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Prevalence and Diagnostic Evaluation of Equine Anaplasmosis in Nineveh Governorate, Iraq

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Abstract

THE PRESENT study was aimed to determine the prevalence of equine anaplasmosis (EA) in equids in Nineveh governorate, using the blood smears examination (BSE) method and indirect enzyme linked immunosorbent assay (i-ELISA), to assess the effectiveness of the two diagnostic techniques, and to investigate some of the risk factors linked with the seroprevalence of EA. From October 2023 to June 2024, a total of 180 blood samples were collected from equids (106 horses and 74 donkeys) of different sexes and ages in Nineveh governorate, Iraq. The epidemiological data were collected through samples collection. Blood smears were made for the detection of Morulae inside white blood cells and serum was used to detect antibodies against *Anaplasma phagocytophilum* using i-ELISA. Results showed that the overall prevalence of EA in equids in Nineveh governorate was 25.5% and 46.1% using BSE and i-ELISA, respectively. A moderate agreement was shown between BSE and i-ELISA according to the Kappa value (0.434), with 86.9%, 67.9%, and 72.7% sensitivity, specificity, and accuracy of i-ELISA. Based on BSE, *A. phagocytophilum* parasitemia was significantly higher in neutrophils and lymphocytes. Moreover, based on i-ELISA, ~~a~~ significant of the risk factors were linked with higher prevalence of the disease, including: donkey type equids, 2years - more than 5 years animals, females, imported horses, pregnant females, infected equids, out of stables animals, presence of ticks on animals and in stables, left coast regions, and the spring and autumn seasons. In conclusion, this study indicates that equine anaplasmosis is widespread in Nineveh governorate, Iraq.

Keywords: Equine anaplasmosis, Blood smears, Indirect-ELISA, Nineveh-Iraq.

Introduction

Equine anaplasmosis (EA) is a one of the emerging and important infectious blood diseases [1]. It is called equine granulocytic anaplasmosis (EGA), previously named equine granulocytic ehrlichiosis (EGE) [2], which was first recorded in California, USA [3]. The EA causes major economic losses as a result of the high mortality rate reaching 5% and the cost of treatment of the infected cases, as well as the control of the vectors that transmit the disease [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]. *Anaplasma phagocytophilum* is one of the obligate intracellular bacteria that infects white blood cells, especially neutrophils [7], lymphocytes, monocytes, basophils, and eosinophils [8, 9]. This bacteria infects various types of animals, including equids [10], sheep, goats, and cows [11, 12], dogs and cats [13, 14], camels and deer [15, 16], as well as humans [17]. The EA mainly transmitted mechanically by hard ticks under the genera of Rhipicephalus, Dermacentor, and Ixodes [9,18], and through contaminated instruments such as syringes and cannulas with the bacteria [15], as well as via blood-sucking insects from the Tabanidae family [19]. It can also be transmitted from mares to fetus by transplacental transmission [20].

The disease is endemic in 39 countries of the world [21], such as in Asia, Africa, Europe, and America [22-26], however, the disease was recorded

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for the first time in horses in 2018 in Nineveh Governorate, Iraq [9].

In addition, there are several risk factors associated with the prevalence of the EA in equids, such as genders, ages, breeds, health condition, pregnancy, management, regions, and seasons [8, 27]. There are different clinical forms of the disease, including acute, chronic, and subclinical forms [15]. The acute form of the disease is mainly characterized by a fever 39-40°C, paleness of mucous membranes, petechial hemorrhage in the third eyelids, anemia, respiratory disturbance, edema in the lower limbs, and nervous signs [28-30].

The clinical signs of the EA are rarely helpful in diagnosing the disease in equids [27], because they are confused with other blood diseases in equids such as babesiosis [31], theileriosis [32], and trypanosomiasis [33]. Therefore, laboratory tests are needed to confirm the diagnosis of the disease, including microscopic examination of the blood smears stained by Giemsa [6]. As well as performing serological tests, such as indirect enzyme-linked immunosorbent assay, and the indirect fluorescent antibody test (IFAT) [24], and can also be using molecular techniques such as conventional-PCR [34], nested-PCR [23], and real-time PCR [32]. Due to the lack of studies on the prevalence of 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 BSE method and i-ELISA, to assess the effectiveness of these two diagnostic techniques, and to investigate some of the risk factors linked with the seroprevalence of EA.

