DIAGNOSTIC STUDY OF EHRLICHIOSIS IN CATTLE OF MOSUL-IRAQ

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ABSTRACT

Ehrlichiosis (Ehrlichia spp.) was detected in 132(70 Iranian,32 Turkish and 30 local breed)calves (10-14 months old) and 18 dairy cows of local breed (3-5 years old). Animals were brought to Veterinary Teaching Hospital, College of Veterinary Medicine, University of Mosul –Iraq from different farms of Mosul city-Iraq, with clinical signs of fever(40-41C^0),anorexia ,emaciation, decrease of milk yield in dairy cows ,paleness of mucous membrane, lymph node enlargement and ticks were detected on different body regions .All blood and buffy coat smears of calves were positive for morula like structures inside the cytoplasm of neutrophils , lymphocytes and monocytes when staining by Giemsa and Wright stains. For all animals , the appearance of Ehrlichia spp. Organisms in leukocytes coincided with erythropenia, leukopenia, and thrombocytopenia and a decrease in hematocrit and hemoglobin concentration. For eighteen dairy cows, Ehrlichia spp. organisms were identified in leukocytes of blood and milk samples. This is apparently the first evidence for Ehrlichia spp. infection in cattle in Iraq. In conclusion, the present study documents that *Ehrlichia* infection exists in Mosul-Iraq and indicates that the bacterium has been present but unnoticed in the area .Further investigation will be needed in order to characterize different species of Ehrlichia infection in cattle, and explain the role of vector and imported animals in introduction of the disease to our country.

INTRODUCTION

Ehrlichiosis is an infection of white blood cells that affects various mammals, including mice, cattle, dogs, deer, horses, sheep, goats and humans (1,2) and usually named according to the host species and the type of white blood cell most often infected. The organisms that cause ehrlichiosis are small pleomorphic Grams-negative obligate intracellular coccobacilli (3).

Species of Ehrlichia are tick- transmitted organisms of the family Anaplasmataceae and order Rickettsials (4). Infection can also transmitted by blood transfusion, and mechanically via biting insects has been suggested as possible mean of spread (3). Ehrlichia spp. are found in leukocytes of a wide range of domestic and wild mammals (4). Although species of these genera cause significant disease in animals, only species of Ehrlichia and Anaplasma phagocytophilium are associated with human infections. The geographical occurrence of the various species of Ehrlichia appear to be limited by the distribution of the appropriate tick vectors and

reservoirs mammalian hosts. White deer and other wild life species or dogs serve as reservoir hosts for most Ehrlichia species (5).

Anaplasma phagocytophilum is the recently designated name replacing three species of granulocytic bacteria, Ehrlichia phagocytophila, Ehrlichia equi and the agent of human granulocytic ehrlichiosis (6,7). Ixoides ricinus has been found to be the main vector of A. phagocytophilum in Europe (8). A. phagocytophilum has also detected in Ixoides ricinus in Iran (9). Recently, A. phagocytophilum has been reported in ruminants and I. ricinus, removed from humans, in the east Black Sea region of Turkey (10,11,12). Tick-born fever (TBF), which is caused by the prototype of Anaplasma phagocytophilum as a disease in cattle in England(13).

In cattle, tick-borne fever usually occurs in dairy animals recently turned out to pasture. The clinical signs are variable in severity. Common symptoms include depression, marked anorexia, decreased milk production, respiratory distress, coughing, abortions and reduced semen quality. The two most prominent syndromes are abortions with a drop in milk yield, and respiratory disease (14,15).

The diagnosis of granulocytic ehrlichiosis in animals in the acute phase is based on clinical findings (fever, anorexia, apathy, limb oedema and petechial hemorrhages), laboratory findings (leukopenia, anemia, thrombocytopenia and detection of intra- cytoplasmic inclusion bodies in leukocytes), tick infestation, season and geographical location (14). In addition, a sensitive and specific PCR for detection of *Ehrlichia* DNA in host blood or in vectors has been recently described (16, 17,18,14). A response to treatment also supports the diagnosis. Ehrlichiosis is usually treated with the tetracycline antibiotics. In dogs, chloramphenicol is also used occasionally (3).

