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Equine Anaplasmosis in Nineveh Governorate: Clinical, Hematological and Biochemical Alterations in Equids

E. Al-Jwari, Q. T. Al-Obaidi.
Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Iraq.

Corresponding Author Email Address: qaestalb1976@uomosul.edu.iq
ORCID ID: https://orcid.org/0000-0002-1334-3745.
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Abstract

The current study was aimed to determine the prevalence of Equine anaplasmosis (EA) in equids in Nineveh Governorate using i-ELISA, and to record clinicalo-hemato-biochemical parameters linked with the EA. A total of 180 blood samples collected randomly from equids (106 horses and 74 donkeys) in Nineveh governorate, Iraq, including, 25 clinically and laboratory healthy equids that served as control equids. Careful clinical examinations for all equids have been done, and samples of blood were collected from all equids for serodiagnosis, hemato-biochemical parameters examination. Results demonstrated that the total prevalence of AE in equids was 46.1% (83/180), representing 30.1% in horses and 68.9% in donkeys using indirect-ELISA. Infected equids were suffering from an acute form of the disease with a significant increase in the body temperature, respiratory rate, heart rate, and capillary refilling time in infected equids compared to the control equids. Hemogram revealed a significant decrease in the TRBCs, Hb, PCV, MCH, and MCHC, with a significant increase in MCV, reflecting a macrocytic hypochromic type of anemia. In addition to significant increase in the ESR. Furthermore, a significant decrease in PLT and TWBCs as a result of a significant decrease in neutrophils and lymphocytes in the infected equids compared to the control equids. Biochemical analysis showed that a significant increase in AST, ALT, ALP, TB, creatinine, and BUN with a significant decrease in TP, phosphorus and calcium, in infected equids compared to the control animal. In conclusion, The EA is widespread in equids in Nineveh Governorate, Iraq along with significant clinico-hemato-biochemical parameter alterations.

Keywords: Equine anaplasmosis, Equids, i-ELISA, hematology analysis, Biochemical analysis.

Introduction

Equine anaplasmosis (EA) is an emerging and important infectious blood disease. Asynoname: equine granulocytic anaplasmosis (EGA) and equine granulocytic ehrlichiosis (EGE) (1,2). It was first discovered in California in 1969 by Gribble, (3). A high mortality rate of EA, which can reach 5%, and the cost of treating affected cases as well as controlling the disease's vectors all result in significant economic losses (4,5). The disease is caused by gram-negative bacteria called *Anaplasma phagocytophilum*, which belongs to the order Rickettsiales, family Anaplasmataceae, and genus *Anaplasma* (6). It is one of the obligate intracellular bacteria that infects leukocytes, especially neutrophils (7), as well as lymphocytes, monocytes, basophils, and eosinophils (8,9). This bacterium infects various types of animals, including: equids, camels. sheep, goats, deer, cows, dogs, and cats (10-16), as well as humans (17). The EA is mainly transmitted mechanically by hard ticks under the genus Rhipicephalus, Dermacentor, and Ixodes (9,18), through blood transfusion and contaminated instruments such as syringes and cannulas with the bacteria (15), It can also be transplacental transmission from the dame to the fetus (19). The disease is endemic in many countries, such as Iraq (9), Iran (20), Turkey (21), Tunis (22), Pakistan (8), South Korea (23), Italy (24), Germany (25), Algeria (26), Virginia State (27), and Brazil (28). The incubation period of EA is from one to three weeks, depending on the severity of the disease, and there are different clinical forms, including acute, chronic, and subclinical forms (15,29). The acute form of the disease is mainly characterized by a fever (39-40°C), paleness of mucous membranes, petechial hemorrhage in the third eyelid, anemia, respiratory disturbance, edema in the lower limbs, nervous signs, and finally death of the animal (30-32). The chronic form of the disease has not been recorded, and subclinical cases are the most common. The presence of *A. phagocytophilum* germs in the blood of infected horses has been recorded for more than four months without any clinical signs being observed (33), and indicated that subacute infections with anaplasmosis in horses are spontaneous recovery (34). Further, EA causes immunosuppression in infected equids which could predispose to secondary bacterial infections, causing tick pyemia, pneumonia, septicemic listeriosis, and encephalitis (15, 35,36).

