

## Microscopic Investigation of Bovine Haemoparasites in Wasit Province, Iraq

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### Abstract

**Background:** Haemoparasites are group of livestock diseases that quit endemic and great important because it causes severe economic losses. **Aims:** Microscopic investigation of haemoparasites in cattle with estimation relationship between positivity and the findings of clinical signs and risk factors. **Materials and methods:** Totally, 118 cattle were selected and subjected for draining of jugular venous blood and preparation of blood smears during December (2021) to February (2022). The slides of blood smears were stained by Giemsa, and examined using the light microscopy. **Results:** The total prevalence rate of haemoparasites was 19.49%; comprising 11.02% *Anaplasma*, 6.78% *Babesia* and 1.69% *Theileria* infections. According to type of infection, there were 70.59% single and 29.41% mixed infections. In single infections, there was a significant increase in prevalence of *Anaplasma*; while in mixed infection, higher elevation was observed in cattle infected with *Anaplasma* and *Babesia* infection. Clinical examination of study cattle reported a significant elevation in values of body temperature, pulse and respiratory rates of infected cattle with *Anaplasma*, *Babesia* and *Theileria*. However, significant higher body temperature, pulse and respiratory rates, enlargement of lymph nodes and paleness of mucous membranes was observed in *Theileria* cases when compared to *Anaplasma* and *Babesia* infections. Regarding values of the risk factors, significant variation in prevalence of haemoparasitic infections was seen among different age groups, sexes and regions. **Conclusions:** This appears as one of the rare Iraqi studies which performed to estimate the prevalence of bovine haemoparasites. Therefore, furthermore annual studies are necessary to detect the prevalence of haemoparasites in cattle livestock of other regions and ruminants (buffaloes, camels, sheep and goats). Also, the prevalence obtained in our study may have been higher if a more sensitive diagnostic assay such as molecular technique was used. Molecular detection of hemoparasites using PCR is more sensitive and specific than examination of blood smears, particularly in cases of low parasitaemia.

**Keywords:** Blood parasite, *Anaplasma*, *Babesia*, *Theileria*, Giemsa stain, Tick

## Introduction

Community interest in raising cattle has increased every year, and the key to success in efforts to increase cattle productivity is the health of cattle itself. These efforts to develop cattle population require a number of measures that known as the preventive measures for the emergence of pathogenesis from disease agents into their host (Sinclair et al., 2019; Meurens et al., 2021). Haemoparasites or blood parasites are a group of the livestock diseases that quit endemic and great important due to their ability to causing severe economic losses in the form of declining work efficiency, reduced reproductive capability, weight loss, lowered in milk production, poor appetite and stunted growth, abortion, and deaths (Thumbi et al., 2013; Bary et al., 2018; Rashid et al., 2019). Animal diseases originating from haemoparasites include *Anaplasma* (Al-gharban and Dhahir, 2015), *Babesia* (Al-Abedi and Al-Al-Amery, 2020), *Theileria* (Hassan et al., 2012) and trypanosomiasis (Al-Badrani, 2012).

*Anaplasma* is an infectious, non-contagious, transmissible arthropod disease that caused mainly by *A. marginale* and *A. central*, and characterized clinically by severe anemia and hemolysis with high body temperature, dehydration and jaundice (Fosgate et al., 2010; Wen et al., 2016). After recovery, cattle remain carrier for their life and may serve as a reservoir for uninfected cattle (Aubry and Geale, 2011). Ticks are a biological amplifier since *Anaplasma* multiply in salivary glands and gut epithelium of this vector to transmit the organism from blood meal (Hajdusek et al., 2013).

*Babesia* is an intraerythrocytic apicomplexan protozoan parasite causing the disease of *Babesia* that responsible on substantial mortalities and morbidities in addition to large economically losses among livestock industry in tropical and subtropical areas (Niu et al., 2015). Worldwide, the principle species of *Babesia* that cause bovine *Babesia* are *B. bovis*, *B. bigemina*, and *B. divergens*. Other *Babesia* that can infect cattle includes *B. major*, *B. ovate*, *B. occultans* and *B. Jakimovi* (Jirapattarasate et al., 2017). Significant increases in prevalence of *Babesia* due to changing of environmental circumstances especially universal warming, favors tick survival and reproduction, and hence great increases in abundances of ticks was observed. In Iraq, *B. bovis*, *B. bigemina* and *B. divergens* are the main detectable *Babesia* spp. among the individuals and herds (Aiz and Sabbar, 2016; Saeed et al., 2017; Al-Abedi and Al-Al-Amery, 2020).

*Theileria* spp. is an obligate intracellular protozoan parasite that closely related to *Babesia* but differs mainly by having of *Theileria* to a developmental stage in leukocytes prior to infection of erythrocytes (extra-erythrocytic multiplication), number of merozoites during multiplication in erythrocytes, and the cycle of transmission in the vector tick (Uilenberg, 2006; Najm et al., 2014). There are several identified *Theileria* spp. that infect cattle, and the most pathogenic and economically importance are *T. parva*, which causes East-Coast fever, and *T. annulata*, which causes tropical *Theileria* (Constable et al., 2016). In Iraq, tropical *Theileria* is the prevalent form of disease, which characterized acutely by high fever, severe depressions and anorexia, enlargement of superficial lymph node (especially the prescapular, submandibular, and pre-

femoral lymph nodes) with paleness (at early stage) and icteration (at the late stages) of mucus membranes (Alfatlawi et al., 2021).