The aim of the present study was to determine whether *Ehrlichia spp*. is detectable in farms of Mosul city-Iraq where the potential vector is not documented.

MATERIAL AND METHODS

From July 2011 to July 2012, a total of 150 cattle included 132 (70 Iranian, 32 Turkish and 30 local breeds) calves (10-14 months old) and 18 dairy cows of local breeds (3-5 years old). Animals were brought to Veterinary Teaching Hospital, College of Veterinary Medicine, University of Mosul– Iraq, from different farms of Mosul city-Iraq with history of outbreak of tick borne diseases. A twenty healthy cattle (15 calves and 5 dairy cows) were used as a control animals for the comparison of the results. A clinical signs of infected cattle were recorded and blood samples were collected from the jugular vein of each animal with ethylene diamine tetra-acetic acide (EDTA) as anticoagulant and examined for estimation of leukocyte, erythrocyte, thrombocyte counts, hematocrit and hemoglobin concentration. Additionally, two thin blood smears were prepared immediately after each blood collection. The blood smears were air dried, fixed in methanol, then stained with Giemsa or Wright stain to detection of DLC: differential leukocyte counts with absolute numbers of each leukocyte type. Five hundred leukocytes were examined for *Ehrlichia* organisms, and the percentage of positive cells was calculated (19). Microhemotocrit buffy coat technique was also used in diagnosis, blood (70µl) is collected into heparinized capillary tubes (75 x1.5 mm) which are then sealed and centrifuged. The buffy coat was collected to make the smears, were fixed by methanol for 3 minutes and Giemsa stained. The slides were visualized by a light microscope at 1000x.

Milk samples (30-ml) was collected from each quarter of dairy cows (lactating cows), by using a sterile technique, in which the milk had a negative California mastitis test and was bacteriologically sterile. The sample was centrifuged at 1.000 rpm for 10 min. The pellet was washed twice with phosphate buffered saline, and smears, were stained with Giemsa stain. Five hundred cells were examined for intracellular *Ehrlichia* organisms (14). Data were analyzed by SPSS statistical program and Means ± SD were determined for the studied parameters. Two-way ANOVA was carried out to find the differences in the parameters. A value of the $P<0.05$ was considered significant in comparison of mean value of control group.

RESULTS

Infected calves showed clinical signs of fever (40-41C^0), tachycardia (165±34 beat/minute) and increase of the respiratory rates (55±19 breath/minute). Sometimes lethargy, anorexia, depression, emaciation, diarrhea, lameness, edema of the hind limbs, lymph nodes enlargement, coughing, dyspnea, serous to purulent oculo-nasal discharge, pale mucous membrane with petachiae and tick infestation. While infected cows showed Pyrexia, decreased milk production, respiratory signs, and tick infestation are the predominant clinical signs. The hematocrit, erythrocyte counts and hemoglobin concentration, were significantly lower than normal value on the control animals. (Table 1). In all infected animals, the leukocyte counts and thrombocyte counts were decreased significantly. The leukopenia was characterized by marked lymphopenia, neutropenia, and eosinopenia, while monocyte numbers showed significant increase (Table 1).

Table (1): Hematological findings for calves and cows infected with *Ehrlichiosis*

Variables(unit)	Control	Infected Cows	Control	Infected Calves
Hematocrit(%)	29.0±1.6	20.3±1.5*	32±3.1	18.7 ±2.7*
Hemoglobin concentration(g/dl)	10.3. ±0.8	8.2±1.0*	11±1.6	7.8±1.4*
Total No. Erythrocyte(10^6/µl)	5.75 ±0.7	4.2±0.6*	9.0±0.8	3.7±0.4*
Thrombocytes(10^3/µl)	376±66	123±70*	279±56	165±83*
Total No. leukocytes(10^3/µl)	6.67±0.7	3.4±0.3*	10.0±1.2	2.8±0.8*
Neutrophil (10^3/µl)	1.53±0.8	0.23±0.03*	3.8±0.02	0.80±0.03*
Lymphocytes(10^3/µl)	2.912±0.2	1.45±0.05*	7.0±0.03	1.02±0.04*
Eosinophils (10^3/µl)	0.34±0.01	0.09±0.02*	0.4±0.01	0.07±0.01*
Monocytes(10^3/µl)	0.54±0.03	1.5±0.01*.	0.2±0.03	1.0±0.03*

All values are mean ± SD.
*means values are significant at $p<0.05$.

