

CLINICAL AND DIAGNOSTIC STUDIES OF HEMOMYCOPLASMOSIS IN DOGS AT BASRAH, IRAQ

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ABSTRACT : *Mycoplasma hemocanis* in dogs at Basra governorate, Iraq has been detected and microscopically diagnosed. Twenty (20) domesticated dogs of both sexes show various clinical manifestations that indicate the infection. Five (5) clinically healthy dogs were used as controls. Diseased dogs showed signs of general wasting, partial or complete loss of appetite, shallow and rapid breathing, accelerated heartbeats, weight loss, the roughness of skin and anemia. A statistically significant increase has been indicated in the body temperature, heart and respiratory rate of diseased dogs than control. *Mycoplasma haemocanis* was detected by Giemsa stained blood smears and appeared as, rings or sometimes rods or cocci shape. However, an isocytosis, poikilocytosis and acanthosis were greatly seen. Results was also revealed a significant decrease of TRBs, HB and PCV, reflecting the macrocytic hypochromic type of anemia. Furthermore, significant Leucocytosis due to significant lymphocytosis was also detected. It has been concluded that canine Hemomycoplasmosis harm dogs leads to emaciation and might be terminated with death.

Key words : *Mycoplasma hemocanis*, dogs, Iraq.

INTRODUCTION

Hemoplasma is a non-implantable bacterium. It has no cell wall and previously classified as Eperythrozoon and Haemobartonella species, that attach to the erythrocyte's surface of its vertebrate host. This microorganism recently involved within the Mycoplasma genus (class Mollicutes) (Rikihisu *et al*, 1997; Messick *et al*, 2002). Diagnosed for the first time in dogs in Germany in 1928 (Kikuth, 1928). It was located on the wall of the infected red blood cells in the form of a ring or a chain surrounding the cell wall (Lumb, 1961).

In recent years, there has been an increased interest in blood-directed mycoplasma that resulting in infectious anemia in a wide range of mammals. The resulting infections from these pathogens can lead to acute hemolysis, which is associated with loss of appetite, lethargy, weight loss dehydration and sudden death in affected animals. The inability to cultivate these causative agent outside its host limited the possibilities of investigating mycoplasma (Willi *et al*, 2007).

Dogs are mainly infected with two haemoplasma species: *Mycoplasma haemocanis* and '*Candidatus Mycoplasma haematoparvum*'. Infection is mostly chronic and subclinical in immunocompetent dog, on the

other side may lead to clinical signs related to haemolytic anaemia following splenectomy, immunosuppression (Sykes *et al*, 2004; Sykes *et al*, 2005).

The disease includes mild symptoms except splenectomized patients which show loss of appetite, pale gums and infertility Diagnosis in dogs may be possible by veterinarians through the case history, biochemical and serological tests and blood smears (Fard *et al*, 2014).

Hematological findings may be different and this difference comes with congruence with the stage of hemoplasma infection. During the acute phase of the disease, the common hematological finding are anemia, anisocytosis, polychromasia and leukocytosis (Pitorri *et al*, 2012; Francisco Soto *et al*, 2016; Messick *et al*, 2004).

After the growing popularity of people to raise dogs in Iraq, especially at Basrah governorate, it became the duty of the veterinarian to take care of them and search for the most important problems that afflict them. This pathogen was one of the most important blood parasites that spread among dogs. Since there is little information of canine hemoplasmosis has been provided at Basrah, Iraq. Therefore, the study was conducted for identifying and knowing this disease and its existence.

MATERIALS AND METHODS

The present study was conducted to examine 25 native dogs show signs of anemia and lethargy. Five native dogs clinically healthy were be considered as control. Full clinical examination was done to all infected and control groups. However, examination of internal parasites has been applied according to the standard methods.

Five (5) milliliters of blood were drained from the Cephalic vein. (2.5) milliliters mixed with EDTA to evaluate total erythrocyte count, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin concentration, total leukocytes count and total thrombocytes count using the Coulter counter form Genies /USA. Moreover, differential leukocyte count and microscopic diagnosis of the microorganism was determine by Giemsa stained blood smears according to Weiss and Wardrop (2010), Salgado *et al* (2011). Other (2.5) milliliters of blood were mixed with trisodium citrate (were plasma separated) used to evaluate the prothrombin time, Fibrinogen time and activated partial thromboplastin time by using kit from Biolabo, France.

Statistical analysis

The significance of variations between infected and healthy dogs were statistically analyzed using (SPSS) student *t*-test (Leech *et al*, 2007).

RESULTS

The clinical investigation of the infected dogs showed several important signs such as complete or Partial loss of appetite (90%), anemia with pale\white gums (75%), shallow and rapid breathing (65%), weight loss (60%), roughness of skin (45%) and general wasting (35%) (Table 1).