During the acute form of disease, direct thin blood smear stained with Giemsa dye remains the most commonly used technique as a quick, cheap and easy to perform diagnostic assay (Shabana et al., 2018). In Iraq, although large numbers of studies were carried out previously and recently, there are continuous annual changes in prevalence of haemoparasites among different regions. Hence, this study was aimed to microscopically investigate of haemoparasites in cattle in Wasit province (Iraq) and to detect the association of clinical signs and risk factors to positive infections.

## Materials and methods

### *Ethical approval*

The current study was licensed by the Scientific Committee of the College of Veterinary Medicine, University of Wasit (Wasit, Iraq). The study animals were involved in this study following an agreement of their owners.

### *Samples and data collection*

An overall 118 cattle, attended to the Veterinary Hospital in Wasit and a number of private veterinary clinics, were selected randomly and subjected aseptically for direct collection 2.ml of jugular venous blood by a disposable syringe (Al-Rawabi, China) into a labeled EDTA plastic tube (AFCO, Jordan), during December (2021) to February (2022). Data of clinical examination [body temperature, pulse and respiratory rates, enlargement of lymph nodes (LN), and paleness / jaundice of mucous membrane (MM)] and risk factors (age, sex and region) were reported.

### *Microscopic examination*

Thin blood smears were prepared from each un-clotted blood sample by transferring and distribution a small drop of blood throughout the area of a slide. After air-drying (1 hour), the slides were fixed with few drops of absolute methanol (BDH, England) and leaved to be dried. and for 3-5 minutes. Then, staining was carried out by Giemsa stain 10% (Syrbio, Syria) using a glass jar (30-60 minutes) and the slides were washed with regular tape water and dried at room temperature. Finally, the slides were examined microscopically at a low (100×) and high (1000×) magnification using the light microscope (Olympus, Japan) to detect haemoparasites within (*Anaplasma* spp., *Babesia* spp. and *Theileria* spp.) and outside (*Trypanosoma* spp.) of erythrocytes (Chagas et al., 2020)

### *Statistical analysis*

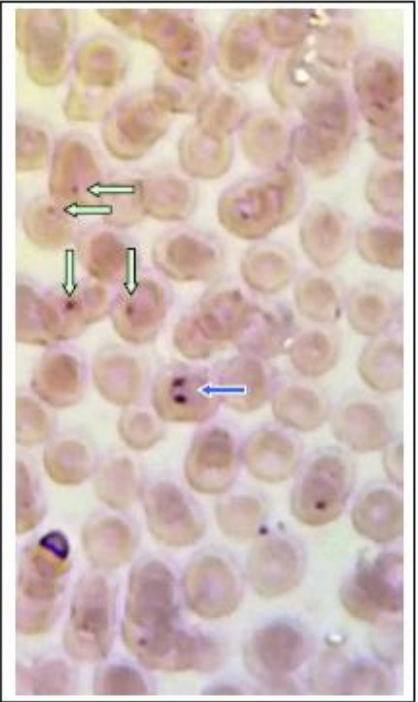
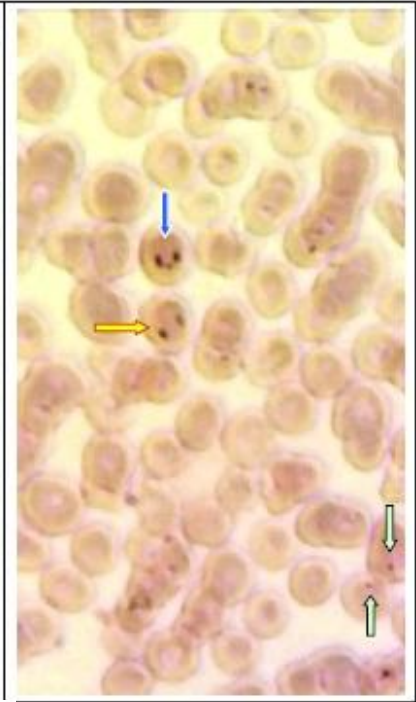
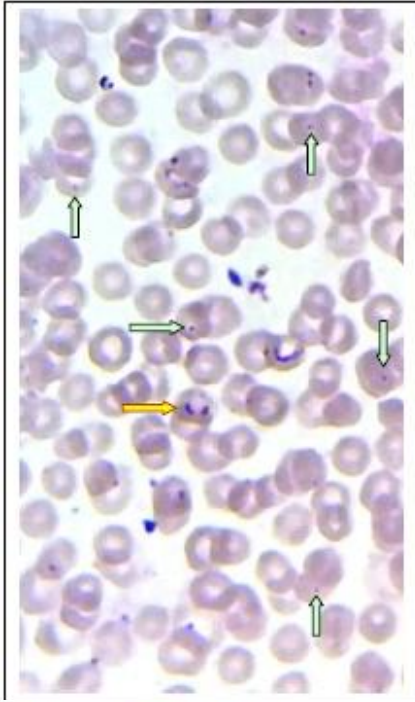
All obtained results were documented and tabled by the Microsoft Office Excel (version 2016), and analyzed statistically by the GraphPad Prism (version 9.0.1) software. The Chi-square ( $\chi^2$ ) and *t*-test were applied to detect significant differences between positive and negative results of each infection, and between the positive results of all infection, respectively. The Odds Ratio (OR) was applied to detect the statistical correlation of positive infections to the clinical data and risk factors at  $P < 0.05$ .

