Epidemiological, clinical, and hematological changes associated with *Theileria annulata* and *Babesia bigemina* infections in water buffaloes (Bubalus bubalis) in Kerbala, Iraq

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Received:23/4/2026 **Accepted:**29/5/2026 **Published:** 15/6/2026

***Abstract*—** The water buffalo (Bubalus bubalis) is regarded as one of the most valuable domesticated species in Asia in general and in Iraq in particular, where it plays a crucial role in the agricultural economy. However, their productivity is greatly influenced by various infectious diseases, of which tick-borne haemoprotozoal diseases are a major constraint to animal health and profitability. The aim of this study was to evaluate the epidemiological and clinical parameters and hematological alterations associated with these infections in buffalo at Kerbala, Iraq. A total of 400 buffaloes of different ages were clinically investigated, and blood samples were collected for microscopic and hematological analysis. The data revealed a total prevalence of piroplasmosis of 37%, with 20.5% and 16.5% of the buffaloes being infected with theileriosis and babesiosis, respectively. Females and buffaloes<1 year of age were more frequently affected, suggesting that sex and age have a significant impact on the infection rate. The clinical symptoms of the infected animals included fever, superficial lymphadenopathy, nasal and ocular discharges, salivation, anemia, respiratory distress, corneal opacity, and haemoglobinuria. (mention of the diagnostic method (microscopy)) The haematological results showed a significant decrease in haemoglobin, packed cell volume (PCV), and red blood cell counts. There were also significant increases in white blood cells, basophils, lymphocytes, and neutrophils. This shows that these parasites cause anemia and stimulate the immune responses of the infected buffalo. The results of this study emphasise the need to monitor the clinical and haematological status of the animals, as well as the need to control tick infestations and implement measures to prevent the spread of these diseases.

Keywords **-** Water buffalo, Hematological changes, *Theileria annulate*, *Babesia bigemina*.

INTRODUCTION

The water buffalo (*Bubalus bubalis*) is regarded as one of the most valuable domesticated species in Asia in general and in Iraq in particular, where it plays a crucial role in the agricultural economy, particularly in the production of milk, meat, and high-quality skin (1). It accounts for around 5% of the total milk production in the world (2). Moreover, buffaloes are employed in agricultural work such as plowing and the transport of heavy loads. The number of buffaloes in Iraq is estimated to be around 400,000, spread over various provinces in the country (3). However, their productivity is greatly influenced by various infectious diseases, of which tick-borne haemoprotozoal diseases are a major constraint to animal health and profitability. Among the most significant tick-borne protozoan pathogens transmitted by Hyalomma spp. The ones that infect cattle are *Theileria annulata*, which causes tropical theileriosis, and *Babesia bigemina*, which is the main causative agent of bovine babesiosis. These two pathogens are a major concern economically because of the serious effects they have on livestock and domestic animals, especially buffalo, because of their high morbidity and mortality rates worldwide (4-7).

Theileria annulata has a complicated life cycle that involves schizogony in leukocytes and piroplasm stages in erythrocytes. This leads to lymphoproliferative disease, systemic inflammation, and severe clinical symptoms such as petechial hemorrhages on the conjunctiva, lymphadenopathy, anemia, fever, anorexia, weight loss, general weakness, neurological symptoms, and dyspnea (8). In the early stages of the infection, the parasite causes the proliferation of lymphocytes, resulting in the enlargement of lymph nodes. As the disease progresses in buffalo, fever occurs along with the destruction of lymphoid cells and severe leukopenia. After the release of merozoites from infected leukocytes, the parasites infect erythrocytes, where they develop into piroplasms, which eventually cause severe anemia (9, 10). On the other hand, *Babesia bigemina* primarily infects red blood cells and results in intravascular hemolysis. This leads to acute hemolytic anemia, hemoglobinuria, fever, and varying degrees of jaundice. All Babesia species known to date are transmitted to vertebrates by ixodid ticks (11).