Material and Methods

Sample size

The calculation of the sample size of this study was determined 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 [35].

$$n \quad \frac{z^2 p\,(1-p)}{d^2}$$

where: n = number of sampled animals, Z = value of the normal distribution for a 5% confidence level, P = expected prevalence, and d

For this study, a minimum of 173 equids were needed. However, 180 samples were collected.

Animals and samples collection

This work was conducted on 180 equids (horses and donkeys), with different sexes, ages, origins, management practices, regions, and seasons. During the period from October 2023 to June 2024, a total of 180 blood samples (10 millilitres) were collected from equids via jugular vein using serial syringes, which were divided in two tubes (5ml for each), the first one with anticoagulant ethylene diamine tetraacetic acid (EDTA) for blood smear examination to primary detection of *A. phagocytophilum* in white blood cells using a light microscope [9]. The second tube is without anticoagulant for serum separation to detect antibodies against *A. phagocytophilum* using i-ELISA [23].

Blood smears examination (BSE)

Three-hundred sixty thick and thin blood smears were prepared from blood with EDTA, stained with ready Giemsa stain (AFCO- Jordan) for five minutes, and then examined under oil immersion (X100 lens) of the light microscope for primary detection of the *A. phagocytophilum* morulae inside the cytoplasm of different types of white blood cells. The parasitemia

$$\text{Parasitemia} = \frac{\text{Number of infected WBCs}}{\text{Number of calculated WBCs (500 cells)}} \text{X100}$$

was calculated according to the following equation [9, 12]:

Indirect enzyme linked immunosorbent assay (i-ELISA)

This test was a performed as 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.

Comparison between two techniques used in this study

According to the Kappa value, the agreement between the ME and i-ELISA was recorded. The results indicate that there is no agreement between the two methods; when the Kappa value is less than 0.00; low agreement occurs; when the Kappa value is between 0.0 and 0.20; fair agreement occurs when the Kappa value is between 0.21 and 0.40; moderate agreement occurs when the Kappa value is between 0.41 and 0.60; substantial agreement occurs when the Kappa value is between 0.61 and 0.80; and almost perfect agreement occurs when the Kappa value is between 0.81 and 1 [36]. Moreover, the ME method's accuracy, sensitivity, and specificity were computed and compared to the N-PCR technique [37, 38].

Statistical analysis

The two-sided *X2*- test and Fischer's exact tests in the IBM-SPSS Statistics (Version 22) Software were utilized to ascertain the variation in the prevalence of the individual risk variables. If the expected cell value in the Chi-square test is less than 5, it is recommended by Fisher exact test P

value results to use factors with P values < 0.05, which are considered significant. Additionally, the odds ratio (OR) and 95% confidence interval for the connection between risk variables for FPV were computed using 2 X 2 tables in the Epi-InfoTM 7 software (Version 7).

Results

The present study observed that the overall prevalence of equine anaplasmosis in the equids (horses and donkeys) in Nineveh Governorate was 25.5% (46/180) and 46.1% (83/180) using thick and thin BSE by light microscopic and i-ELISA, respectively (Table 1). Furthermore, there was a moderate agreement between the BSE method and the i-ELISA in diagnosing equine anaplasmosis based on the Kappa value, which was 0.434, with the sensitivity 86.9%, specificity 67.9%, and accuracy 72.7% of the i-ELISA compared to the BSE method (Table 2).

The initial results of thin and thick blood smears examination using a light microscope showed the appearance of inclusion bodies (Morulae) of *A. phagocytophilum* bacteria in all types of white blood cells (neutrophils, lymphocytes, mononuclear cells, basophils, and eosinophils), often individually, and sometimes the presence of more than one Morulae in the cytoplasm for the same mentioned cells (Fig. 1). Moreover, the total parasitemia of *A. phagocytophilum* in white blood cells in blood smears was calculated, which ranged between 3 - 32%, with a mean of 16%. It included the highest significant parasitemia (P<0.05) in neutrophil cells, which ranged between 10 - 65%, with a mean of 35%., followed by lymphocytes ranging between 8-60%, with a mean of 30%, compared to mononuclear cells, basophils, and eosinophils, in which the parasitism did not show a significant change among them (Table 3).