Results

Microscopic examination of stained blood smears revealed that the total prevalence rate of haemoparasites among 118 study cattle was 23 (19.49%), comprising 13 (11.02%), 8 (6.78%) and 2 (1.69%) with *Anaplasma*, *Babesia* and *Theileria*, respectively (Table 1, Figure 1).

Table (1): Total results of Giemsa’s stained blood smears examined by light microscopy

Total No.	Positive	Negative
118	23 (19.49%)	95 (80.51%)



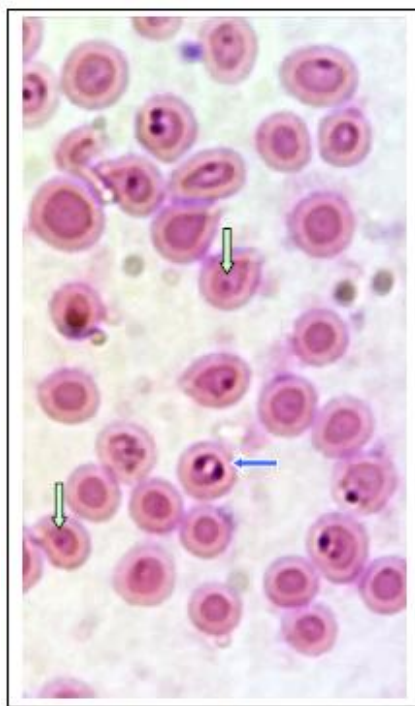


Figure (1): Positive results of light microscopy at a magnification of 100×  
(→): *Anaplasma*  
(→): *Babesia*  
(→): *Theileria*

Among positive infections, there were 56.52% (13/23), 34.78% (8/23) and 8.7% (2/23) infected cattle with *Anaplasma*, *Babesia* and *Theileria*, respectively (Figure 3). Additional classification of infected cattle according to type of infection revealed totally on 70.59% (12/17) single and 29.41% (5/17) mixed infections. Statistically, the findings of single infection were reported a significant increase ( $P<0.0235$ ) in prevalence of *Anaplasma* (66.67%) when compared to *Babesia* (33.33%) and *Theileria* (0%), (Figure 4). For mixed infection, higher significant elevation ( $P<0.0153$ ) were observed in values of *Anaplasma* and *Babesia* infection (60%), while significant decreases were seen in values of *Anaplasma* and *Theileria* (20%) as well as *Anaplasma*, *Babesia* and *Theileria* (20%), but not in values of *Babesia* and *Theileria* infection which recoded the absence of positive samples (0%), (Figure 5).