The most important tick families are the hard ticks of the family Ixodidae, of which there are 713 recognized species (12). The life cycle of ixodid ticks is regulated by several intrinsic and extrinsic factors, including tick behavior during feeding, relationships between hosts and parasites, host survival rates, and the effects of weather and climate (13,14). Both pathogens are known to cause significant mortality and morbidity in water buffalo, leading to economic losses due to

reduced productivity, treatment costs, and animal deaths. Several studies in Iraq have investigated the prevalence of blood-borne parasites in water buffalo (Bubalus bubalis). A molecular study reported the presence of *Babesia bigemina* in 32.86% of *Rhipicephalus (Boophilus) annulatus* ticks collected from water buffalo in Al-Qadisiyah, Babylon, and Najaf governorates. Additionally, 34.38% of water buffalo in these areas were infested with ticks, highlighting the significant role of ticks in the epidemiology of babesiosis (15). Hasanpour et al. (16) investigated naturally infected buffaloes with *Theileria annulata* and reported a significant reduction in red blood cell (RBC) and white blood cell (WBC) counts, as well as the packed cell volume (PCV), compared to healthy ones. The study also revealed a reduction in some mineral constituents, especially potassium, with a significant rise in serum AST and ALT activities. Mahmoud et al. (6) performed molecular detection and genetic analysis of *Theileria annulata*, *Babesia bovis*, and *Babesia bigemina* in cattle and buffalo. From 300 blood samples examined, the PCR analysis revealed an overall infection rate of 20% in piroplasms, with 10.7% in *T. annulata*, 5.3% in *B. bovis*, and 4% in *B. bigemina*.

Alautaish *et al*. (17) studied the *B. bigemina* infection among buffaloes in northern Basrah Province. They found a 16% clinical infection rate and 41% subclinical infection. Infected animals showed considerable hematological and serological changes. The infection rates were higher among animals in summer and females. Al-Shammari et al. (18) studied the prevalence of blood protozoan infections among cattle in Babylon Governorate, Iraq. Among 232 blood samples examined microscopically for protozoan infections, the overall infection rate among animals was 19.82%, of which 7.75% were *B. bigemina*, 6.89% were *Theileria annulata*, and 5.17% were *Anaplasma marginale*. Infected animals showed considerable reductions in RBCs, hemoglobin, PCV, MCH, platelet count, calcium, copper, iron, selenium, total protein, albumin, globulin, whereas ESR, AST, ALT, LDH, bilirubin, creatinine, glucose was significantly increased. However, despite these studies, there is still a lack of field and epidemiological information on the combined clinical and hematological effects of *Theileria annulata* and *Babesia bigemina* infections in water buffalo in Iraq, especially with regard to the hematological findings and epidemiological aspects of these infections. Thus, the aim of the present study is to determine the epidemiological and clinical assessment, and to study the hematological findings of *Theileria annulata* and *Babesia bigemina* infections in water buffalo.

This study aimed to investigate the epidemiological patterns, clinical manifestations, and hematological alterations associated with infection by *Theileria annulata* and *Babesia bigemina* in water buffaloes (Bubalus bubalis) in Kerbala, Iraq, and to assess their impact on animal health and disease diagnosis.

MATERIALS AND METHODS

Animals

A total of 400 buffaloes of various ages from different areas of Kerbala city (cross-sectional), suspected of piroplasmosis (*Theileria annulata* and *Babesia bigemina*), were clinically examined.

Blood samples

Were obtained using a sterile syringe from the ear vein of clinically suspected piroplasmosis cases and other contact animals (who appeared to be in good condition) on anticoagulant (sodium salt of EDTA) for hematological analysis and blood smears.

Blood examination

According to (19), blood samples were used to create thin and thick films, which were then allowed to air dry, fixed with 100% methyl alcohol for 1-3 minutes, and stained with 10% Giemsa stain for 30 minutes before being studied under a light microscope with an x40 lens.

The hematological analysis:

Red and white blood cell counts (RBCs and WBCs), packed cell volume (PCV), hemoglobin concentration (Hb%), and differential leukocyte counts were measured using the BCC-3600-Duruy-South Korea automated hematology analyzer.

The search was conducted under the ethics number UOK.VET.ME.2026.180

Statistical analysis

Data were analyzed using SAS (Statistical Analysis System, version 9.1). Proportions were compared using the chi-square test, and means were compared using the unpaired t-test. A p-value<0.05 was considered statistically significant (20).

RESULTS AND DISCUSSION

Epidemiological aspects of bovine piroplasmosis

A total of 148 animals infected, or (37%) of the 400 animals analyzed, had piroplasmosis infections. A total of 82 animals, or (20.5%), were determined to have theileriosis. However, as indicated, 66 animals, or 16.5% of the total, were confirmed to be infected with babesiosis, Table 1. The prevalence of babesiosis documented in the present investigation (16.5%) closely aligns with the findings reported in buffaloes from northern Basrah, Iraq, where a clinical prevalence rate of 16% was recorded (17). This similarity may be attributed to certain ecological and climatic similarities between the two regions, especially in areas with a tropical or subtropical climate, where high temperatures and high humidity provide a suitable environment for the survival and reproduction of ticks, which are the main carriers of babesiosis parasites. This explanation is consistent with the observations of Alsaad *et al*. (21) and Sobhy *et al*. (22), who emphasized the role of climatic factors in increasing tick infestation rates and consequently enhancing the spread of babesiosis. Interestingly, a significant rate of subclinical infections was identified in buffaloes from Northern Basrah, Iraq, at 41%, thus playing a significant role in the epidemiology of babesiosis, thereby acting as a carrier of disease (17).