Results according to the i-ELISA observed that the prevalence of EA was significantly higher (P<0.0000) among donkeys' type 68.9% (OR: 5.12 times, CI: 2.69-9.76), compared to horses type 30.1% (Table 4). The prevalence of EA was significantly (P< 0.0381 and 0.0021) higher in 2 years and > 5 years old equids 41.6 % (OR: 5.00 times, CI: 1.02-24.46) and 54.8% (OR: 5.00 times, CI: 1.81-39.87) compared to < 1 year and 2 years old 12.5% and 35.2% respectively (Table 4). Regarding the gender factor, the prevalence of EA was significantly (P< 0.0000) higher among female equids 64.7% (OR: 4.66times, CI: 2.48-8.76) compared to male equids 28.2% (Table 4). The study also revealed that the prevalence of EA was significantly (P<0.0001) higher in imported horses 66.6% (OR: 7.44 times, CI: 2.61-21.18), compared to local horses 21.1% (Table 4). The prevalence was also significantly (P< 0.0166) higher among pregnant mares 93.7% (OR: 10.71, CI: 1.34-85.57), compared to non-pregnant mares 58.3%. In this study, the prevalence of EA was significantly (P<0.0000) higher in clinically infected equids 66.6% (OR: 5.44 times, CI: 2.87-10.31), compared to clinically healthy equids 26.8% (Table 4). Moreover**,** the prevalence of EA was significantly (P<0.0000) higher among equids in grazing 75.3% (OR: 7.89 times, CI: 3.97-15.68), compared to equids in stable 27.9% (Table 4). The prevalence of EA was significantly (P<0.0001) higher in equids infested with ticks and when ticks were found in the stables 81.4**%** (OR: 6.63 times, CI: 3.97-15.68), compared to equids non- infested with ticks and when ticks were non-found in the stables 39.8% (Table 4).

In addition, the prevalence of the EA was significantly higher (P<0.0094), (P<0.0239) in the left coast of Nineveh regions 61.4% (OR: 2.38 times, CI: 1.27-4.46), compared to the right coast of Nineveh regions 36.3% (Table 5). The present study also showed that the prevalence of the EA disease was significantly (P< 0.0145 and 0.0003) higher in the spring and autumn months was 67.5% (OR: 4.88, CI: 2.09-11.09) and 49.3% (OR: 2.28, CI: 1.14-4.58) respectively, compared to the winter months 43.4% (Table 6).

Discussion

In the present study, the overall prevalence of EA in equids (horses and donkeys) in Nineveh Governorate was 25.5% and 46.1% using the ME of blood smears and the i-ELISA, respectively. This result was near or higher than the prevalence recorded in previous studies in Iraq. Albadrani and Al-Iraqi, [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, Mohand and Saleem, [39] reported a prevalence of the EA in Duhok and Erbil Governorates in donkeys of 12% and 16% using the nested PCR technique, respectively. There are numerous studies that have reported the prevalence of EA in equids in different countries, including Turkey, it was 8.6% and 6.4% in horses using i-ELISA and multiplex-PCR technique, respectively [23], 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 [40], in Italian horses, it was 51% using the indirect fluorescent antibody test (IFAT) [25], in Germany, it was 15.2% in the horses using real time-PCR (41), and in Algeria, 19.5% and

25.9% in the horses using IFAT and i-ELISA, respectively [24]. The prevalence of equine anaplasmosis 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, presence of ticks on equids and in stables, seasonal variations and climatic conditions, as well as vector control program differ between countries [8,10, 23, 40, 42].

