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FIRST IDENTIFICATION *OF ANAPLASMA PLATYS* AND *ANAPLASMA PHAGOCYTOPHLIUM* IN THE BLOOD OF DOGS IN BAGHDAD GOVERNORATE

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Abstract

150 blood samples were collected from dogs in hospital Sahat Adan in Bagdad, Iraq. The blood samples were examined for staining with Giemsa stain and clinical signs and a hematological diagram were used for the diagnosis of infection. The results of this study revealed significant differences between temperature, pulse rats and the respiratory rats of infected dogs :other accompanied clinical signs showed significant Anorexia, diarrhea, loss body weight, and vomiting , The hematological diagram displayed significant differences in infected dogs as compared to non-infected the total red blood cell count, Hemoglobin concentration, platelet count, total white blood cell count, lymphocytes and neutrophil were significant decreased (p<0.05), while monocytes, eosinophil and basophils showed non-significant changes. the result of Blood smear staining was prepared and examined microscopically for the presence of morula and revealed 9/150 (6%) as the total infection rate, as were 4/12 (44.44%) *A. platys* and 5/12 (55.56%) *A. phagocytophlium.*
Keywords: *A.platys-A.phagocytophlium* –Canine - Diagnosis -Iraq.

Introduction

Dog is one of the almost common pets which influenced by many tick-borne diseases of parasitic, bacterial, and viral origins resulting in considerable impacts on the health (Otranto *et al*., 2009; Jaarsma *et al*., 2019). *Anaplasma phagocytophilum* and *A. platys*, two bacterial pathogenic agents belonging to Anaplasmataceae Family of Rickettsiales Order, are known to infect livestock and domestic animals including dogs (Kocan *et al.*, 2004). Canine anaplasmosis caused by *A. phagocytophilum* is characterized primarily by granulocytic vacuoles (canine granulocytic anaplasmosis); whereas, *A. platys* is specifically infect the platelets resulting in cyclic thrombocytopenic (canine thrombocytopenic anaplasmosis), (Alberti and Sparagano, 2006; Severo *et al*., 2015). Clinical manifestations caused by infection with each one are also variable; nonetheless, it is still controversial whether both pathogens responsible on clinical symptoms (Otranto *et al*., 2009; Carrade *et al*., 2009; Sainz *et al*., 2015). Blood-smear microscopy is one of the most routinely used tools during the acute phase of illness to diagnosis of canine anaplasmosis as it easy to performed, simple to interpreted and costly inexpensive. The presence of morulae in cytoplasm of infected granulocytes suggests a positive infection; therefore, the test is relatively has low sensitivity when relied solely due to difficulty of observing the inclusion bodies in infected blood cells especially in chronic infection as well as it requires a highly experience (Tarello, 2005; Wardrop *et al*., 2005; Kubelova *et al*., 2013). The most common laboratory finding in canine anaplasmosis is thrombocytopenia (Sainz *et al*., 2015). For first time in Iraq, the current study aimed for :- Microscopic examination of Giemsa's stained blood smears to detect of inclusion bodies blood cells particularly in granulocytes and platelets, Evaluation of clinical and hematological changes in positive dogs with anaplasmosis and compare them with that in negative dogs.

Materials and Methods

Study site and dog samples

The number of sick cases of domestic and police dogs admitted to the Bagdade veterinary hospital were 150 animals in different Ages, sex and breed with variable cases. during the period at the December to May (2018-2019).

Clinical Examination

Clinical examination was carried out to all animals prior to sample collection, which includes pulse rate, rectal temperature, respiratory rate and mucous membranes, The case history was taken which include appetite and other signs. feeding type, age, Sex, breed type, management, recorded for each animal in a special forma chart designed for this purposes.

Blood sample collection and preparation of Blood smear

Whole blood samples with EDTA anticoagulants tubes were collected from 150 dog from cephalic vein and make a thin film which was stained with Giemsa stain (Santamaria *et al*., 2014). The 2ml of blood was draw from the cephalic vein by a vacutainer tube with an(EDTA).the blood samples was used for hematological parameters The hematological tests were done according to (Humacount analyser veterinary software hematology), the samples were transferred in a cooling box to the laboratory research at the veterinary Baghdad hospital department of internal and preventive veterinary medicine.