This carrier effect may be a contributory reason for the relatively higher prevalence rate of piroplasmosis, at 37%, identified in the present investigation. In a previous investigation carried out in cattle from Babylon Governorate, Iraq, a relatively lower rate of protozoan infections was identified, at 19.82%, where *Theileria annulata* and *Babesia*

bigemina were identified at a rate of 6.89% and 7.75%, respectively (18). The relatively higher prevalence rate in buffaloes could possibly be due to differences in susceptibility among species, differences in management practices, and exposure to tick infestation (9). Further, the grazing habits, humidity, and seasonal activity of ticks may also affect the epidemiological pattern of such hemoprotozoan infections in different host species.

Table 1. Number and percentage of infected buffaloes with *Theileria annulata* and *Babesia bigemina*

% Of infected animals	No. of infected animals	No. of examined animals	**Haemoprotozoal diseases**
20.5%	82	400	***Theileria annulata***
16.5%	66	400	***Babesia bigemina***
37%	148	400	**Total number of diseased**

Table 2 shows the number and percentage of infected animals with piroplasmosis and its forms based on sex. The data indicate that the overall infection rate is higher in females (39.1%) than in males (29.5%). With respect to the forms of the parasite, the overall infection rate is also higher in females for both Theileria (21.4%) and Babesia (17.6%) than in males (17% and 12.5%, respectively). Analysis of the data reveals that the differences in infection rates between males and females are significant, indicating that sex is a factor in infection susceptibility. In general, Theileria is more common than Babesia, and females are more susceptible to both forms of infection. These results are consistent with study (9, 17, 28), but differ from a number of other studies (18).

Table 2. Numbers and percentage of infected buffaloes according to sex

Babesia infested animal	**Theileria infected animal**	**No. of animals infected with piroplasmosis**	**Total no. of animals**	**Sex**
55 (17.6%)	67 (21.4%)	122 (39.1%)	312	**Female**
11 (12.5%)	15 (17%)	26 (29.5%)	88	**Male**
66 (16.5%)	82 (20.5%)	148 (37%)	400	**Total**
Significant difference among the infected and sex (P<0.05)				

Table 3 presents the percentage of animals infected with piroplasmosis based on age and demonstrates the impact of age on the infection rate. The data show that the age group with the highest susceptibility is below 1 year, where 74 out of 104 animals were infected, accounting for 71.1%. In this category, the infection rates were 47.1% for *Theileria annulata* and 24% for *Babesia bigemina*. Animals between 1-3 years had 43 infected out of 129, accounting for 33.3%, with 13.9% infected by Theileria and 19.3% by Babesia. Animals above 3 years had 31 out of 167 infected, accounting for 18.5%, with 8.9% infected by Theileria and 9.5% by Babesia. This could be due to the fact that young animals that are less than one year old are more susceptible to infection because of their low resistance and absence of acquired immunity to parasites. As the animals get older, they become more resistant to infection because of acquired immunity due to repeated exposure to parasites; thus, the low infection rate in animals that are more than three years old. These results are consistent with study (23,24), but differ from a number of other studies (18,25).

Table 3. The age susceptibility of bovine piroplasmosis

Babesia bigemina	*Theileria annulata*	No. of infected animals	No. of animals	**Age group**
25 (24%) *	49 (47.1%)	74 (71.1%)	104	**Less than 1 year**
25 (19.3%)	18 (13.9%)	43 (33.3%)	129	**1-3 years**
16 (9.5%)	15 (8.9%)	31 (18.5%)	167	**>3 years**
66 (16.5%)	82 (20.5%)	148 (37%)	400	**Total**
*** Significant differences among the infected and age (P<0.05)**				

The table clearly shows that infection rates decrease with increasing age, meaning younger animals are more susceptible than older ones. Statistical analysis indicated significant differences among the age groups (P<0.05), demonstrating that age is an important factor influencing the infection rate of piroplasmosis and its associated parasites.