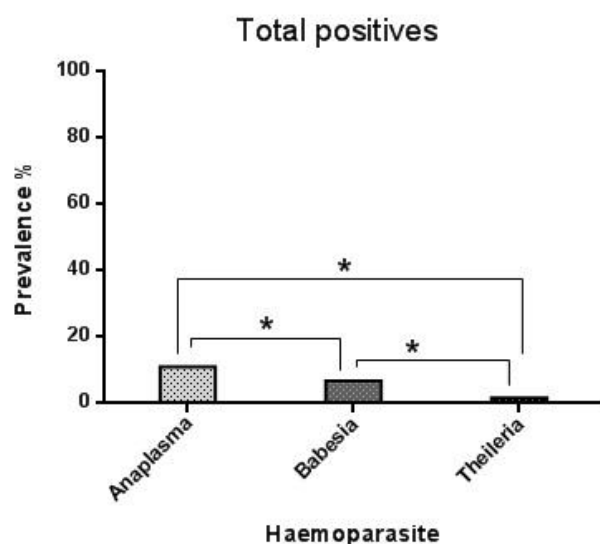


Figure (2): Distribution of haemoparasites among the total examined cattle

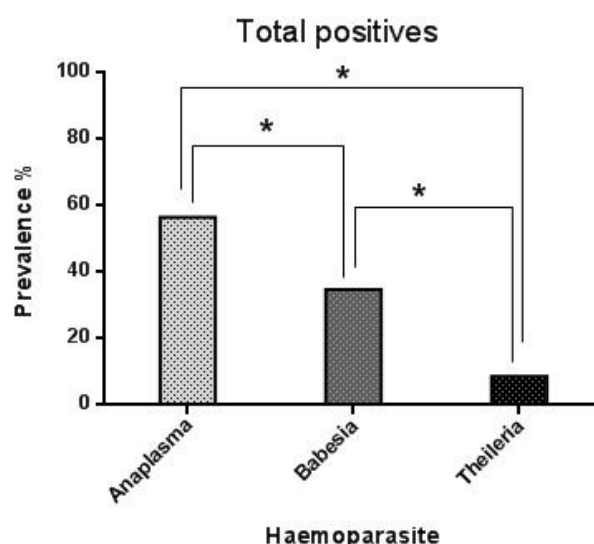


Figure (3): Distribution of haemoparasites among positive infected cattle

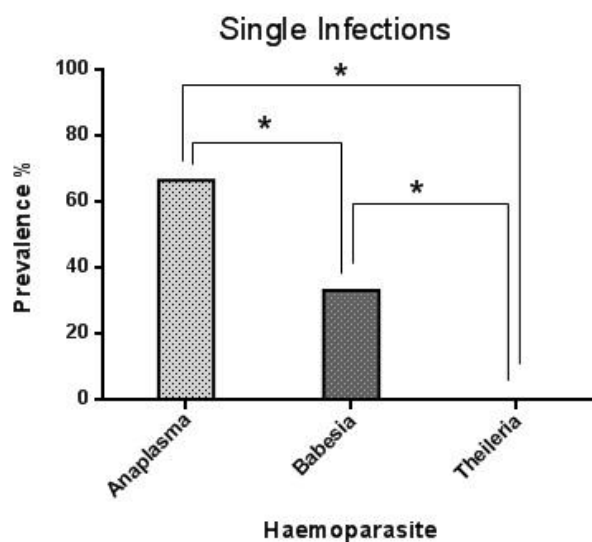
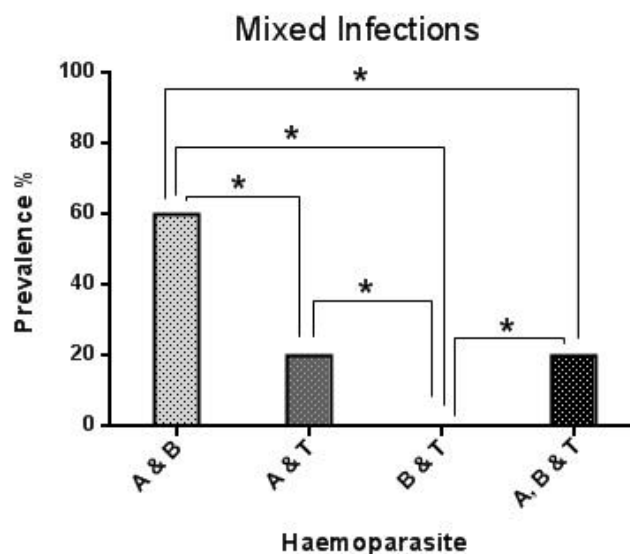


Figure (4): Distribution of single infection according to genus of haemoparasite



**Figure (5): Distribution of mixed infection according to genus of haemoparasite**

(A & B): *Anaplasma* and *Babesia*; (A & T): *Anaplasma* and *Theileria*  
 (B & T): *Babesia* and *Theileria*; (A, B & T): *Anaplasma*, *Babesia* and *Theileria*

The findings of clinical examination of positive cattle with *Anaplasma* showed that there were significant increases ( $P < 0.05$ ) in values of body temperature ( $38.64 \pm 0.35$ ), pulse ( $69.92 \pm 2.87$ ) and respiratory ( $33.85 \pm 1.05$ ) rates when compared to values of negative cattle ( $37.81 \pm 0.23$ ,  $62.92 \pm 2.01$ ,  $27.44 \pm 0.91$ , respectively), (Table 2). For *Babesia*, results of infected cattle were showed a significant elevation ( $P < 0.05$ ) in values of temperature ( $39.34 \pm 0.37$ ), pulse ( $71.25 \pm 3.34$ ) and respiratory ( $35.25 \pm 0.72$ ) rates in comparison with those of non-infected animals ( $37.83 \pm 0.25$ ,  $63.24 \pm 2.16$ ,  $28.07 \pm 1.14$ , respectively), (Table 3). For *Theileria*, there were significant increases ( $P < 0.05$ ) in values of temperature ( $40.12 \pm 0.94$ ), pulse ( $80 \pm 12$ ) and respiratory ( $39.5 \pm 1.5$ ) rates of infected cattle when compared to values of non-infected ones ( $37.91 \pm 0.21$ ,  $64.15 \pm 2.03$ ,  $29.02 \pm 1$ , respectively), (Table 4).

**Table (2): Results of vital signs in positive cattle with *Anaplasma***

Sign (Unit)	Positive	Negative	<i>p</i> -value
Body temperature (°C)	$38.64 \pm 0.35$ *	$37.81 \pm 0.23$	<b>0.033</b>
Pulse rate (Beat / Minute)	$69.92 \pm 2.87$ *	$62.92 \pm 2.01$	<b>0.042</b>
Respiratory rate (Breath / Minute)	$33.85 \pm 1.05$ *	$27.44 \pm 0.91$	<b>0.048</b>

Significance \* ( $P < 0.05$ )