Usually, the clinical signs of the EA are rarely helpful in diagnosing the disease in equids (37), because they are confused with other blood diseases in equids such as babesiosis (38), theileriosis (39), and trypanosomiasis (40). Therefore, laboratory tests are needed to confirm the diagnosis of the disease, including microscopic examination of the blood smears stained by Romanowsky stains (6). As well as performing serological tests, such as indirect enzyme-linked immunosorbent assay, and the indirect fluorescent antibody test (IFAT) (26), and can also be using molecular techniques such as conventional-PCR (41), nested-PCR (23), real-time PCR (32), and the loop-mediated isothermal amplification (LAMP) method (42). Due to the lack of studies on the EA in equids in the Nineveh Governorate, Therefore, this study was designed to determine the prevalence of EA in equids in Nineveh Governorate, using i-ELISA, and to record clinical, hematological, and some biochemical parameters linked with the EA.

Materials and Methods

Ethical approval: The University of Mosul, College of Veterinary Medicine's Animal Ethics Committee permits this work on August 20, 2023 (UM. VET. 2023. 088).

Sample size: The sample size was calculated in this study by relying on an epidemiological statistical equation based on the previous study on the molecular prevalence of equine anaplasmosis in Baghdad province, which was 13% (10), with a confidence level of 95% and an absolute error of 0.05, according to (43). For this study, a minimum of 173 equids were needed. However, 180 samples were collected.

Animals and sample obtained: A total of 180 equids were conducted in this study, comprising 106 horses and 74 donkeys of both sexes and different ages, including 25 clinically and laboratory-healthy equids that were considered as control group. All equids were obtained from different areas in Nineveh Governorate. During the period from October 2023 to August 2024, 180 blood samples (10 milliliters) were collected from equids via a jugular vein puncture using serial syringes, which were divided into two tubes (5ml for each), the first one with the anticoagulant EDTA- ethylene diamine tetraacetic acid- for the hematology parameters analysis and the second tube is without anticoagulant for serum separation using a centrifuge at a speed of 2500 cycles per minute for 10 minutes, after which the serum was kept in the refrigerator at -20°C until the indirect enzyme-linked immunosorbent assay (i-ELISA) and biochemical parameters analysis. Moreover, all equids were examined for internal and external parasites based on Zajac *et al*. (44), and the positives for these parasites were excluded from this study**.**

Indirect enzyme-linked immunosorbent assay (i-ELISA): This test was done as a conforming assay to identify the IgG antibodies against *A. phagocytophilum* in 180 serum samples using the Horse Ap-IgG ELISA kit (Shanghai Ideal Medical Technology Co., Ltd., China), according to manufacturer guidelines.

Hematology parameters analysis: A hematology analyzer (Genex-California, USA) was utilized to evaluate the total red blood cell counts (TRBCs), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet counts, total and differential white blood cell counts (TLCs and DLCs), as well as the erythrocyte sedimentation rate (ESR) according to (45).

Biochemical analysis: A chemistry analyzer (IDEXX-Vet Test, Arachem/USA) was used to estimate the aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, creatinine, blood urea nitrogen (BUN), total protein (TP), phosphorus and calcium, using special cassettes for each test and 40 microliters per serum sample to perform tests as instructed by the manufacturer.

Statistical analysis: The two-sided *X2* test in the IBM-SPSS Statistics (Version 22) software was utilized to ascertain the variation in the prevalence between the type of equids, a P value was deemed significant if it was less than 0.05. Additionally, a *t*-student test was used to find out the significant difference in hematological and biochemical parameters between infected equids and control equids using the same program as above.

Results

The present study observed that the total prevalence of EA in the equids in Nineveh Governorate was 46.1% (83/180) using i-ELISA. Furthermore, there was significantly higher (P<0.0000) among donkeys, which was 68.9% (51/74), compared to horses, which was 30.1% (32/106) (Figure 1). Additionally, almost all of the equids infected with EA had the acute form of the disease, which was characterized by fever, emaciation, pale mucous membranes in the eyes, petechial hemorrhages, and jaundice in certain cases. Other equids with the disease had respiratory disorders, nasal discharge, coughing, edema in the lower limbs, lameness, the appearance of nervous signs such as stiffness and/or ataxia during walking with recumbency, and ticks was present on various parts of their bodies, with different frequency and percentage (Table 1), (Figure 2). The results also indicate that significant increase (P<0.05) in the body temperature, respiratory rate, heart rate, and capillary refilling time in infected equids compared to control equids (Table 2).

