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Research article

Detection of *Anaplasma phagocytophilum* infection in sheep in some provinces of Iraq

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Abstract:

The study was designed to record the prevalence of Anaplasma phagocytophilum infection within sheep in Iraq. A total of 297 blood samples were collected from sheep in three provinces: Babylon, Misan and Wasit. Diagnosis of infection was done on the basis of examination of stained smear. Which was prepared and examined microscopically of blood stained smear for the presence of morulae, and revealed 8/297 (2.69%) as the total infection rate. Vital clinical examination revealed non-significant differences between temperature, pulsation and the respiratory rates of infected sheep; other significant clinical signs were emaciation at 5/8 (62.50%) and pale mucous membrane at 3/8 (37.50%). The haematological diagram displayed significant differences in infected sheep compared to non-infected sheep in some haematological parameters. The platelet count, lymphocytes and neutrophil were significantly decreased (P<0.05), while total white blood cell count, monocytes, eosinophils and basophils were decreased non-significantly. The results obtained are the first blood smear diagnosis of A. phagocytophilum infection within sheep in Iraq.

Keywords: *Anaplasma phagocytophilum*, blood smear, Infection rates, Iraq, Sheep.

Introduction:

Tick-borne fever in sheep caused by *Anaplasma phagocytophilum* is widespread and has been recorded in many European countries (1, 2), the United States (3), Turkey (4) and in Iran (5). *A. phagocytophilum* is endemic in 42 countries across the world (6). *A. phagocytophilum* is the zoonotic agent of human granulocytic anaplasmosis (7). *A. phagocytophilum* is recorded in a wide range of animal hosts such as sheep, cows, dogs, horses, wild deer and rodents (8, 9). *A. phagocytophilum* clearly replicates within cytoplasmic vacuoles of neutrophils, seen as morulae, which can be detected by using Giemsa staining (10). The distribution of the organism is determined by the populations of tick vectors, hosts and reservoir host species (11). *A. phagocytophilum* is mainly transmitted by *Ixodes ricinus* ticks (12) "*A. phagocytophilum* infects and multiplies in the organs of ticks, particularly the salivary glands, which enables transmission to other hosts during feeding" (8). Tick-borne fever is manifested as a febrile disease; the clinical signs may varied from mild to severe disease, sometimes predisposing to other secondary infections (13). Infection appears in sheep without developing clinical signs of the disease (14, 15). Natural infections of *A. phagocytophilum* are identified by serology, blood chemistry, haematology and "polymerase chain reaction (PCR)", as well as genetic variation of distinct genes (16). Therefore, the aim of this study is to record the infection rates and presence of *Anaplasma phagocytophilum* infection within sheep in Iraq in which the potential vectors are not recorded.

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Materials and Methods:

Ethical approval

The present study was approved by the Animal Ethical Committee.

Animals and data collection:

The study included 297 sheep of mixed breeds (male and female) with ages ranging from less than 1 year to more than 5 years. These were selected randomly from different areas of Babylon, Wasit and Misan provinces of Iraq, from February 2018 to October 2018. Case history, clinical examination and clinical signs were recorded for each sheep.

Blood collection:

The blood samples were collected from the tip of the ear to make a thin film, which was stained with Giemsa stain. Then, 5 ml of blood was collected from the jugular vein by a vacutainer tube with an anticoagulant, which was ethylene diamine tetra acetic acid (EDTA) for haematological parameters according to (17). All samples were transferred in a cooling condition (cooling box) to the research laboratory at the College of Veterinary Medicine, Al-Qasim Green University, to perform all laboratory tests.

Blood smear preparation:

Thin blood smears were prepared on a clean glass slide, fixed with methanol for three minutes and stained with Giemsa. Finally, the stained slides were observed under 100x magnification of an Olympus microscope for *Anaplasma phagocytophilum* inclusion bodies (18).

Blood analysis: The haematological parameters were analysed by a blood analyser instrument (abacus unior vet) that can determine 15 parameters.

Statistical analysis:

The results were analysed by a SPSS statistical program. A chi-square test was used to assess the association between the percentage results of the variables. Means ± SE were determined for the haematological parameters by using an independent t-test. A *p* value of $p \leq 0.05$ was considered significant (19).

