISA highlights its value in detecting carrier animals and subclinical infections, which are often missed by conventional approaches. The limited sensitivity of microscopy, particularly in low parasitemia situations, and ELISA's inability to distinguish between past and present infections, result in lower detection rates than PCR[18,19].

The present study also demonstrated that females were significantly more affected than males (46.9% vs. 33.6%). This difference may be explained by physiological and hormonal factors unique to females, particularly pregnancy and lactation, which impose considerable metabolic and nutritional demands. Such stressors are well documented to suppress immune function, making females more susceptible to haemoparasitic infections; in addition, repeated pregnancies may lead to a cumulative decline in immune competence over time [20]. Behavioural and management factors may also contribute since females are often retained longer for breeding and milk production, increasing their cumulative exposure to tick vectors compared with males, who are usually sold or slaughtered earlier [21]. Earlier studies have reported similar sex-linked predispositions in bovine anaplasmosis and other tick- disorders, supporting our findings [22].

Older cattle (>3 years) showed a significantly higher infection rate (45.2%) compared to younger animals (≤3 years, 35.0%); this trend supports the idea of cumulative exposure, where repeated contact with tick vectors over multiple seasons increases the risk of infection. Furthermore, older animals are more prone to repeated infestations, leading to chronic carrier states that sensitive diagnostic methods like PCR can readily identify [23]. Conversely, younger cattle may have limited exposure because of protective management practices, like housing or restricted grazing. In some cases, they may retain partial maternal immunity acquired during the early postnatal period. As a result, younger animals may initially resist, but their susceptibility can increase as they age and encounter more frequent tick challenges. These results align with earlier reports identifying age as a significant risk factor in the epidemiology of tick-borne infections[24].

A pronounced seasonal variation in *A. marginale* prevalence was observed, with infection rates peaking in summer (62.6%) and reaching their lowest level in winter (17.3%). This pattern matches the ecology of tick vectors, especially Rhipicephalus species, which flourish in the hot and moderately humid Iraqi summers. Winter's cooler and drier conditions reduce tick survival and questing activity, leading to a marked decline in transmission; similar seasonal dynamics have been reported in other arid and semi-arid regions, where tick-borne diseases show strong temporal clustering during warmer months [25].

These observations emphasise the need for strategic vector control measures, including acaricide application, pasture management and farmer awareness programs, to be intensified during high-risk seasons [26]. Preventive measures implemented in early summer, before peak tick activity, may substantially reduce transmission and the subsequent clinical disease burden. The findings match reports from other Iraqi regions [27, 28].

Infected cattle exhibited significant hematological changes, including lower haemoglobin level, PCV, RBC counts, and higher WBC counts; these alterations signify regenerative anaemia and an inflammatory response, reflecting the pathophysiology of *A. marginale* infection [29]. Parasites enter red blood cells, causing harm and resulting in anemia. At the same time, compensatory bone marrow activity attempts to restore erythrocyte [30]. The leukocytosis observed may respond to tissue damage and secondary bacterial infections associated with tick infestations[31]; comparable findings have been documented in Egypt [32] and Pakistan [33] studies, supporting the consistency of these haematological signatures across various epidemiological settings.

The findings of this research emphasise the importance of targeted management of bovine anaplasmosis in western Iraq. Given the higher prevalence among females, older animals, and during the summer season, control strategies should focus on these risk groups. Seasonal tick management, including the timely use of acaricides and improved grazing activities, is essential to reduce transmission. Farmer education on disease recognition and proper tick control is equally important, as many rely on traditional methods; integrating PCR into surveillance programmes, at least for breeding females and suspected cases, would enhance the early detection of carriers often missed by microscopy or ELISA. These measures, combined with closer coordination between farmers and veterinary authorities, will be critical for reducing the burden of *A. marginale* and limiting its economic impact in the region.

The study is not without limitations. Molecular confirmation was limited to PCR without sequencing of amplicons, which could provide more definitive strain characterisation. Additionally, management practices and tick infestation levels were not systematically recorded, which may have influenced prevalence estimates. Expanding the study to include molecular typing and vector surveillance would improve understanding of regional transmission dynamics.

CONCLUSION

This research is the first comprehensive molecular and haematological investigation of *A. marginale* in cattle from western Iraq, providing essential baseline data for Anbar Province; the higher prevalence in females, older cattle, and during summer highlights the importance of host and environmental factors in disease dynamics. The clear superiority of PCR highlights its significance as a reference tool for surveillance and early detection. Observed haematological changes further confirm the clinical relevance of the infection. These findings emphasise the need to incorporate molecular diagnostics, strengthen seasonal tick control, and prioritise high-risk groups alongside farmer education to reduce transmission and economic losses.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Authors' Contributions

S. S. Shahatha: carried out the experiments, discussed the results, drafted the manuscript, and approved the final version. **E.A.K. ALABBAS:** Project management, validation, and resources. **H.Y. Khalaf:** Supervision, writing, and editing. H.H. Mutlaq: validation, writing, and editing. **A.J. Dhulkefl:** Conducted the experiments, gathered resources, and performed statistical analysis. **N.T. Abd:** Planned and supervised the experiments and the critical review. Each author contributed equally to this work, carefully reviewed the manuscript's final draft, and pledged to take responsibility for every facet of the research.

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Ethical Approval

The study protocol was reviewed and authorised by the University of Anbar's Institutional Ethics Committee (Approval No. 15: Jan. 2024). All animal handling and sampling procedures complied with institutional and international guidelines for the care and use of animals in research. Informed consent was obtained from cattle owners before sampling.

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