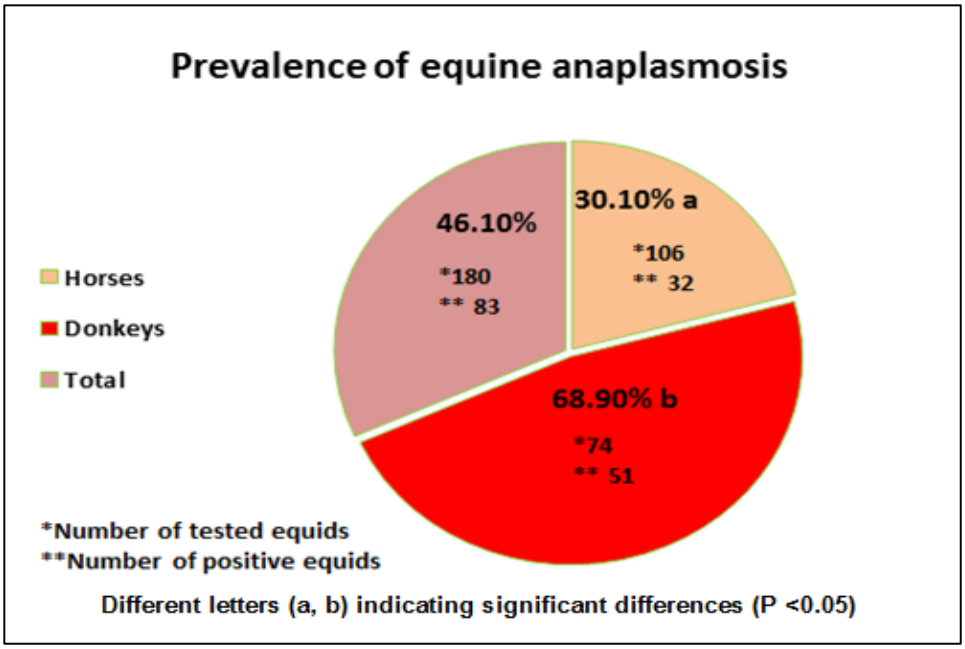

Figure 1: Prevalence of equine anaplasmosis in equids in Nineveh Governorate using Indirect ELISA.

Table 1: The frequency and percentages of the clinical signs in horses and donkeys infected with anaplasmosis (58 infected animals)

Clinical signs	Frequency	Percentage %
Fever	55	94.8
Loss of appetite	53	91.3
Emaciation	49	84.4
Paleness of the mucous membranes	29	50
Petechial haemorrhages	16	27.5
Jaundice	13	22.4
Nasal discharge and cough	18	31.0
Edema of the limbs	17	29.3
Stiffness walking	13	22.4
Recumbency	11	18.9
The presence of ticks on equids	10	17.2

Table 2: Clinical parameters in infected equids with anaplasmosis compared to control equids

Clinical parameters	Control equids (n=25) Mean ± Standard error	Infected equids (n=58) Mean ± Standard error
Body temperature rate (ºC)	37.62 ± 0.36 [a]	39.98 ± 0.80 [b]
Respiratory rate / min	17.36 ± 3.22 [a]	42.65 ± 1.76 [b]
Heart rate/min	48.75 ± 1.65 [a]	72.22 ± 4.53 [b]
capillary refilling time /sec	1.13 ± 0.11 [a]	3.82 ± 0.17 [b]

Different horizontally subscripted letters (a, b) indicate a significant difference (P <0.05).

In this study, the hemogram of the horses and donkeys infected with EA demonstrated a significant decrease (P<0.05) in TRBCs, Hb, and PCV, with a significant increase (P<0.05) in the MCV and a significant decrease (P<0.05) in the MCH and MCHC, reflecting a macrocytic hypochromic type of anemia, along with a significant increase (P<0.05) in the ESR compared to the control equids (Table 3). Moreover, there was a significant decrease (P<0.05) in PLT and TWBCs as a result of a significant decrease in neutrophils and lymphocytes compared to the control equids (Table 4).

In addition, the biochemical parameters analysis of the horses and donkeys infected with anaplasmosis showed a significant increase (P<0.05) in the AST, ALT, ALP, TB, creatinine, and BUN, with a significant decrease (P<0.05) in TP, phosphorus, and calcium compared to the control equids (Table 5).

Discussion

In the present study, the overall prevalence of EA in equids (horses and donkeys) in Nineveh Governorate was 46.1% using the i-ELISA, respectively. This result was near or higher than the prevalence recorded in previous studies in Iraq. (9) recorded that the prevalence of anaplasmosis in horses in Nineveh Governorate was 28.9% using microscopic examination of blood smears. Furthermore, in Baghdad Governorate, the prevalence of the disease in horses was 6.87% and 13.125% using microscopic examination and conventional PCR techniques, respectively (10). Also, (46) reported a prevalence of the EA in Duhok and Erbil Governorates in donkeys of 12% and 16% using the nested PCR technique, respectively.