For all calves and only eight cows, *Ehrlichia* organisms were seen in blood smears that were carefully examined and screened under the microscope. *Ehrlichia* morulae were observed as vacuoles-bound clusters of organisms which appears as a

abaophilic inclusions in the cytoplasm of neutrphils (Figures 1-A and B). Some leukocytes contained more than one morula in the cytoplasms and frequently present in the neutrophil (Figures 2-A, B) and lymphocytes (Figures 3-A,B,C) but no frequency of morulae in the monocytes (Figures 4-A,B,C). There are many forms of *Ehrlichia* seen inside leukocytes which indicates that more than one species can be present (Figures 5-A,B). The percentage of parasitemia of infected leukocytes in blood of calves ranged from 1% to 25%. *Ehrlichia* organisms were identified predominantly in neutrophils (87%) and lymphocytes(8%), but monocytes and eosinophils were also infected to a smaller degree 2% and 3% respectively (Table 2). Large numbers of a zurophilic granules were also seen in the cytoplasm of lymphocytes and monocytes of the peripheral blood smears (Figures 6- A,B,c)

The buffy coat layer, which is predominantly composed of mononuclear cells, was revealed rare basophilic inclusion bodies (morulae) about 10-50%, were seen in neutrophils(90%), lymphocyte(7%) and monocytes(2%) (Table 2) (Figures 7- A,B,C).

Table(2): The severity of leukocytes infection within Ehrlichiosis in blood of Calves

Cells	Blood smears	Buffy coat smears
Leukocytes	1-25%	10-50%
Neutophils	87%	90%
Lymphocytes	8%	7%
Monocytes	2%	3%
Eosinophils	3%	0%

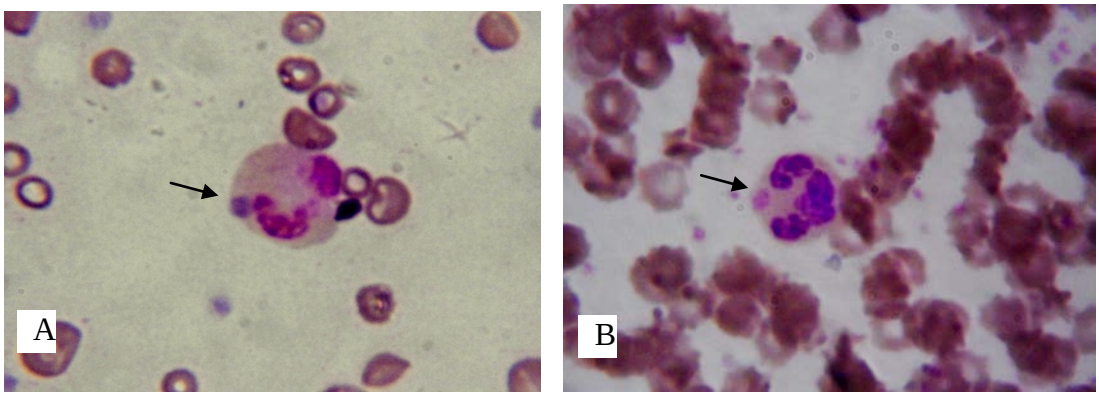


Figure 1; a peripheral blood smear from a calf with an granulocytic ehrlichiosis, a typical round ehrlichial morula is seen at the cytoplasm of the neutrophil, (arrow). Some platelets can be seen below and to the right of the neutrophil, for comparison.; original magnification: x 1000. (A: Giemsa stain, B: Wright stain)