This study also showed a moderate agreement between the ME of the blood smears and the i-ELISA in the diagnosis of equine anaplasmosis in horses and donkeys, based on the Kappa value, with moderate sensitivity, specificity, and accuracy of the i-ELISA compared to the ME method. This indicates that the ME of the blood smears and i-ELISA are suitable for diagnosing the disease in equids. These results agree with Albadrani and Al-Iraqi, [9] and Alani and Yousif, [10] who stated that the ME of the blood smears is an easy, fast, and cheap field test and can be used to diagnose acute cases of disease. However, this test is low-sensitive and rarely produces real results in chronic, subclinical, and latent infection, with very low parasitemia, so its results must be confirmed using more sensitive and accurate techniques such as serological tests and molecular techniques [8]. Furthermore, Laamari *et al*. [24] indicated the efficiency of the i-ELISA through sensitivity, specificity, and high accuracy in diagnosing equine anaplasmosis compared to the ME method. Oğuz, [23] added that the use of i-ELISA is one of the most sensitive tests in the detection of the antibodies for *A. phagocytophilum* in horses. On the other hand, Schvartz *et al*. [43] mentioned that the antibodies against *A. phagocytophilium* appear only after 19 days of infection, which explains the false negative results in the i-ELISA. There is also no distinction between acute infections and previous exposures [26, 44].

Results according to ME of Giemsa-stained blood smears observed the morulae of *A. phagocytophilum* in the different types of white blood cells, often individually, and sometimes the presence of more than one morulae in the cytoplasm of cells, with the significant highest percentage of parasitemia in neutrophils, followed by lymphocytes. This finding is consistent with Albadrani and Al-Iraqi, [9], Alani and Yousif, [10] and Mohand and Saleem, [39] who noted *A. phagocytophilum* inside some types of white blood cells, including neutrophils, lymphocytes, eosinophils, and monocytes.

The current study showed that the prevalence of EA was significantly higher in donkeys compared to infected horses. This result agreed with [45] 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 the ticks more than mules and horses. On the contrary, Mohand and Saleem, [39] indicated that there is no significant difference in the prevalence of the EA among mules, donkeys, and horses.

The study also demonstrated that the prevalence of EA was significantly higher in female equids compared to male equids. These results are consistent with Hinson *et al*. [26], the reasons may be attributed to physiological factors such as estrus, pregnancy, and lactation period in females, which are considered stress factors and reduction the animal immunity [46, 47]. While, Laamari *et al*. [24] and Drážovská *et al*. [48] indicated that there was no effect of sex on the prevalence of EA in horses. Additionally, results showed that the prevalence of EA was significantly higher in pregnant mares compared to non-pregnant mares. Similar result observed by (46).

Moreover, results showed that the prevalence of EA increased with the age of the equids. This result agreed with Laamari *et al.* [23], Oğuz, [24], and Alani and Yousif, (2023b) [42]. The reason may be due to the fact that foals after birth are less susceptible to the disease because they receive temporary immunity from their mares through colostrum, which contains antibodies, and thus will protect them from the disease, in the first months after birth [20]. On the other hand, Drážovská *et al*. [48] stated that there were no significant differences in the prevalence of EA between the different ages of the equids.

The study also found that the prevalence of EA was significantly higher in imported horses compared to local horses. This result was identical to that recorded by Lee *et al*. [44]. The reason may be that some of the horses in this study were imported from different countries where the disease is endemic or due to bad management practices such as overcrowding in stables, which leads to an increase and rapid spread of the disease [8, 23, 49]. In addition, the prevalence of the disease was significantly higher in equids outside stables than in equids inside stables. This result is consistent with Saleem *et al*. [8]. The reason is due to the fact that animals inside stables receive better care compared to draft horses and race horses, which are more exposed to ticks, stress, and low immunity [50].

The present study showed that the prevalence of EA was significantly higher in animals infested with ticks compared to those not infested with ticks. This result was consistent with Costa *et al.* [50] and Alali *et al*. [51]. The reason may be attributed to the fact that ticks are the main vector of this disease [9, 52].

The study also showed that the prevalence of EA was significantly higher in the spring and autumn months compared to the winter months, these results were in agreement with Alani and Yousif, [10] and Razzaq *et al*. [53]. The reason might be due to the increase in weather temperature during that period, which led to an increase in the spread of ticks that transmit the disease [44, 54].