Statistical Analysis method of the study data

Microsoft Office Excels and IBM SPSS programs were used in current study to collect of obtained data and for statistical analysis. Chi-square (x^2) and *t-test* were applied to detect the significant differences between the microscopic and molecular results, and to detect relationship between the positive PCR-results with the risk factors, tick infestation, as well as the clinical and hematological findings. Differences were considered significant at a level of P<0.05, (Onwuegbuzie *et al*., 2007).

Results

Clinical examination

In (table 1) of 150 study dogs submitted for clinical examination, the findings were revealed on significant variation (P<0.05). However, significant increases (P<0.05) were detected in the cases that showed the symptoms of depression, diarrhea, weakness, and vomiting (15.79%); and those with anorexia, diarrhea, loss body weight, and vomiting (14.63%).

Table 1 : Results of clinical examination among study dogs

Clinical symptoms	Total No.	Positives		Negatives
		No.	%	
Anorexia, diarrhea, loss body weight, and vomiting	41	6	14.63 *	35
Anorexia, ataxia, depression, chronic vomiting, and weakness	5	0	0	5
Anorexia, abdominal pain, and constipation	17	0	0	17
Anorexia, depression, jaundice, and weakness	12	0	0	12
Anorexia, depression, jaundice, loss body weight, and weakness	18	1	5.56	17
Ataxia, depression, nasal discharge, and respiratory signs	15	1	6.67	14
Ataxia, depression, obesity, and respiratory signs	8	0	0	8
Depression, diarrhea, weakness, and vomiting	19	3	15.79 *	16
Depression, loss of appetite, weakness, and vomiting	12	1	8.33	11
Depression, weakness, and chronic pneumonia	3	0	0	3
Total No.	150	12	8	138

Significant increases * (P<0.05)

In (table 2), Regarding Vital signs to findings of clinical examination, significant elevation (P<0.05) was showed among the values of temperature (38.85 ± 0.15), pulse (103.58 ± 4.99) and respiratory (51.64 ± 5.83) rates of PCR-positive dogs with *Anaplasma* spp., (Table 2).

Table 2 : Total results of vital signs among positive dogs by PCR

Vital signs	Positives	Negatives
Temperature	38.85 ± 0.15 * (38.3 – 39.7)	38.27 ± 0.23 (37.3 – 39.6)
Pulse rate	103.58 ± 4.99 * (70 – 136)	86.08 ± 2.32 (51-141)
Respiratory rate	51.64 ± 5.83 * (30-87)	43.21 ± 2.43 (27-107)

Values [M±SE (R)], Significant increases * (P<0.05)

In (table 3) Among the positive dogs with anaplasmosis, although there is a slight elevation in values of temperature, pulse and respiratory rates of infected dogs with *A. phagocytophilum* in comparative with infected dogs with *A. platys*; however, differences were insignificant (P>0.05), (Table 3).

Table 3: Results of vital signs among positive dogs

Vital signs	Positives	
	A. platys	*A. phagocytophilum*
Temperature	38.62 ± 0.22 (38.3 – 39.5)	39.01 ± 0.19 (38.4 – 39.7)
Pulse rate	91.4 ± 7.24 (70 – 115)	96.14 ± 6.95 (81-136)
Respiratory rate	45.8 ± 10.49 (32-87)	49.86 ± 6.51 (30-82)

Values [M±SE (R)], Significant increases * (P<0.05)

In (Table 4), a totally of 150 dog were submitted for this study. The result of microscopic examination for blood smears test by using Giemsa stain of positive dogs. Nine blood samples appeared positive for anaplasmosis with infection rate 6%.

Table 4 : Total results of 150 dogs examined by microscopy

Test	Total No.	Result	
		Positives	Negatives
Microscopy	150	9 (6%)	141 (94%)

In (Table 5) Types of *Anaplasma* spp Based on the findings of microscopic examination, the study showed that there significant increases (P<0.05) in prevalence of *A. phagocytophilum* (55.56%), among positive dogs, compared to *A. platys* (44.44%).

Table 5 : Types of *Anaplasma* spp. based on microscopic examination

Type	No.	%	Total Positives
A. platys	4	44.44	9
A. phagocytophilum	5	55.56 *	

Significant increases * (P<0.05)

The examination of staind blood smear shows intracytoplasmic inclusion bodies (morulae) in neutrophial Figure (1).