Results:

Infection rates:

The 297 blood samples that were collected from sheep in the three provinces in Iraq (Babylon, Misan and Wasit), and examined by staining with Giemsa stain, revealed a total infection rate of 2.69% (Table 1). There was a non-significant variance among infection rate in the provinces. The examined stained blood smear shows intracytoplasmic inclusion bodies (morulae) in neutrophil. Figure 1(A, B).

Table (1). Infection rates of *Anaplasma phagocytophilum* according to blood smear staining

Province	No. of blood samples	Infected	(%)
Babylon	162	4	2.46^{A}
Misan	71	2	2.81^{A}
Wasit	64	2	3.12^{A}
Total	297	8	2.69^{A}

Chi square value (X2=0.081).
Similar letters denote non-significant differences at p<0.05

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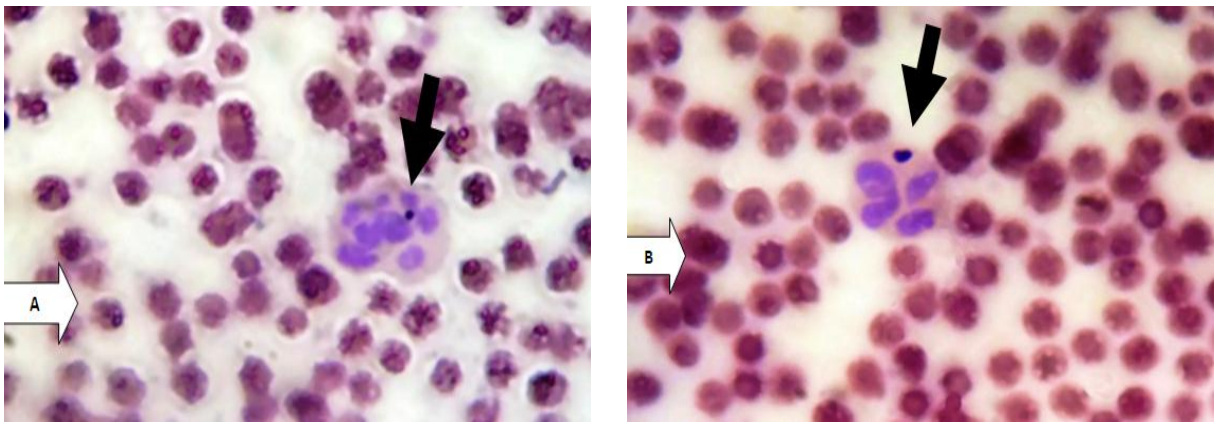

Figure (1) (A, B): *Anaplasma phagocytophilum* intracytoplasmic inclusion bodies (morulae) in neutrophil, (black row). (Giemsa stain, X100).

Clinical examination:

All sheep were clinically examined. The variable clinical signs were observed and are compared in Table 2 and Figure 2 below. Sheep infected with *Anaplasma phagocytophilum* showed emaciation as predominant signs 5/8 (62.50%), with a pale mucous membrane of infected sheep 3/8 (37.50%). Fever was recorded in 3/8 of infected sheep, but vital clinical examination revealed non-significant differences between temperature, pulsation and respiratory rates in infected sheep with *A. phagocytophilum* when compared with other negative control sheep (Table 3).

Table 2: Clinical signs of infected sheep with *Anaplasma phagocytophilum*.

Clinical signs	No. of blood samples	%
Fever	3	21.42 ^{A}
Pale mucous membrane	3	37.50 ^{BD}
Loss of appetite	2	25.00 ^{AB}
Emaciation	5	62.50 ^{C}

X2: chi square value (X2=20.25).
Different letters denote significant differences at p<0.05
Similar letters denote non-significant differences at p<0.05