**Table (3): Results of vital signs in positive cattle with *Babesia***

Sign	Positive	Negative	<i>p</i> -value
Body temperature (°C)	$39.34 \pm 0.37$ *	$37.83 \pm 0.25$	<b>0.021</b>
Pulse rate (Beat / Minute)	$71.25 \pm 3.34$ *	$63.24 \pm 2.16$	<b>0.036</b>
Respiratory rate (Breath / Minute)	$35.25 \pm 0.72$ *	$28.07 \pm 1.14$	<b>0.04</b>

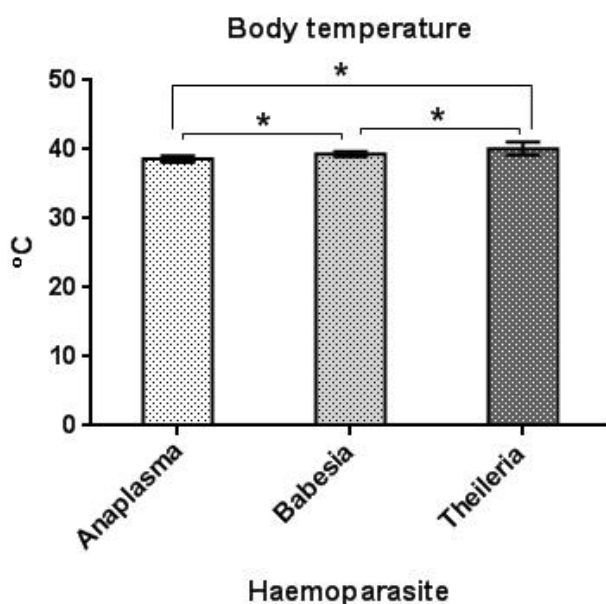
Significance \* ( $P < 0.05$ )

**Table (4): Results of vital signs in positive cattle with *Theileria***

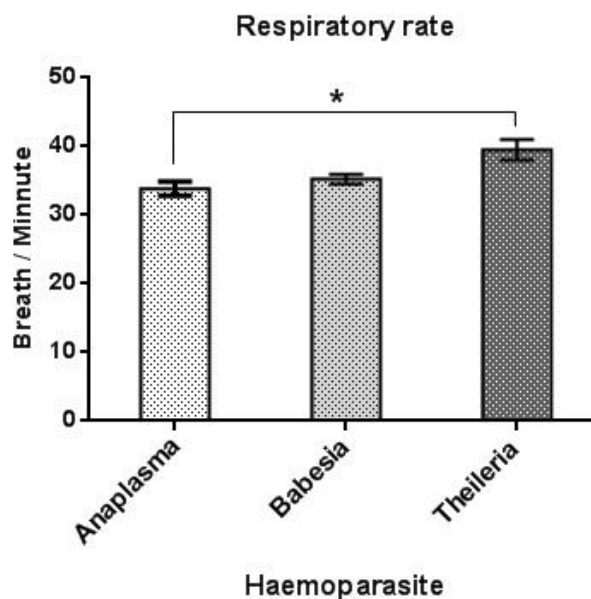
Sign	Positive	Negative	<i>p</i> -value
Body temperature (°C)	$40.12 \pm 0.94$ *	$37.91 \pm 0.21$	<b>0.15</b>
Pulse rate (Beat / Minute)	$80 \pm 12$ *	$64.15 \pm 2.03$	<b>0.019</b>
Respiratory rate (Breath / Minute)	$39.5 \pm 1.5$ *	$29.02 \pm 1$	<b>0.023</b>

Significance \* ( $P < 0.05$ )

In comparison between the results of infected cattle, significant differences ( $P < 0.029$ ) in values of body temperature (°C) were observed between cattle with *Anaplasma* ( $38.64 \pm 0.35$ ), *Babesia* ( $39.34 \pm 0.37$ ) and *Theileria* ( $40.12 \pm 0.94$ ), (Figure 6). For pulse (b/m) and respiratory (b/m) rates, no significant differences ( $P > 0.05$ ) were seen between values of infected cattle with *Anaplasma* ( $69.92 \pm 2.87$  and  $33.85 \pm 1.05$ , respectively) and *Babesia* ( $71.25 \pm 3.34$  and  $35.25 \pm 0.72$ , respectively); however, significant increases ( $P < 0.045$ ) were detected in cattle with *Theileria* ( $80 \pm 12$  and  $39.5 \pm 1.5$ , respectively), (Figures 7, 8).



**Figure (6): Temperature of infected cattle with haemoparasite**



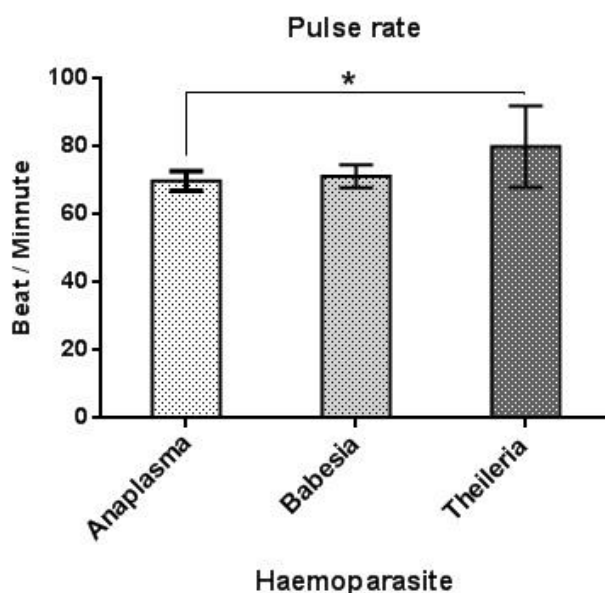
**Figure (7): Respiratory rate of infected cattle with haemoparasite**