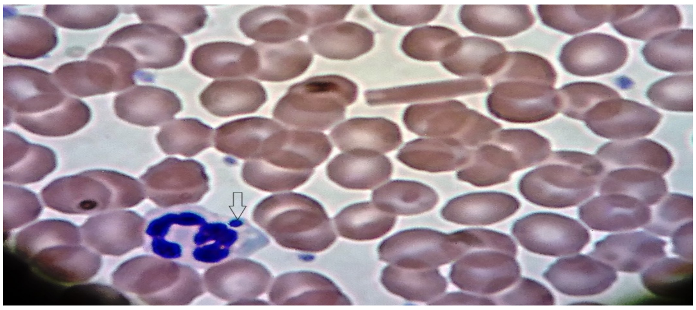

Fig. 1 : Positives blood smear from *Anaplasma phagocytophlium* infected dog. The inclusions were identified with arrow head as purple stained bodies within the neutrophil cytoplasm. (Giemsa stain, X100)

The examination of stand blood smear shows intracytoplasmic inclusion bodies (morulae) in platelet figure (2)

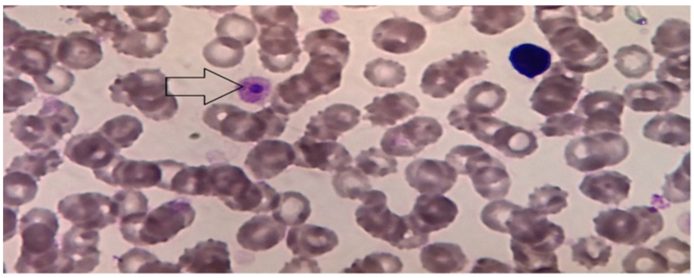

Fig. 2 : Positives blood smear from Anaplasma platys infected dog. The inclusions were identified with arrow head as purple stained bodies within the Platelet cytoplasm. (Giemsa stain,X100).

In Table 6 regarding to RBCs indices, significant decreases (P<0.05) in values (M ± S) of positive dogs by PCR were detected in total RBCs (4.98 ± 0.27), Hb (13.27 ± 0.73), and platelets (179.92 ± 21.47); whereas, no significant differences (P>0.05) were observed in values of PCV, MCV, MCH, and MCHC.

Table 6 : Total results of RBCs indices among study dogs

Indices	Positives M±SE (R)	Negatives M±SE (R)
Total RBCs 10^6/µl	4.98 ± 0.27 * (3.19 – 6.85)	5.87 ± 0.89 (3.06 – 7.23)
PCV %	40.05 ± 2.01 (24.12 – 46.7)	44.61 ± 2.67 (31.56 – 54.39)
Hb g/dl	13.27 ± 0.73 * (9.2 – 17.3)	15.29 ± 0.85 (9.69 – 18.42)
MCV Fl	79.83 ± 14.27 (67.51-89.43)	76.24 ± 5.38 (68.93-83.25)
MCH Pg	26.52 ± 3.16 (22.73-28.85)	26.34 ± 1.48 (19.95-31.66)
MCHC g/dl	33.42 ± 3.37 (26.78-41.23)	35.04 ± 3.71 (25.85-39.72)
Platelets 10^3/µl	179.92 ± 21.47 * (89 – 310)	286.85 ± 7.27 (160-487)

Significant decreases * (P<0.05)

In (Table 7) Among positive dogs by PCR, the values of RBCs indices showed that there no significant differences (P>0.05) were found between the results of dogs infected with *A. platys* and *A. phagocytophilum.*