A
B
C
D
E
F
G
H
I
J

Figure 2: Diseased equids shown different clinical signs: A&B; Emaciation and nasal discharge, C&D; Petechial hemorrhages in the third eyelid of the eye and paleness in the mucous membrane lining the upper lip, E&F; Jaundice of the mucous membrane lining the third eyelid and the lower lip, G&H; Edema in the lower limb and recumbency with stiffness in the hind legs, and I&J; presence of ticks on various parts of the body.

Table 3: Hematological parameters in equids infected with anaplasmosis compared to control equids.

Parameters	Control equids (n=25) Mean ± Standard error	Infected equids (n=58) Mean ± Standard error
TRBCs (10^{*6}/µL)	7.98 ± 0.60[a]	5.42 ± 0.30 [b]
Hb (g/dL)	12.11 ± 0.13 [a]	7.25 ± 0.34 [b]
PCV (%)	34.55 ± 0.75 [a]	22.17 ± 0.33 [b]
MCV (fl)	32.39 ± 0.49 [a]	36.22 ± 0.97 [b]
MCH (pg)	17.36 ± 3.22 [a]	13.95 ± 0.22 [b]
MCHC (g/dL)	37.86 ± 0.92 [a]	31.68 ± 0.30 [b]
PLT (10^{*3}/µL)	448.75 ± 19.28 [a]	266.60 ± 11.53 [b]
ESR (mm/20 min)	22.31 ± 1.79 [a]	58.42 ± 2.18 [b]

Different horizontally subscripted letters (a, b) indicate a significant difference (P <0.05).

Table 4: Total and differential white blood cells in equids infected with anaplasmosis compared to control equids.

Parameters	Control equids (n=25) Mean ± Standard error	Infected equids (n=58) Mean ± Standard error
TWBCs (10^{*3}/µL)	9.96 ± 0.40 [a]	6.22 ± 0.37 [b]
Lymphocytes (10^{*3}/µL)	4.21 ± 0.45 [a]	2.98 ± 0.25 [b]
%	(1.33 ± 43.62)	(0.22±32.66)
Neutrophils (10^{*3}/µL)	5.17 ± 0.33 [b]	3.25 ± 0.18 [b]
%	(0.76 ± 46.52)	(0.26 ± 35.72)
Mononuclear cells (10^{*3}/µL)	0.76 ± 0.16 [a]	0.72 ± 0.11[a]
%	(0.10 ± 5.43)	(0.14 ± 5.61)
Eosinophils (10^{*3}/µL)	0.42 ± 0.04 [a]	0.40 ± 0.05 [a]
%	(0.20 ± 3.37)	(0.10 ± 3.31)
basophils (10^{*3}/µL)	0.66 ± 0.37 [a]	0.64 ± 0.32 [a]
%	(0.10 ± 7.48)	(0.12 ± 7.51)

Table 5: Biochemical parameters in equids infected with anaplasmosis compared to control equids

Parameters	Control equids (n=25) Mean ± Standard error	Infected equids (n=58) Mean ± Standard error
AST (IU/L)	233.22 ± 7.47[a]	346.11 ± 9.50 [b]
ALT (IU/L)	19.98 ± 0.51[a]	37.32 ± 0.77 [b]
ALP (IU/L)	139.31 ± 6.16[a]	222.13 ± 8.12 [b]
TB (mg/dL)	1.80 ± 0.45[a]	2.93± 0.10 [b]
Creatinine (mg/dL)	1.59 ±0.10[a]	0.57 ±0.05 [b]
BUN (mg/dL)	20.35 ± 0.62[a]	42.18 ± 1.83 [b]
TP (g/dL)	7.29± 0.05[a]	4.22± 0.01 [b]
Phosphorus (mg/dL)	6.55± 0.23[a]	3.63 ±0.011 [b]
Calcium (mg/dL)	12.16± 0.28[a]	7.09± 0.42 [b]

Different horizontally subscripted letters (a, b) indicate a significant difference (P <0.05).