Also, infected cattle with *Theileria* were showed significant higher prevalence ( $P < 0.05$ ) in values of enlargement of LN (100%) and paleness of MM (100%) than cattle with *Anaplasma* (30.77% and 46.15%, respectively) and *Babesia* (37.5% and 50%, respectively), (Table 5).

**Table (5): Results of clinical examination in positive cattle with *Theileria***

Symptom	Genus of haemoparasite (Total positives)			<i>p-value</i>
	<i>Anaplasma</i> (13)	<i>Babesia</i> (8)	<i>Theileria</i> (2)	
Enlargement of LN	4 (30.77%)	3 (37.5%)	2 (100%)	<b>0.031</b>
Paleness/jaundice of MM	6 (46.15%)	4 (50%)	2 (100%)	<b>0.04</b>

Significance \* ( $P < 0.05$ )



**Figure (8): Results of pulse rate among microscopically infected cattle with haemoparasite**

The association of risk factors to positive haemoparasitic findings demonstrated a significant variation ( $P < 0.05$ ) in their values (Tables 6-8). Concerning *Anaplasma*, the findings of risk factors were showed a significant increase ( $P < 0.025$ ) of infection in cattle aged  $< 4$  (14.75%) and 4-8 (9.3%) years old when compared to those of  $> 8$  years (0%), and in females (12.97%) more than males (4.76%), ( $P < 0.03$ ). Among study regions, higher significant values were reported in Al-Kut (12.96%), Al-Hai (11.11%) and Al-Numaniyah (10.53%); while lowered value was seen in Badra (5.88%), ( $P < 0.041$ ). Statistically, the risk of infection was appeared more significance in cattle aged  $< 4$  (2.114) years old, females (2.583), and those found at Al-Kut (1.383) and Al-Numaniyah (0.929).

For the genus of *Babesia*, prevalence rate on infection was increased significantly ( $P < 0.031$ ) in cattle aged  $< 4$  (9.84%) and 4-8 (4.65%) years old when compared to those of  $> 8$  years (0%); however, insignificant variation ( $P < 0.059$ ) was observed between values of females (7.22%) and males (4.76%). Among study regions, values of Al-Hai (11.11%) and Al-Kut (7.41%) were increased significantly ( $P < 0.016$ ) when compared to values of Badra (5.88%) and Al-Numaniyah (5.26%). The risk of infection was elevated significantly in cattle aged  $< 4$  (2.722), females (1.517), and animals existed at Al-Hai (1.719) and Al-Kut (1.175) regions.



Regarding theileria's infection, no significant differences ( $P>0.05$ ) were detected between the age groups;  $<4$  (3.28%), 4-8 (0%) and  $>8$  (0%) years, females (2.06%) and males (0%), as well as between different study regions; Badra (0%), Al-Hai (0%), Al-Kut (1.85%) and Al-Numaniyah (2.63%). Although, the values of risk of infection were differed insignificantly between study age groups;  $<4$  (0.0339), 4-8 (0%) and  $>8$  (0%) as well as between females (0.021) and males (0), significant increase was recorded in cattle existed at Al-Numaniyah (2.08) and less significantly in Al-Kut (1.186) but not in Badra (0) and Al-Hai (0).

**Table (6): Statistical correlation of risk factor to positives cattle with *Anaplasma* (No.: 13)**

Factor (Group)	Total	Positive	Prevalence (%)	Negative	OR	Risk
<b>Age (Year)</b>						
$<4$	61	9	14.75	52	2.31	2.114
4-8	43	4	9.3	39	0.76	0.778
$>8$	14	0	0	14	0	0
<b>p-value</b>	-	-	<b>0.025</b>	-	<b>0.01</b>	<b>0.0096</b>
<b>Sex</b>						
Female	97	12	12.37	85	2.94	2.583
Male	21	1	4.76	20	0.34	0.387
<b>p-value</b>	-	-	<b>0.03</b>	-	<b>0.0051</b>	<b>0.0074</b>
<b>Region</b>						
Badra	17	1	5.88	16	0.46	0.496
Al-Hai	9	1	11.11	8	1.01	1
Al-Kut	54	7	12.96	47	1.45	1.383
Al-Numaniyah	38	4	10.53	34	0.93	0.929
<b>p-value</b>	-	-	<b>0.041</b>	-	<b>0.015</b>	<b>0.018</b>

Significance \* ( $P<0.05$ )

**Table (7): Statistical correlation of risk factor to positives cattle with *Babesia* (No.: 8)**

Factor (Group)	Total	Positive	Prevalence (%)	Negative	OR	Risk
<b>Age (Year)</b>						
$<4$	61	7	9.84	55	3.06	2.722
4-8	43	1	4.65	41	0.613	0.581
$>8$	14	0	0	14	0	0
<b>p-value</b>	-	-	<b>0.031</b>	-	<b>0.0053</b>	<b>0.0091</b>
<b>Sex</b>						
Female	97	7	7.22	90	1.56	1.517