Clinical findings

The animals infected with Theileriosis exhibited clinical signs including fever, ranging from 40 to 41.7°C, enlargement of superficial lymph nodes, nasal and ocular discharge, salivation, anemia, respiratory distress, and eye lesions, as shown in Figure 1. In contrast, animals infected with Babesiosis presented with fever (40–41°C), loss of appetite, cessation of rumination, laboured breathing, and varying degrees of jaundice (icterus), ranging from mild paleness to yellow discolouration of conjunctival and vaginal mucous membranes in severe cases, along with corneal opacity and haemoglobinuria, as depicted in Figure 2. These findings are consistent with those of Alautaish et al. (17), and the rise in body temperature (40-41.7°C) in buffaloes infected with Theileriosis and Babesiosis is regarded as a normal response to the invasion of parasites (26,27). In Babesiosis, especially in B. bigemina infection, the lysis of red blood cells caused severe anemia, resulting in jaundice of the mucous membranes and haemoglobinuria because of the large amount of haemoglobin released, which could have a negative impact on the kidneys. Anorexia was also noticed, leading to loss of weight, emaciation, and reduction in milk secretion. The corneal opacity noticed in some infections might be related to alterations in plasma proteins and blood flow (26, 28).

Figure 1. Enlargement of superficial lymph nodes

Figure 2. corneal opacity of eye

Hematological analysis Theiler

Table 4 showed significant changes in the haematological values between normal and infected buffaloes, including increased (P<0.05) basophils, lymphocytes, neutrophils, and WBCs in infected animals, indicating an active immune response. These results correspond to (17, 26) results. Conversely, a significant decrease (P<0.05) was observed in hemoglobin, PCV, and RBC count, indicating that both *Theileria annulata* and *Babesia bigemina* caused anemia in infected buffalo. These results are consistent with (17,29,30,31). This reduction can be attributed to a number of interlinked processes. Firstly, the direct hemolysis of red blood cells due to the replication of the parasites within them results in hemolytic anemia (32). In addition, autoimmune mechanisms may also be responsible for the reduction in red blood cells, as the production of antibodies against erythrocyte components results in auto-agglutination and the subsequent destruction of infected cells. In addition, cellular immunity also results in the phagocytosis and clearance of infected red blood cells via the reticuloendothelial system, thereby leading to a reduction in the total number of RBCs and contributing to anemia (33,34). Thus, the reduction in Hb, PCV, and RBCs in infected buffaloes can be attributed to both the direct action of the parasites as well as the immune response of the host.

Table 4. Illustrates the significant differences in the hematological values among the infected and Normal buffaloes.

Infected animals Mean±S.E	Normal animals Mean±S.E	Parameters
2.0 ± 6.1 *	1.42± 0.2	Basophils (×10³/µl)
3.65 ± 1.0	3.7 ± 1.8	Eosinophils (×10³/µl)
7.44 ± 1.2 *	12.2 ± 1.3	Hb concentration g/dL
44.8± 1.8 *	40.7± 4.14	Lymphocytes (×10³/µl)
3.1 ± 63	3.3 ± 1.28	Monocytes (×10³/µl)
47.25 ±3.22 *	44.42 ± 3.32	Neutrophils (×10³/µl)
26.4± 2.13 *	34.2 ± 2.3	PCV %
4.8 ± 0.9 *	7.4 ± 0.72	RBCs count X10⁶µl
14.2± 0.9 *	10.64 ± 2.12	WBCs count X 10³µl
*** Significant differences among the infected and Normal buffaloes (P<0.05).**		

Blood film examination and diagnosis

The Giemsa-stained blood smears clearly distinguish between the two types of blood parasites. In the case of theileriosis, the smears showed the intra-erythrocytic stage of the parasites, appearing in various shapes such as comma-shaped, oval, and spherical. This indicates active parasitic development within red blood cells and represents an active stage of infection. This pattern is characteristic of theileriosis and aids in accurate diagnosis, as seen in Figure 3. In the case of babesiosis, the blood smears revealed red blood cells containing pear-shaped parasites, which is a distinctive feature of this type of infection. This shape reflects the specific manner in which the parasite invades red blood cells, allowing differentiation between babesiosis and theileriosis in laboratory diagnosis, as seen in Figure 4.