Moreover, there are numerous studies that have reported the prevalence of EA in equids in different countries, including Turkey, where it was 8.6% and 6.4% in horses using i-ELISA and multiplex-PCR technique, respectively (21), in Pakistan, the prevalence of disease in horses, donkeys, and mules was 11.86%, 9.43% and 10.53% respectively, using the quantitive-PCR technique (8), in South Korea, it was 0.2% in horses using the nested-PCR technique (47), in Algeria, 19.5% and 25.9% in the horses using IFAT and i-ELISA, respectively (26), in Italian horses, it was 51% using the indirect fluorescent antibody test (IFAT) (24), and in Germany, it was 15.2% in the horses using real time-PCR (41). The prevalence of EA in equids varies from country to country, as a result of management practices, differences in the sensitivity and accuracy of diagnostic tests used, sample size, the presence of ticks on equids and in stables, the susceptibility of equids, seasonal variations and climatic conditions, as well as the vector control program, which differ between countries (8,10,23, 47,48).

The current study showed that the prevalence of EA was significantly higher in donkeys compared to infected horses. This result agreed with (29) who indicated that the prevalence of the EA was significantly higher in donkeys compared to mules. This may be due to the fact that most donkeys are used for work, which exposes them to constant stress, decreased immunity, and exposure to ticks more than mules and horses. On the contrary, (46) indicated that there is no significant difference in the prevalence of the EA among mules, donkeys, and horses.

In this study, infected equids with anaplasmosis were suffering from an acute form of the disease, with different clinical manifestations.

These findings were consistent with (9, 32,48,49,50), The rise in body temperature of infected equids with anaplasmosis may result from the liberation of pyrogens from distracted infected WBCs, and phagocytes, which stimulate the thermoregulation center in the hypothalamus of the brain, and may also be due to the release of the inflammatory cytokines (gIL-1α, IL-1β, IL-6 and TNF-α) of infected WBCs (51,52). Furthermore, some equids infected with anaplasmosis showed paleness of the mucous membranes lining the conjunctiva of the eye and gums; the reason may be attributed to the development of anemia as a result of a decrease in the TRBCs, Hb concentration, and WBCs (15,52). Other infected equids showed petechial hemorrhage on the third eyelid, probably due to thrombosis in the small capillary blood vessels as a result of the thrombocytopenia (37). Respiratory disorders can also be observed in infected equids. This may be due to secondary bacterial infections in the lung as a result of animal immunosuppression (15,36).

Moreover, the edema in the lower limbs with lameness may result from inflammation of the arteries and small veins as a result of the adhesion of the *A. phagocytophilum* in the cells lining of them, which leads to the release of chemical mediators, which increases their escaping of fluid around the tissues, and the lameness may result from damage to the nerves in the distal extremities (50). The presence of ticks on the different body parts of the infected equids indicates that they are the main vectors of the disease (9,15). Most equids affected by anaplasmosis observed an increase in respiratory rate, heart rate, and capillary filling time. The reason may be attributed to the decrease in TRBCs and Hb concentration, which leads to hypoxic anemia, which occurs as a compensatory mechanism (15,53).

The results of the study observed that equids infected with anaplasmosis, significantly decreased TRBCs, Hb concentration and PCV, which lead to anemia. This agrees with (9,54). There are several causes of anemia in the cases of EA, including the production of inflammatory mediators, which can inhibit the production of red blood cells (erythropoiesis) and shorten their lifespan; bone marrow depletion and suppression; and the release of interleukin-6, which leads to iron sequestration to an amount that is not enough to form reticulocytes; and the formation of abnormal RBC shapes such as piokilocytosis, acanthocytes, schistocytes, hypochromasia, and keratocytes (8,9,54,55). Furthermore, in this study, the type of anemia was macrocytic hypochromic anemia due to the significant increase in the MCV and the significant decrease in the MCH and MCHC. On the

contrary, (46,48,53) indicated that the type of anemia in the case of EA was normocytic normochromic anemia. Infected equids also exhibit thrombocytopenia and leukopenia. A similar finding was reported by (25,56,57).