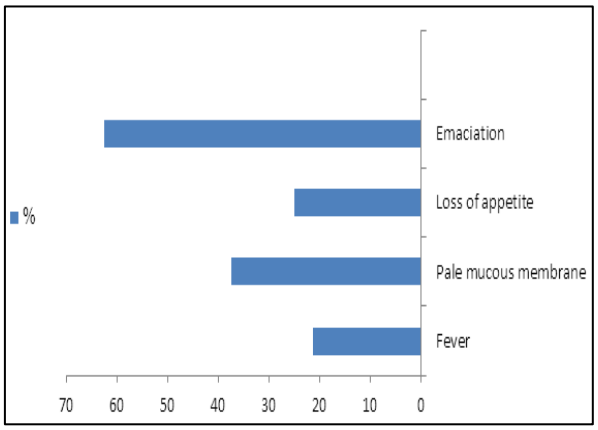

Figure (2): Clinical signs of infected sheep with Anaplasma phagocytophilum.

Table (3): Vital clinical signs of infected and non-infected sheep with *Anaplasma phagocytophilum*

Signs	*Anaplasma phagocytophilum.* +Ve		*Anaplasma phagocytophilum.* –Ve		T value
	No.	(Mean ±SE)	No.	(Mean ±SE)	
Temperature (^{o}C)	8	(39.56 ±0.12)^{A}	289	(39.48 ±0.02)^{A}	0.661
Pulse rate (beats/minute)		(79±1.27)^{A}		(78.20 ±0.41)^{A}	0.419
Respiratory rate (rate/minute)		24.78±1.03^{A}		26.44±0.18^{A}	1.994

Similar letters denote the non-significant differences at (P<0.05)

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Haematological analyses:

Total platelets count (PLT):

The mean of total platelet count (PLT) of infected sheep with *Anaplasma phagocytophilum* was recorded as 1.283 ±7.90 (×103/µL). The mean of non-infected sheep was recorded as 3.076 ±8.80 (×103/µL). These results show that the PLT count of infected sheep significantly decreased compared with non-infected sheep (Table 4).

Table 4. Total platelets count of *Anaplasma phagocytophilum* in infected and non-infected sheep.

State	Anaplasma phagocytophilum. +Ve		Anaplasma phagocytophilum. –Ve	
Platelets count (×103/µL)	Number of blood samples	(Mean ±SE)	Number of blood samples	(Mean ±SE)
	8	(1283 ±7.90)^{A}	289	(3076 ±8.80)^{B}
T value	4.519			

Different letters denote significant differences at p<0.05

Total white blood cells count (WBCs):

The mean of the total white blood cells count (WBCs) of infected sheep with *Anaplasma phagocytophilum* was 5.893 ±521 (/µL). This was less than the mean of the WBCs count of non-infected sheep at 8.370 ±156 (/µL), but non-significant Table (5).

Table (5):Total leukocytic count of infected and non-infected sheep with *Anaplasma phagocytophilum*.

State	*Anaplasma phagocytophilum.* +Ve		*Anaplasma phagocytophilum.* –Ve	
	No. of blood sample	(Mean ±SE)	No. of blood sample	(Mean ±SE)
Total WBCs count (/µL)	8	(5893 ±521)^{A}	289	(8370 ±156)^{A}
T value	3.476			

Similar letters denote non-significant differences at (P<0.05)

Differential white blood cells count (WBCs)

The results of the differential white blood cells count (WBCs) showed a significant decrease in lymphocytes and neutrophils in sheep infected by *Anaplasma phagocytophilum* compared with non-infected sheep. However, monocytes, eosinophils and basophils in infected sheep were slightly decreased and non-significant compared to those cells in non-infected sheep (Table 6).

Table (6): Differential leukocytes count of infected sheep compared with non-infected sheep with *Anaplasma phagocytophilum*.