Male	21	1	4.76	20	0.641	0.659
<b><i>p-value</i></b>	-	-	<b>0.059</b>	-	<b>0.026</b>	<b>0.036</b>
<b>Region</b>						
Badra	17	1	5.88	16	0.83	0.855
Al-Hai	9	1	11.11	8	1.81	1.719
Al-Kut	54	4	7.41	50	1.19	1.175
Al-Numaniyah	38	2	5.26	36	0.7	0.707
<b><i>p-value</i></b>	-	-	<b>0.016</b>	-	<b>0.023</b>	<b>0.027</b>

**Significance \* (P<0.05)**

**Table (8): Statistical correlation of risk factor to positives cattle with *Theileria* (No.: 2)**

<b>Factor (Group)</b>	<b>Total</b>	<b>Positive</b>	<b>Prevalence (%)</b>	<b>Negative</b>	<b>OR</b>	<b>Risk</b>
<b>Age (Year)</b>						
< 4	61	2	3.28	59	0.0339	0.0328
4-8	43	0	0	43	0	0
>8	14	0	0	14	0	0
<b><i>p-value</i></b>	-	-	-	-	<b>0.094</b>	<b>0.098</b>
<b>Sex</b>						
Female	97	2	2.06	95	0.021	0.022
Male	21	0	0	21	0	0
<b><i>p-value</i></b>	-	-	<b>0.0098</b>	-	<b>0.068</b>	<b>0.061</b>
<b>Region</b>						
Badra	17	0	0	17	0	0
Al-Hai	9	0	0	9	0	0
Al-Kut	54	1	1.85	53	1.212	1.186
Al-Numaniyah	38	1	2.63	37	2.126	2.08
<b><i>p-value</i></b>	-	-	<b>0.084</b>	-	<b>0.0077</b>	<b>0.0074</b>

**Significance \* (P<0.05)**

## Discussion

Haemoparasites generally constitute a serious challenge to the health and wellbeing of livestock in the tropical and sub-tropical regions, including Iraq. Our findings showed that the total positive rate of bovine haemoparasites was 19.49%, with significant prevalence of *Anaplasma* (11.02%) when compared to *Babesia* (6.78%) and *Theileria* (1.69%). Higher prevalence of cattle haemoparasitism has been documented in Nigeria (Ola-Fadunsin, 2017), Iran (Rajabi et al., 2017), and Egypt (Taha et al., 2018). Disparity in time of study, breeds of animals sampled, differences in sample size, the diagnostic tool used, and the management and nutritional status of

animals sampled could have resulted in the inconsistency of prevalence recorded in this study compared to those by other researchers (**Abdullah et al., 2019**). We observed *Anaplasma* to be the most prevalent hemoparasites-affecting cattle. The fact that *Anaplasma* infection can be transmitted by numerous means (biologically by ticks, mechanically by biting flies, through blood-contaminated fomites and shearing instruments) could have been the reason for our finding.

The existence of single and mixed infection is very dependent on the condition of livestock (host), other infectious agents, vectors and environment that composed socio-cultural and socioeconomic areas as well as weather climate and geographical conditions (**Al-gharban and Dhahir, 2015**). The effect of rainfall, temperature and relative humidity on the occurrence of hemoparasites of cattle is not strange, although our finding appears to be one of the first studies in Iraq. *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. studied are all tick-borne parasites, so their occurrence will be determined by the abundance the vector responsible for their transmission. In a recent study conducted in Kenya (**Keesing et al., 2018**), the findings showed that rainfall was negatively influenced the abundance of *Rhipicephalus pulchellus* and *R. praetextatus* larvae. **Tokarevich et al. (2017)** reported that temperature favors the increase of tick population because temperature determines the duration of the different stages of the tick life cycle. The ability of ticks to lay eggs and hatch in a season depends greatly on the sum of effective temperatures (**Estrada-Peña et al., 2011; Diyes et al., 2021**). Generally, environmental factors such as temperature and humidity have been shown to influence tick abundance, availability of hosts, their survival and disease transmission thereby influencing the prevalence of diseases transmitted by ticks (**Dumic and Severnini, 2018**).