This study observed alterations in some biochemical parameters in equids infected with anaplasmosis**,** including an increase in the AST, ALT, ALK, TB, BUN, and creatinine while decreasing in the TP, calcium, and phosphorus. These findings agree with (20,54, 58), The reasons for the increase in AST, ALT, ALK, and TB may be attributed to a decrease in blood flow for the liver, which leads to degenerations of the central lobule in the liver cells, liver cell damage, systemic infections, and muscle damage (59). Furthermore, the increase in ALK may be due to chronic renal failure, insufficient diet, and hyperactivity of the parathyroid gland (37). The increase in the level of BUN might be due to the effect of bacteria on kidney function (60,61). Other studies referred to the reasons for the decrease in the level of TP in the cases infected with equine anaplasmosis as the continuation of immunosuppression, the inability to increase the immune response, and the decrease of albumin and globulin in the blood, which may be caused by a loss of animal appetite, a lack of protein production due to liver tissue damage, or poor digestion and absorption of proteins**,** in addition to high body temperature resulting in protein destruction (20,54,59). Moreover, the decrease in the level of calcium and phosphorus in this study is attributed to animal starvation, poor digestion and absorption, and their depletion from the liver (62). On the other hand, other studies demonstrated that there were no significant changes in the level of the above-mentioned biochemical parameters in the infected equids. The reason may be due to the difference in the virulence of *A. phagocytophilum* or the sample size of the study animals (48,63,64).

Conclusion

It has been concluded that EA is widespread and still circulating in all animals environment in Nineveh Governorate, Iraq, Therefore, early detection of the diseased is more important as well as the advice for the application of control measures. Moreover, the disease is accompanied by clinical, hematological, and some biochemical parameter alterations in equids infected with the disease.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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أنابلازموسز الفصيلة الخيلية في محافظة نينوى: التغيرات السريرية والدموية والكيموحيوية في الفصيلة الخيلية

أحمد ألياس الجواري، قيس طالب العبيدي.

فرع الطب الباطني والوقائي- كلية الطب البيطري - جامعة الموصل، العراق.

الخلاصة

تهدف الدراسة الحالية إلى تحديد نسبة انتشار مرض الأنابلازما في الفصيلة الخيلية في محافظة نينوى، وذلك باستخدام اختبار الممتز المناعي غير المباشر. تم جمع 180 عينة دم تم جمعها عشوائياً من االفصيلة الخيلية (106 خيول و 74 حمير) في محافظة نينوى، العراق، ومن ضمنها 25 من الفصيلة الخيلية السليمة سريرياً ومختبريا عدت حيوانات سيطرة. تم إجراء جميع الفحوصات السريرية على جميع الخيليات، ثم تم جمع عينات الدم من جميع الخيليات وتم اجراء فحوصات المعايير الدموية وبعض المعايير الكيموحيوية. أشارت النتائج إلى أن نسبة الانتشار الكلية لمرض انابلازموسز الفصيلة الخيلية في محافظة نينوى 46.1% (180/83)، مثلت النسبة في الخيول 30.1% (106/32) وفي الحمير 68.9% (74/51) باستخدام اختبار الممتز المناعي غير المباشرة. وعانت الخيليات المصابة من الشكل الحاد من المرض مع الارتفاع المعنوي في درجة حرارة الجسم، ومعدل التنفس، ومعدل ضربات القلب، ووقت إعادة ملئ الشعيرات الدموية في الخيليات المصابة مقارنة بحيوانات السيطرة. أظهر تحليل الصورة الدموية انخفاضًا معنويا في العدد الكلي لكريات الدم الحمر وتركيز خضاب الدم وحجم خلايا الدم المرصوصة ومعدل خضاب الدم الكروي ومعدل تركيز خضاب الدم الكروي، مع الارتفاع المعنوي في معدل حجم الخلايا الكروي، وكان فقر الدم من النوع ذي الكريات كبير الحجم قليل الصباغ، مع الارتفاع المعنوي في معدل ترسيب كريات الدم الحمر. فضلا عن ذلك، لوحظ الانخفاض المعنوي في اعداد الصفائح الدموية والعدد الكلي لخلايا الدم البيض نتيجة للانخفاض المعنوي في الخلايا العدلة والخلايا اللمفية في الخيليات المصابة مقارنة بحيوانات السيطرة. كما أظهر التحليل الكيموحيوي وجود ارتفاع معنوية في خميرة الاسبارتيت ناقلة الامين وخميرة الالنين ناقلة الامين والفوسفات القاعدي والصفراوين الكلي ويوريا نيتروجين الدم والكرياتنين، مع الانخفاض المعنوي في البروتين الكلي والكالسيوم والفسفور في الخيليات المصابة مقارنة بحيوان السيطرة. واستنتج، ان مرض انابلازموسز الفصيلة الخيلية واسع الانتشار في محافظة نينوى، العراق مع وجود تغيرات معنوية في المعايير السريرية والدمية والكيموحيوية.

كلمات المفتاحية: أنابلازموسز الفصيلة الخيلية، الخيليات، اختبار الممتز المناعي غير المباشر، التحليل الدمي، التحليل الكيموحيوي.