Table 7 : Results of RBCs indices among positive dogs

Indices	Positives [M±SE (R)]	
	A. platys	*A. phagocytophilum*
Total RBCs 10^6/µl	4.95 ± 0.49 (4.19 – 6.85)	5 ± 0.35 (3.19 – 5.8)
PCV %	39.98 ± 2.5 (31.72 – 46.03)	40.11 ± 3.11 (24.12 – 46.7)
Hb g/dl	13.06 ± 1.47 (9.2 – 17.3)	13.41 ± 0.79 (9.69 –15.6)
MCV Fl	77.96 ± 17.01 (67.51– 85.24)	80.41 ± 15.22 (75.85 – 89.43)
MCH Pg	25.89 ± 3.44 (22.05 – 26.47)	26.22 ± 2.09 (19.95–31.66)
MCHC g/dl	32.71 ± 5.73 (28.64 – 36.49)	33.58 ± 3.46 (26.78 – 41.23)
Platelets 10^3/µl	158.8 ± 35.4 (89 – 278)	195 ± 26.54 (118 – 310)

Significant decreases * (P<0.05)

In (table 8) Concerning to WBCs indices of positive dogs, the findings were revealed on significant decreases (P<0.05) in values (M±SE) of total WBC (10.61 ± 0.54) and lymphocytes (33.42 ± 2.38); whereas, no significant variation (P>0.05) was seen among the values of monocytes, neutrophils, basophils, and eosinophils.

Table 8 : Total results of WBCs indices among study dogs

Indices	Positives M±SE (Range)	Negatives M±SE (Range)
Total WBCs 10^3/µl	10.61 ± 0.54 * (7.16-13.09)	12.69 ± 0.22 (11.08-16.47)
Lymphocytes %	33.42 ± 2.38 * (23-47)	38.53 ± 1.15 (22 – 51)
Monocytes %	3.67 ± 0.48 (1-7)	3.97 ± 0.37) (1-9)
Neutrophils %	61.08 ± 4.16 (34 - 76)	56.49 ± 1.33 (30-79)
Basophils %	0.83 ± 0.11 (0-1)	0.49 ± 0.01 (0-1)
Eosinophils %	3.92 ± 0.51 (1-7)	3.54 ± 0.32 (0-9)

Significant decreases * (P<0.05)

In (table 9) The findings of WBCs indices were showed that there in significant decreases in values of neutrophils among positive dogs with *A. phagocytophilum* (58.2 ± 6.84) in comparison with *A. platys* (63.74 ± 5.59). However, there no significant differences (P<0.05) in values of total WBCs, lymphocytes, monocytes, basophils, and eosinophils among the positive dogs with *A. platys* and *A. phagocytophilum.*

Table 9 : Results of WBCs indices among positives

Indices	Positives [M±SE (Range)]	
	A. platys	*A. phagocytophilum*
Total WBCs 10^3/µl	10.57 ± 0.83 (7.16 – 12.81)	10.65 ± 0.72 (9.08 – 13.09)
Lymphocytes %	31.57 ± 2.78 (24 – 42)	36 ± 4.28 (23 – 47)
Monocytes %	3.57 ± 0.61 (1 – 5)	3.8 ± 0.86 (2 – 7)
Neutrophils %	63.74 ± 5.59 (35 – 76)	58.2 ± 6.84 * (34 – 74)
Basophils %	0.86 ± 0.14 (0 – 1)	0.8 ± 0.2 (0 – 1)
Eosinophils %	3.86 ± 0.71 (1 – 7)	4 ± 0.84 (2 – 7)

Significant decreases * (P<0.05)