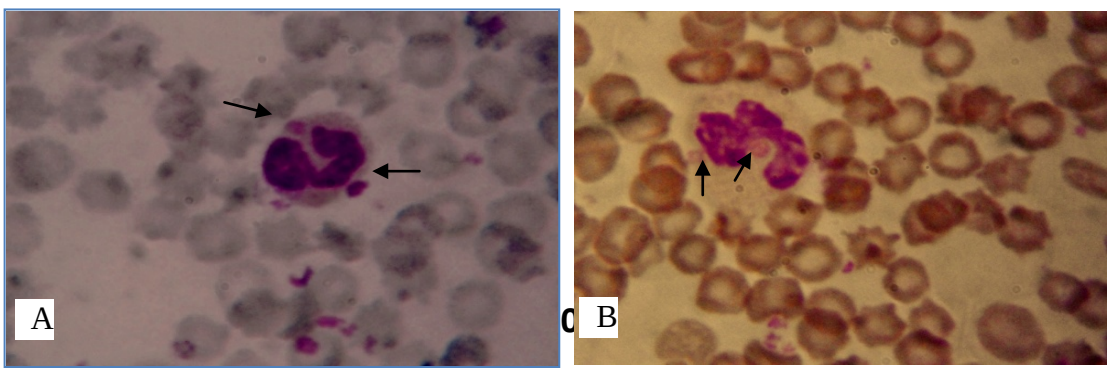
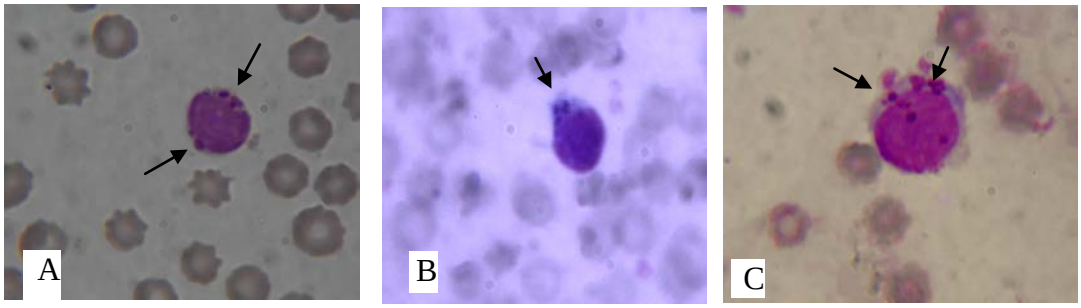
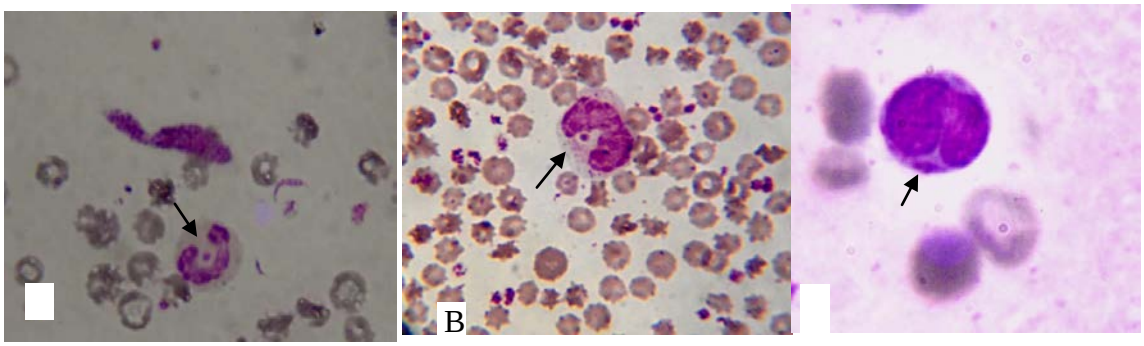