State	Number of blood samples	WBCs (/µL) (mean ±SE)				
		Lymphocytes (/µL)	Neutrophils (/µL)	Monocytes (/µL)	Eosinophils (/µL)	Basophils (/µL)
Anaplasma phagocytophilum. +Ve	8	2835± 146 a	2483± 175 a	306± 27 a	322± 38 a	38.5± 2.12 a
Anaplasma phagocytophilum. -Ve	289	4364± 108 b	4482± 99 b	410± 8 a	459± 12 a	44± 1.10 a
T value		3.125	4.437	2.732	2.482	1.149

Different letters denote significant differences at (P<0.05)
Similar letters denote non-significant differences at (P<0.05)

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Discussion

Anaplasma phagocytophilum- organisms were first identified microscopically by the examination of stained blood smears in this study of *A. Phagocytophilum* infection in sheep in Iraq. (10) recorded that *A. phagocytophilum* clearly replicates within cytoplasmic vacuoles of neutrophils, seen as morulae, which can be detected by using Giemsa staining. The results revealed non-significant differences between provinces. All provinces studied were located in the same geographical area, which has a similar climate and populations of tick species (18). The infection rate in the three provinces with *A. phagocytophilum* was 8/297 (2.69%) according to blood smear staining (morulae). The result agreed with (20) in the Czech Republic and the Slovakia Republic, and with (18) in Turkey, who recorded (9.86%) were positive for blood smears of sheep and disagreed with (21) in China (where the rate was 28.8%). "Differences in the infection rate of *A. phagocytophilum* from area to area may be due to many factors, such as a seasonal variation of tick vectors and hematophagous flies, climatic conditions, and breed susceptibility" (22). Variable clinical signs were observed in the sheep infected with *A. phagocytophilum*. Clinically, they showed several signs including emaciation, fever, pale mucous membrane and loss of appetite,. The study's result agreed with (23) (11), (24), who also reported fever, anorexia, emaciation, and reduced milk production. Some sheep infected with *A. phagocytophilum* did not show clinical signs. These results agreed with other authors who found *A. phagocytophilum* infection has been found in small ruminants in Tunisia, but "these infected animals have not shown any clinical signs relating to tick-borne fever"(25, 26) (27). Clinical signs of tick-borne fever caused by *A. phagocytophilum* are subclinical or heavy symptoms (28). Sheep may develop infection of *A. phagocytophilum* without clinical signs of the disease (13, 15). Sheep that gave positive results showed variable differences in their clinical signs. Some infected sheep without clinical signs may be due to the phase of infection (acute, persistent and carrier), immune status, age, infective dose, climate and management (5). An absence of clinical signs does not mean absence of infection (25).The results showed that sheep infected with *A. phagocytophilum* decrease in PLT count compared with non-infected sheep. The results agreed with (24) who recorded that changes which accompany *A. phagocytophilum* include a reduction in the number of circulating platelets (thrombocytopenia), and with (29) who recorded the main haematological effects are thrombocytopenia and changes in the haematological diagram. (30) also stated that thrombocytopenia is one common haematological abnormality useful for diagnostic tests of *A. phagocytophilum* infection. Haematological abnormalities and presence of morulae are also considered a diagnostic method for the detection of *A. phagocytophilum*. The results showed that infected sheep show a decrease in WBCs but there are non-significant differences between infected and non-infected sheep. The results agreed with (31) who recorded that infection with *A. Phagocytophilum* developed mild and transient leukopenia. (8) also recorded that the clinical pathology associated with *A. Phagocytophilum* infection occurs in the differential WBCs count and platelets. The results of differential WBCs showed a significant decrease in lymphocytes in *A. phagocytophilum* infected sheep, while there was a significant decrease in neutrophils in infected sheep. The results agreed with (24) who recorded the effects of TBF infection in sheep on the haematological diagram and manifested with thrombocytopenia and leukopenia due to lymphocytopenia and neutropenia. This shows a slight decrease in the total WBCs due to *A. phagocytophilum* infection and is associated with a decrease in differential leucocyte count during infection

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(32). The results agreed with (33) who recorded additional indicators for the detection of TBF in sheep which are leukopenia and prolonged neutropenia. The result is in accordance with other results where *A. phagocytophilum* is present in sheep with the onset of a fever, and develop a severe but transient thrombocytopenia and is followed by lymphocytopenia and more prolonged neutropenia (8).

Conclusion

Blood smear staining (morulae) as well as thrombocytopenia, lymphopenia and neutropenia are considered as fast screening test for detection of *A. phagocytophilum* infection. This is the first record of *A. phagocytophilum* infection within sheep in Iraq; the disease is zoonotic and could transmit to humans.

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