The diversity of clinical signs may depend on the severity of the infection, infective dose and willingness of the animal to infection. **Belkahia et al. (2017)** and **Said et al. (2018)** mentioned that significant increases in body temperature, pulse rate and respiratory rate in acute cases when compared to healthy ones or carriers could occur with minimal variations according to age, genetic factors, country and season. The elevation noticed in acute infection may be associated with peak parasitemia and anemia, where a transient febrile response occurs concurrently with increased pulse and respiratory rate (**Al-gharban and Dhahir, 2015**). The febrile crisis may be a result of pyrogens releasing from destruction of WBCs, these pyrogens affect on the hypothalamus, causing elevation of the body temperature. The increasing in body temperature causes an elevation in respiratory rate; while, increasing in pulse rate could result from anemia and dehydration (**Deroost et al., 2016**). Furthermore, the increased respiratory rate may be ascribed to hypoxemia and subsequent tissue hypoxia. Anemia is usually accompanied by increase cardiac output, pulse rate and respiratory rate (**Mohammed and Salman, 2016**). These signs may also be affected by several factors, including stress, environment, species, age, sex, pregnancy, lactation and trace mineral deficient diet (**Kahn et al., 2005; Radositits et al., 2007; Nejres et al., 2010**). Other researchers concluded that dyspnea might occur due to presence of anemia and related decrease in oxygen transport capacity of the blood and physiologic

adjustment to increase the efficiency of the erythron and reduce the workload on the heart (**Birdance et al., 2006; Constable et al., 2016**). In acute cases, fever, dullness, depression, lack of appetite, decrease of milk production, stop rumination, constipation as well as paleness of mucous membranes and general weakness were mentioned by other investigators in different countries of the world (**Wang et al., 2003; Liu et al., 2005; Fosgate et al., 2010**).

A number of studies have confirmed that the occurrence of acute phase of infections is more prevalent in young and adult cattle in particular after 2 years of age, and mild or subclinical in calves of < 12 months, yearlings and recovered cattle (**Constable et al., 2016; Al-Abedi and Al-Amery, 2020; Sray, 2021**). In regarding to age factor, our study reported that the higher rates of infection were seen in cattle aged < 4 years. These findings are similar to those reported by other studies (**Figuerola et al., 2010; Ziam et al., 2020**) who attributed these results to a natural resistance of younger animals in particular those aged 6-9 months to different infection due to the role of maternal immunity that transferred throughout the colostrum and provided a temporal protection from different infections. This protection could last for about 3 months, and in most cases, is followed by an age resistance that lasts until the animals are about 9 to 12 months of age (**Tassi et al., 2002**). However, **Kahn et al. (2005)** suggested that in animals of < 1 year old, *Anaplasma* is usually subclinical, and in yearlings and 2 years old, infection is moderately severe; while in older cattle, haemoparasitic infection is severe and often fatal. **Kasozi et al. (2014)** concluded that clinical infection is significantly observed in cattle, and the age resistance in calves is gradually wane after one year of age and these animals become increasingly susceptible to the disease in the regions that had no endemic stability. Other studies confirmed that the low chance of exposure to exophilic ticks is associated with the low infestation rates (**Zintl et al., 2017; Niaz et al., 2021**). On the other hand, lower prevalence rate of haemoparasites in older animals may be due to their multiple recurrent infections and the development of concomitant immunity during their lifetime (**Suarez et al., 2019**). An association of positivity to other risk factors had showed a significant difference in their values. In concerning with sex factor, results showed that the rate of prevalence of infection among females and males was differed significantly in *Anaplasma* infection and insignificantly in *Babesia* and *Theileria* infections. The low number of examined male animals could cause the lack of significance in our findings, or to that, both sexes exposed to a same level of infection. However, higher prevalence of infections among females than males is in agreement with that detected by other researchers (**Tuli et al., 2015; Goyal, 2018; Ziam et al., 2020**). This might be explained by the fact that the milch animals have higher hormonal stress, carry more ticks, stress due to milk production and calving, and are at higher risk of exposure to the infection during milking time. Variation in prevalence of haemoparasite, in particular *Anaplasma*, was observed in this study. **Bobby Fokidis et al. (2008)** suggested that interspecific differences in parasitic infection cannot be attributed entirely to differences in vector abundance or body condition. Interactions between immune function, parasite infection risk, and resource availability may contribute to determining the relative ability of certain species to adapt to regions. However, there are a number of factors that actively influencing animal population, and have received a considerable

attention in different animals. Compared to animals in native rural environments, urban animals are exposed to many potentially detrimental factors including increased brood parasitism, human disturbance, noise, feral predation, introduced competitors, exposure to toxins, artificial lighting, and warmer temperatures (Omudu and Amuta, 2007; Bobby Fokidis et al., 2008; Blanco et al., 2017).

## Conclusions

Since almost previously and recently studies in Iraq were carried out to detect the haemoparasites solely, this appears to be the one of the rare Iraqi studies which aimed to detect the prevalence rate of different haemoparasites in cattle. Our findings demonstrated that single and mixed infection could be existed at the same host, and that *Anaplasma* infection was significantly more prevalent than *Babesia* and *Theileria* infections. Data of clinical examination reported that the higher changes in vital signs and severe systemic reactions were found in cattle infected with *Theileria*, and that age was the most commonly related risk factor for haemoparasites. However, furthermore annual studies are necessary to detect the rate of prevalence of haemoparasites in cattle livestock of other regions, as well as in other ruminants (buffaloes, camels, sheep and goats) with application of the advanced diagnostic techniques.

## Authors' contributions

ZRA and AFJ: Collection of blood samples and preparation the slides of blood smears; HAJG and SJA: Clinical examination, data collection, and Giemsa's staining the slides of blood smears with the statistical analysis of obtained results; and GJKA: Microscopic examination of stained smears to detect of haemoparasites.

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## Competing interests

No

## References

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