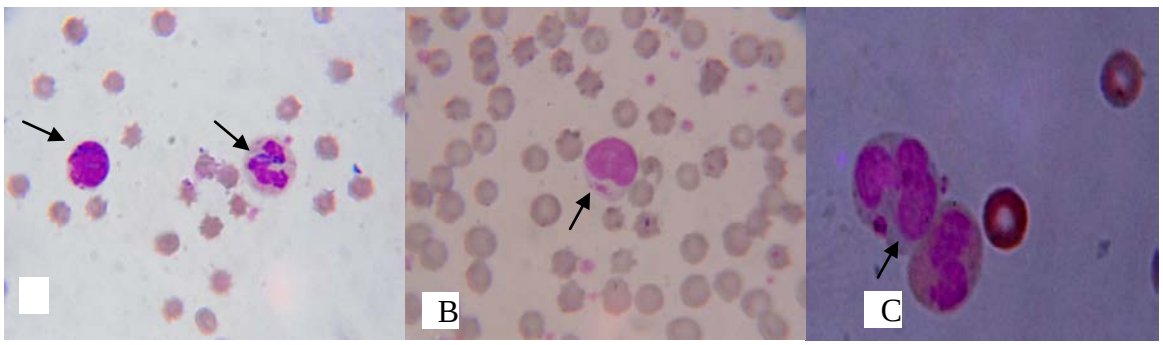
Figure 2; a peripheral blood smear from acow with an granulocytic ehrlichiosis, A: ehrlichia morula is seen at the cytoplasm of the neutrophil. Giemsa stain was used; original magnification: x 1000. B: a typical round ehrlichial morulae is seen at the center of the Wright stain was used; original magnification: x 1600.neutrophil, adjacent to the nuclear lobes (arrow).



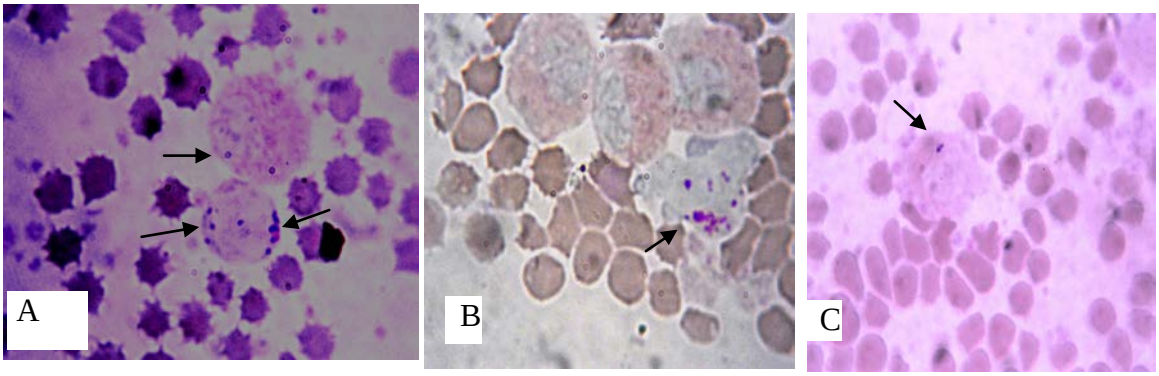
Figure(3) Photomicrography of blood smears of calf showing more than one morula in the cytoplasm of lymphocyte in A,B,C . Giemsa stain x1000



Figure(4) Photomicrography of blood smears of calf showing a typical morula in the center of mononuclear cells in A,B . Giemsa stained x1000 and C:Ehrlichia morula at the cytoplasm of monocyte. Wright stain x1600.