Conclusion

This study concludes that EA is widely distributed in equids in Nineveh Governorate, Iraq. The two techniques used in this study are efficient for detecting the disease based on the moderate agreement between them, and the risk factors associated with the high prevalence of the disease.

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Conflict of Interest

The author declares that there is no conflict of interest.

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).

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TABLE 1. Prevalence of Equine anaplasmosis in horses and donkeys at Nineveh Governorate using BSE method and i-ELISA

Type of technique	No. of tested equids	No. of positive equids	Prevalence %
BSE method	180	46	25.5
i-ELISA		83	46.1

TABLE 2. Agreement between BSE method and i-ELISA according to kappa value, with the calculating the ratio of the i-ELISA sensitivity, specificity, and accuracy for Equine anaplasmosis diagnosis

		BSE method		
		Infected	**Uninfected**	**Total No.**
i-ELISA	Infected	40 a	43 b	83
	Uninfected	6 c	91 d	97
Total		46	134	180

(a)True positive samples, (b) False positive samples, (c) False negative samples,(d) True negative samples. Kappa value was (0.434). Sensitivity = a/(a+c)x 100= 86.9%. Specificity= d/(b+d)x 100= 67.9%, Accuracy=(a+d)/(a+c+b+d) = 72.7%.

TABLE 3. Parasitemia of *A. phagocytophilum* -Morulae in total and different white blood cells in blood smears of infected equids (Number of calculated cells = 500)

Cells type	Parasitemia
	Range% (Mean ± Standard Error)
White blood cells	3-32% (16 ± 1.18)
Neutrophil cells	10-65% (35 ± 2.34)^{a}
Lymphocyte cells	8-60% (30 ± 1.55)^{a}
Monocyte cells	2-6% (6 ± 0.39)^{b}
Basophile cells	0-5% (4 ± 0.48)^{b}
Eosinophil cells	0-5% (4 ± 0.22)^{b}

Superscript different letters (a, b) indicating significant differences (P <0.05)

TABLE 4. Odds ratio of equids risk factors associated with prevalence of equine anaplasmosis based on indirect enzyme linked immunosorbent assay

Category	No. of tested equids	Indirect enzyme linked immunosorbent assay			
		No. of positive equids (%)	Odds Ratio	95% Confidence intervals	P value
Type of equids					
Horses	106	32 (30.1)[a]	1		
Donkeys	74	51 (68.9)[b]	5.12	2.69-9.76	0.0000
Ages					
< 1 year	16	2 (12.5)[a]	1		
1 - 2 years	34	16 (35.2)[a]	6.22	1.22-31.67	0.0263
2 - 5 years	48	20 (41.6)[b]	5.00	1.02-24.46	0.0381
> 5 years	82	45 (54.8)[b]	8.51	1.81-39.87	0.0021
Gender					
Males	92	26 (28.2)[a]	1		
Females	88	57 (64.7)[b]	4.66	2.48-8.76	0.0000
Origin					
Local	85	18 (21.1)[a]	1		
Imported	21	14 (66.6)[b]	7.44	2.61-21.18	0.0001
Pregnancy					
Non-pregnant	72	11 (58.3)[a]	1		
Pregnant	16	15 (93.7)[b]	10.71	1.34-85.57	0.0166
Clinical status					
Clinically healthy	93	25 (26.8)[a]	1		
Clinically infected	87	58 (66.6)[b]	5.44	2.87-10.31	0.0000
Management					
In stable	111	31 (27.9)[a]	1		
In grazing	69	52 (75.3)[a]	7.89	3.97-15.68	0.0000
Presence of ticks on equids					
No	153	61 (39.8)[a]	1		
Yes	27	22 (81.4)[b]	6.63	3.97-15.68	0.0001
Ticks found in stables					
No	52*/153	61 (39.8)[a]	1		
Yes	18*/27	22 (81.4)[b]	16.36	3.97-15.68	0.0001

Superscript different letters (a, b) indicating significant differences (P <0.05

TABLE 5 Odds ratio of regions risk factors associated with prevalence of equine anaplasmosis based on indirect enzyme linked immunosorbent assay