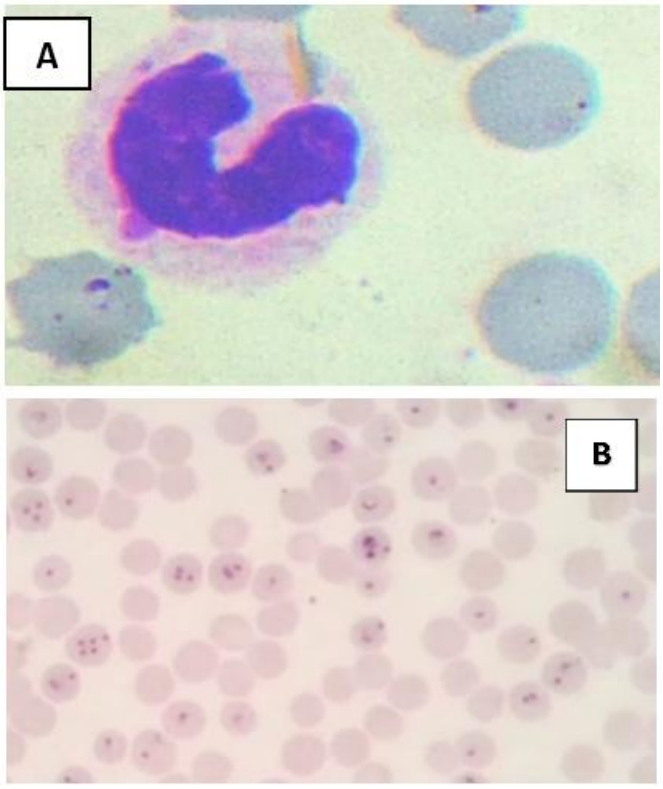

Figure 3. A, B. *Theileria annulata* in buffaloes in the form of comma, oval and round shape 40X.

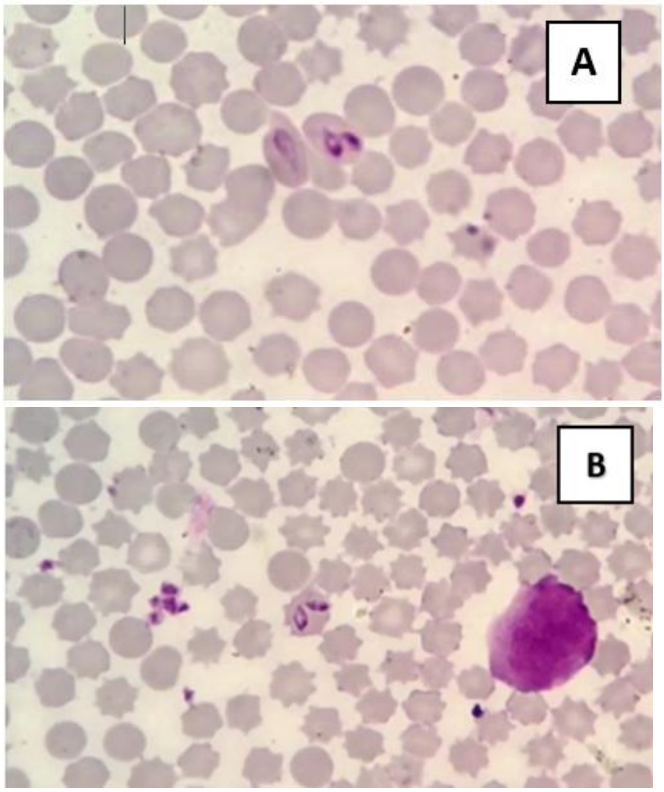

Figure 4. A, B. *Babesia bigemina* in buffalo's pear shaped inside RBCs 40X

CONCLUSION

The overall prevalence of piroplasmosis in water buffalo in Karbala, Iraq, was 37%, with *Theileria annulata* affecting 20.5% and *Babesia bigemina* affecting 16.5%. Female and younger animals (<1 year) were more susceptible, indicating that sex and age play important roles in infection rates. Clinically, infected animals exhibited fever, superficial lymphadenopathy, nasal and ocular discharges, salivation, anemia, respiratory distress, corneal opacity, and haemoglobinuria. Haematological analysis showed significant reductions in haemoglobin, PCV, and RBC counts, along with significant increases in WBCs, basophils, lymphocytes, and neutrophils, indicating that these parasites cause anemia and stimulate the immune system in affected buffaloes. These findings also stress the need for regular clinical and haematological monitoring, tick control, and prevention measures to prevent the spread of these diseases.

Conflict of Interest

The authors declare there is no conflict of interest.

Funding

The authors did not receive any funds for this study.

Acknowledgment

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