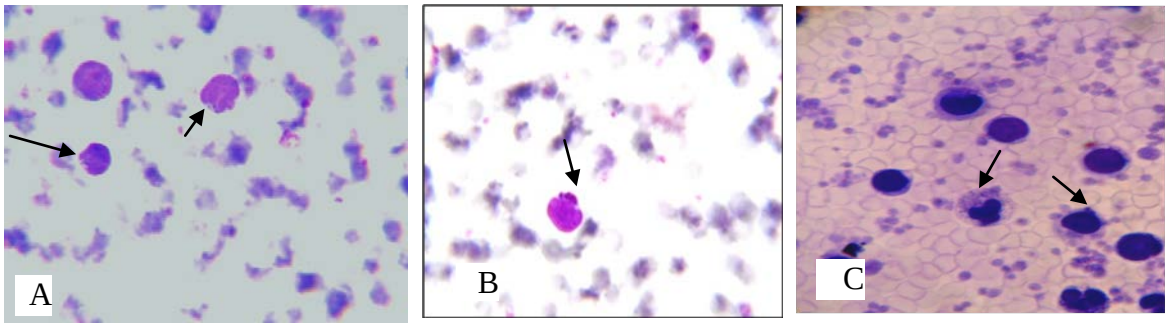


Figure(5) Photomicrography of blood smears of calf showing more than one morula in the cytoplasm of neutrophil and lymphocyte in A . and Ehrlichia inclusions in the cytoplaaasm of monocyte in B . Giemsa stain x1000



Figure(6) Photomicrography of buffy coat smears of calf showing more than one morula in the cytoplasm of neutrophil and lymphocyte in A . and Ehrlichia inclusions in the cytoplasm of monocyte in B and C . Giemsa stain .x1000

Ehrlichia organisms were identified in 1 to 5% of leukocytes in milk smears of eighteen lactating cows (Figure 7-A,B,C). The individual cell count in milk was between 90,000 and 130,000/ml, with a proportion of neutrophils of less than 25%.Ehrlichia inclusions were predominantly in milk neutrophils(81%) in comparison with lymphocyte(11%) and monocytes(8%) (Table 3).



Figure(7) Photomicrography of milk smears of cows showing more than one morula in the cytoplasm of neutrophil and lymphocyte in A . and Ehrlichia inclusions in the cytoplasm of macrophages in B and C . Giemsa stain .x1000

Table(3): The severity of leukocytes infection within Ehrlichiosis in blood and milk of dairy cows

Type of cells	Blood smears	Buffy coat smears	Milk smears
Leukocytes	3-18%	10-33%	1 -5%
Neutrophils	50%	70%	81%
Lymphocytes	10%	5%	11%
Monocytes/Macrophages	20%	20%	8%
Eosinophils	20%	5%	0%

DISCUSSION

Ehrlichia organisms were first idenified microscopically in this study in cattle of Iraq. These morulae arise as a result of intracellular replication of the reickettsia.(20) showed that *Anaplasma phagocytophilum* clearly replicates within neutrophils, as seen by the abundance of morulae seen within cytoplasmic vacuoles of neutrophils. In blood smears, the morula is considered as diagnostic for ehrlichiosis (3). They are three intra-cytoplasmic forms; initial body, elementary body and morula (a vacuole-bound tightly packed cluster of organisms) that appears as basophilic inclusions within the cytoplasm of granulocytes or monocytes. Morulae are regularly found in neutrphils during the acute stage of infection (21).

The results found in this study have shown that morulae can be searched for in peripheral blood smear and milk smear for Ehrlichia detection. The ability to identify morulae in the circulating leukocytes of infected cattle is enhanced by preparing and microscopically evaluating a buffy coat smear. Other studies have also observed a low frequency of morulae in peripheral blood smears and they have attributed this feature to the low parasitemia that occurs under natural infection. The low parasitemia could be due to the bacteria remaining lodged in the spleen longer than in the blood, justifying the higher number of morulae in this organ (21, 22, 23, 24). Some researchers believe that the spleen could be the shelter for ehrlichial organisms for periods longer than the bacterial time in the blood (25,26).

The present study showed that among the assays for the diagnosis of naturally occurring bovine ehrlichiosis, the search for morulae in buffy coat smears is more efficient than in peripheral blood smears when it is intended to provide parasitological diagnosis of bovine ehrlichiosis. It is important to note that the search for morulae in the milk is a low-cost assay with easy execution, and which can be safely applied by the clinician to the diagnosis of bovine ehrlichiosis. There were smaller numbers of infected leukocytes in milk than in blood, and they appeared when high parasitemia (14).