Seasons	Indirect enzyme linked immunosorbent assay				
	No. of tested equids	No. of positive equids (%)	Odds Ratio	95% Confidence intervals	P Value
Right coast of Nineveh	110	40 (36.3)[a]	1		
Left coast of Nineveh	70	43 (61.4)[b]	2.38	1.27-4.46	0.0094

Superscript different letters (a, b) indicating significant differences (P <0.05)

TABLE 6. Odds ratio of season's risk factors associated with prevalence equine anaplasmosis based on indirect enzyme linked immunosorbent assay

Seasons	Indirect enzyme linked immunosorbent assay				
	No. of tested equids	No. of positive equids (%)	Odds Ratio	95% Confidence intervals	P Value
Winter 2024 (December, January & February)	67	20 (29.8)[a]	1		
Autumn 2023 (October & November)	73	36 (49.3)[b]	2.28	1.14-4.58	0.0145
Spring 2024 (March, April & May)	40	27 (67.5)[b]	4.88	2.09-11.09	0.0003

Superscript different letters (a, b) indicating significant differences (P <0.05)

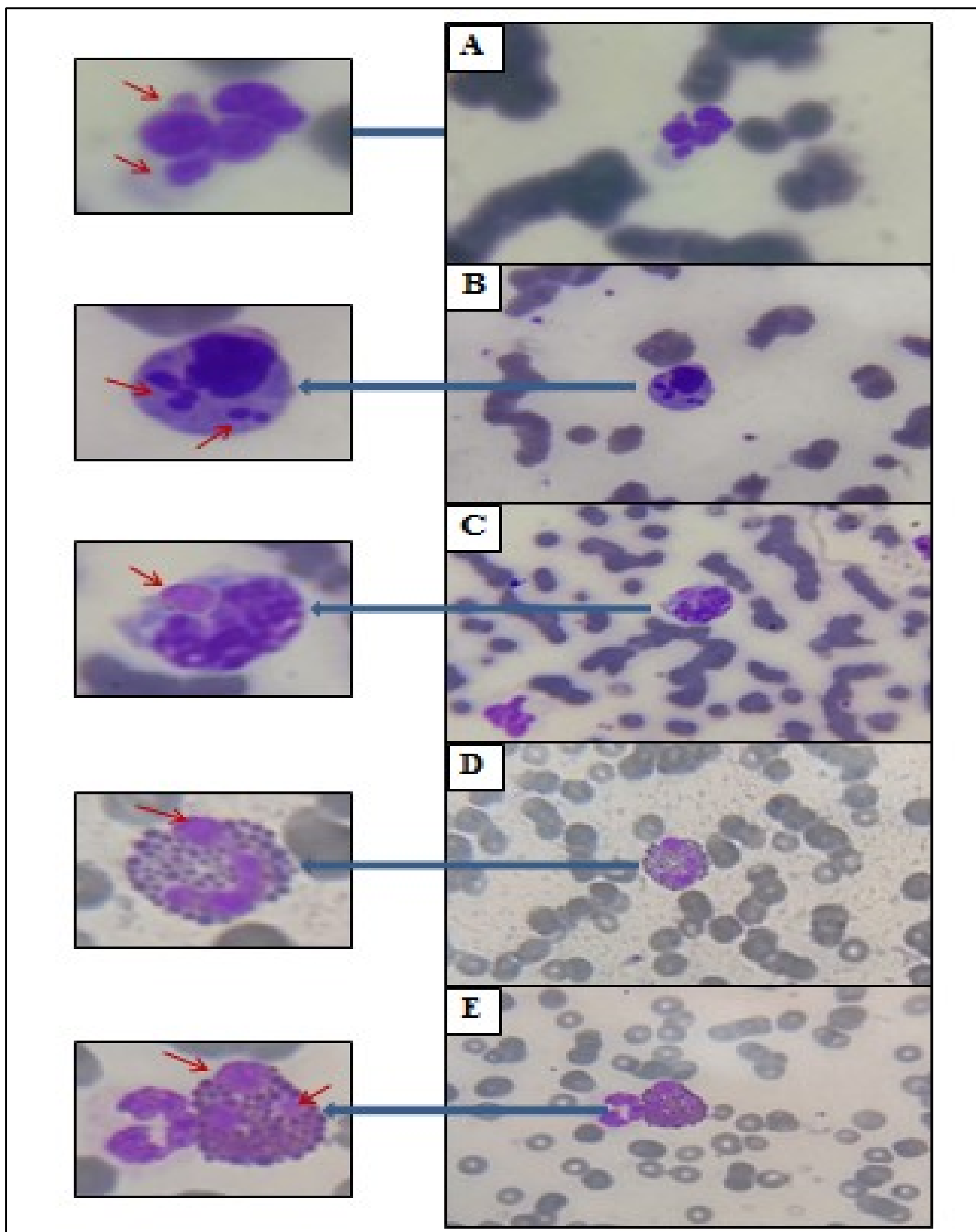

Fig. 1. Blood smears stained with ready Giemsa stain showed inclusion bodies (Morulae) of *A. phagocytophilum*: A- Inside horse neutrophil; B- Inside donkey lymphocyte; C- Inside donkey monocyte and; D- Inside horse basophil; E- Inside horse eosinophil, examined using a light microscope under oil immersion at (1000X).

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أنتشار وتقييم تشخيص داء الأنابلازما في الفصيلة الخيلية في محافظة نينوى، العراق

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الملخص