Discussion

In the genus of *Anaplasma, A. phagocytophlium* and *A. platys* are consider as tick-borne pathogens with great clinical importance in dogs (Brown *et al*., 2006; Stuen *et al*., 2013). Out of 150 dogs which presented to sahat adan hospital suffering from variable clinical signs including, Anorexia, diarrhea, loss body weight, and vomiting (14.63%). Also depression, diarrhea, loss body weight, and other signs (15.79%). Significant increases (P<0.05) frequent among the dogs infected with anaplasma spp.when compared with dogs without this infection also there are detected another signs like pale mucus membrane, emaciation, ataxia, depression, weakness, abdominal pain, and constipation, jaundice, nasal discharge, respiratory signs, chronic pneumonia , obesity. No hemorrhagic signs were recorded during present studies. Physical examination during this study showed similarities with many authors as a result of comparison with them (Cockwill *et al*., 2009; Vargas-Hernandez *et al*., 2016; Ybanez *et al*., 2018). The are varying degrees of clinical signs ranging from mild asymptomatic infection to acute severe disease depending on the host, immunity, pathogen virulence, infective dose, the route of infection and environmental factors (Irwin and Jeffries, 2004; Watanabe, 2012; Mokhtar *et al*., 2013; Nazari *et al*., 2013; Sykes and Foley, 2014). The peripheral blood smear examination is the simplest and most accessible diagnostic test for most veterinarians in detecting intercellular parasites (Ybanez, 2014). The infection rate in the Baghdade city with *A. platys* was 4/150 (44.44 %) and *A. phagocytophlium* 5/150 (55. 56%). according to blood smear staining (morulae). The result agreed with (Cockwill *et al*., 2009) recorded (3.5%) the infection rate with *A. phagocytophlium* in dog in Saskatoon, Saskatchewan. However, the result of this study disagreed with findings of (Jensen *et al*., 2007) who showed a low infection rate 1.8% by buffy coat examination morulae in granulocytes were detected in only two dog out of 111 dogs. in addition some study in Iraqi done by (Alfattli *et al*., 2017), the detection of morulae in the genus Anplasma in dogs does not identify the agent to the species level and further testing is needed .As registered by (Ghirbi *et al*., 2009) in Tunsia, Diagnostics based solely on the presence of inclusions in cytoplasmic Anaplasma species by blood smear exams shows poor sensitivity because of the low and transient bacteremia presented by this agent. However when the chronic or subclinical phase of these diseases, these inclusions are not detected, only detects in acute phase of the disease (Ybanez, 2014; Beaufils *et al*., 2002). Hematological abnormalities such as anemia ,thrombocytopenia and leukopenia are the common disorders in canine anaplasmosis (Kohn *et al*., 2008; Ranik *et al*., 2011; Ozata and Ural, 2014). In the present study show that the infected dogs with canine anaplasmosis derceased in PLT count, RBCs, Hb constration and PCV compared with non-infected, the result agreed with (Hendrix *et al*., 2002; Ulutas *et al*., 2007; Gaunt *et al*., 2010). The causes and pathogenesis of anemia remain unspecified in our causes ,anemia could be related to many mechanisms, including hemolysis, transient bone marrow dysplasia or reduced even absent proliferation of precursors following cytokine suppression of hematopoiesis within bone marrow ,the importance of immune –mediated erythrocyte destruction in dogs with anaplasmosis warrants further investigation, hemorrhage due to an increased bleeding tendency could also be a mechanism of anamia (Poitont *et al*., 2005; Eberts *et al*., 2011). The results of infected dogs showed a non-significant slight decreased in WBCs with anaplasmosis, this result agrees with (Franzen, 2008; Kohn *et al*., 2011), Hematological parameters has been reported both leukopenia and leukocytosis were observed in dogs infected with anaplasmosis, depending on the phase of the diseases or may not be the anaplasmosis main cause of leukocytosis but of other disease with similar laboratory values, (Kohen *et al*., 2008; Ranik *et al*., 2011; Ozata and Ural, 2014), The results of differential leukocytic count showed a significant decrease in neutrophils infected dogs with *A. phagocytophlium* compared with non-infected dogs, a significant decrease with lymphocytes positive compared with non-infected, the result agreed (Egenrall *et al*., 1997; Poitont *et al*., 2005), Explain the causes of Neutropenia in dogs can be due to increased use (during sever supportive inflammation/infection), decreased production as a result of primary or secondary insults to bone marrow neutrophilic precursor cell), or accelerated destruction through immune- mediated mechanism (Schnelle and Barger, 2012). Anaplasmosis deprive the hosts innate immune response, causing carrier infection via antigenic variations and modulating lymphocytes response, due to lymphocyopenia because during the lymphocytopenia the subpopulations of T cells (CD_4+CD_8) are reduced (Woldhiwet, 2008). The present results showed non-significant differences in Monocytes, Eosinophils and Basophils. This result agrees with (Whist *et al*., 2003; Henkelbach *et al*., 2006; Pilger *et al*., 2011).

Conclusion

- We have diagnosis and identified of Anaplasmatacea Family *A. platys* and *A. phagocytophlium* in Iraqi dog with significant prevalence.
- The clinical examination did not show specific clinical signs and a certain diagnosis requires the combination of clinic pathological.
- The blood smear (Morulae) is particular methods for detection acute form of infection.

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