The most commonly infected leukocytes were neutrophils, followed by lymphocytes and monocytes. Neutrophils were predominantly infected during the acute phase of the disease, whereas monocytes were infected towards the end. (27)found that neutrophils and eosinophils are the cells in which the organisms primarily multiply, whereas monocytes and lymphocytes are secondary host cells for *E. phagocytophila*.

The hematological changes were a decrease in the hematocrit, erythrocyte count, and concentration of hemoglobin, similar to findings by(28), which may be the result of an increased rate of destruction or impaired erythropoiesis. This observation is similar to what has been described for disease caused by other species of *Ehrlichia* (29). The significant leukopenia was characterized by lymphopenia, neutropenia and

eosinopena. Leukopenia, thrombocytopenia and anemia of animals infected with ehrichiosis have been attributed to possible suppression of bone marrow production (30,31) or to the autoimmune destruction of infected leukocytes, platelets and perhaps red blood cells, and the appearance of large granular lymphocytes in the peripheral blood smear during ehrlichiosis which can be also associated with unexplained immunological abnormalities (27).

To our knowledge, this is the first reported evidence in cattle of Mosul-Iraq of a disease caused by a member of the genus *Ehrlichia*, It seems to be there are more than one species of Ehrlichia in cattle in this study, we need further investigations to confirm the presence of *A. Phagocytophilum* and determination the transmitting vectors, animal reservoirs and pathogenesis of *A. phagocytophilum* in animals and possible risk of transmitting this infection to animals in Iraq.

CONCLUSIONS

Ehrlichia spp. of cattle had been introduced to Mosul-Iraq and their probable vectors are present in the region, which endangers the livestock of the area. Surveys of the *Ehrlichia* species and their possible vectors are recommended to assess their distribution and infection rates in all regions of Iraq, and to plane control measures against their vectors.

ACKNOWLEDGMENTS

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دراسة تشخيصية للايرليخيوسز في الماشية في مدينة الموصل –العراق

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الخلاصة

تضمنت الدراسة تشخيص الاصابة بمرض الايرليخيوسز في 132 عجل من السلالات المحلية(30حيوان) والمستوردة من ايران(70حيوان) وتركيا(32حيوان) تراوحت اعمارها بين 10-14 شهر، و18 بقرة حلوبة من السلالات المحلية بعمر 3-5 سنوات 0جلبت الحيوانات من حقول مختلفة حول مدينة الموصل الى المستشفى البيطري التعليمي/جامعة الموصل /العراق 0 استخدم 20حيوان (15عجل و5 ابقار من السلالات المحلية والمستوردة) كانت سليمة سريريا ومختبريا كمجموعة سيطرة في الدراسة 0اظهرت الحيوانات المصابة علامات سريرية تمثلت بالحمى(40-41 درجة مئوية) وفقدان الشهية والهزال وشحوب الاغشية المخاطية وتضخم العقد اللمفية والاصابة بالقراد فضلا عن انخفاض في انتاج الحليب في الابقار 0 لوحظ اثناء الفحص المجهري للمسحات الدموية المحيطية ومسحات الغلالة البيضاء وجود اشتمالات الايرليخيا (التوتية) داخل هيولى خلايا الدم البيض المتمثلة بالعدلات والخلايا اللمفية وخلايا وحيدة النواة واحيانا الحمضات، اذ اصطبغت بلون ازرق قاعدي بصبغتي الكيمزا والرايت 0 شوهدت الاجسام الاشتمالية للايرليخيا في هيولى خلايا الدم البيض للمسحات الموية ومسحات الحليب للابقار المصابة 0 ترافقت الاصابة بالايرليخيا بانخفاض معنوي في اعداد كريات الدم الحمر وخلايا الدم البيض واعداد الصفيحات الموية فضلا عن انخفاض معنوي في حجم خلايا الدم المرصوصة وتركيز خضاب الدم 0 اثبتت هذه الدراسة وجود الاصابة بلايرليخيا في الماشية في مدينة

الموصل-العراق لاول مرة نحتاج الى دراسات اخرى لتصنيف الانواع المختلفة للايرليخيا ومعرفة دور القراد والحيوانات المستوردة في ادخال هذا المرض الى بلدنا.

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