تهدف الدراسة الحالية إلى تحديد نسبة انتشار مرض الأنابلازما في الفصيلة الخيلية (الخيول و الحمير) في محافظة نينوى، وذلك باستخدام فحص المسحات الدمية واختبار الممتز المناعي غير المباشر ، وتقييم كفاءة التقنيات التشخيصية المختلفة المستخدمة في هذه الدراسة، والتحري عن بعض عوامل الخطورة المرتبطة بالانتشار المصلي للانابلازما في الفصيلة الخيلية. من الفترة الممتدة من تشرين الأول 2023 إلى آب 2024، تم جمع 180 عينة دم من الفصيلة الخيلية شملت (106 خيول و 74 حمير) من مختلف الأجناس والأعمار في محافظة نينوى، العراق. وتم أخذ المعلومات الوبائية خلال جمع العينات. كما تم عمل المسحات الدموية للكشف عن جراثيم الانابلازما داخل خلايا الدم البيضاء واستخدام مصل الدم للكشف عن الأجسام المضادة ضد للانابلازما في الفصيلة الخيلية باستخدام تقنية الممتز المناعي غير المباشر. أظهرت النتائج ان نسبة الانتشار الكلية لمرض الانابلازما في الفصيلة الخيلية في محافظة نينوى بلغت 25.5% و46.1% باستخدام فحص المسحات الدموية واختبار الممتز المناعي غير المباشر على التوالي. كما لوحظ وجود توافق معتدل بين الفحص المجهري للمسحات الدموية واختبار الممتز المناعي غير المباشر بالاعتماد على قيمة كابا والتي بلغت ((0.434، وبنسبة حساسية وخصوصية ودقة لاختبار الممتز المناعي الغير المباشر بلغت 86.9%و 67.9% و72.7% على التوالي . اعتمادا على فحص المسحات الدموية الرقيقة والسميكة، تم الكشف عن جراثيم الانابلازما في الفصيلة الخيلية وكانت نسبة التطفل اعلى معنويا في الخلايا العدلة والخلايا اللمفية. واعتمادا على اختبار الممتز المناعي غير المباشر لوحظ وجود عوامل خطورة معنوية مرتبطة بارتفاع نسبة انتشار المرض شملت: الفصيلة الخيلية نوع الحمير والحيوانات التي عمرها من سنتين إلى أكثر من 5 سنوات والإناث، والخيول المستوردة والإناث الحوامل والحيوانات المصابة والحيوانات خارج الإسطبلات ووجود القراد على الحيوانات وفي الاسطبلات ومناطق الساحل الأيسر وفي فصلي الربيع والخريف. في الختام تشير هذه الدراسة إلى أن مرض الأنابلازما في الفصيلة الخيلية واسع الانتشار في محافظة نينوى، العراق.

الكلمات الدالة: انابلازما الخيول- مسحات الدم- الاليزا الغير مباشرة-