Table 1 :Clinical signs of diseased dogs.

Clinical signs	Diseased dogs n=20	%
Complete or Partial loss of appetite	18	90
Anemia with pale\white gums	15	75
Shallow and rapid breathing	13	65
Weight loss	12	60
Roughness of skin	9	45
General wasting	7	35

A statistical increase has been noticed in body temperature, respiratory and heart rate of diseased dogs than in controls (Table 2).

Microscopic examinations of blood smears appeared that *Mycoplasma haemocanis* appears as ring, rod or cocci shape (Fig. 1).

Table 2 : Vital signs of infected dogs with Hemomycoplasmosis.

Vital signs	Controls n=5	Diseased n=20
Body temperature C°	38.06±0.28	40.31±0.17*
Respiratory rate/ mint	60±0.70	89±1.23 *
Heart rate/ mint	88±2.54	151±4.92*

In addition to that, the blood smears showed a lot of changes in the wall of RBCs and the red blood cell itself, anisocytosis, poikilocytosis and acanthocytes, it was the most common abnormalities (Fig. 2). Furthermore, evidence of anemia is also noticed in the blood smear and marked by the presence of macrocytic hypochromic red blood cells.

The current study indicated a significant decrease (*P*<0.05) in the RBC, HB and PCV values if diseased dogs compared with the controls which reflected macrocytic hypochromic type of anemia (Table 3).

Table 3 : Blood parameters of diseased dogs and control.

parameters	Control n=5	Diseased n=20
RBC×10 ⁶	6.32±0.28	4.14±0.10*
Hb g/L	156.8±3.56	80±3.22*
PCV%	35.2±1.65	25±0.75
MCV/fl	61.2±1.49	65.8±0.81
MCHC g\dl	34±1.78	29.45±1.06

On the other hand, results revealed a significant increase in total leukocyte count as a result of a significant lymphocytosis (Table 4).

Table 4 : Total and absolute differential leukocyte count of diseased dogs and controls.

parameters	Controls n=5	Diseased n=20
TLC x 10 ³	13.88±1.95	19.54 ±1.60*
Lymphocyte / absolute	4070±52.44	9755.95±175.67*
Neutrophils / absolute	8146±98.87	8361.25±100.67
Monocytes / absolute	532±16.42	539.05±10.42
Eosinophils / absolute	347.6±2.78	342.25±7.84
Basophiles / absolute	31.6±0.81	32.9± 0.88

Results of clotting factors indicates of diseased dogs and controls indicated a significant decrease (*P*<0.05) in total platelets count. Whereas, a significant increase (*P*<0.05) has been encountered in the values of Mean platelets' volume. Platelets distribution width, Prothrombin time and Activated partial thromboplastin time in diseased dogs compared with the controls (Table 5).

Table 5 : Clotting factor indices of diseased dogs and controls.

Parameters	Controls (n = 5)	Diseased (n=20)
Total platelets count x10 ³	422.13±5.17	223.34±22.44*
Mean platelets' volume/fl	10.77±2.78	15.11±3.56*
Platelets distribution width %	16.82±1.88	19.78±4.76*
Prothrombin time/Sec	12.11±2.77	17.45±5.71*
Activated partial thromboplastin time/Sec	60.82±2.45	68.44±7.34*

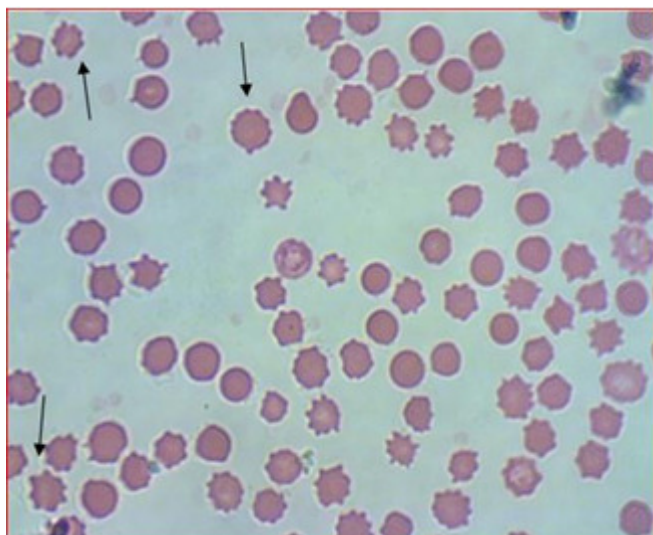


Fig. 1 : Mycoplasma haemocanis on the cell wall of dog erythrocyte.

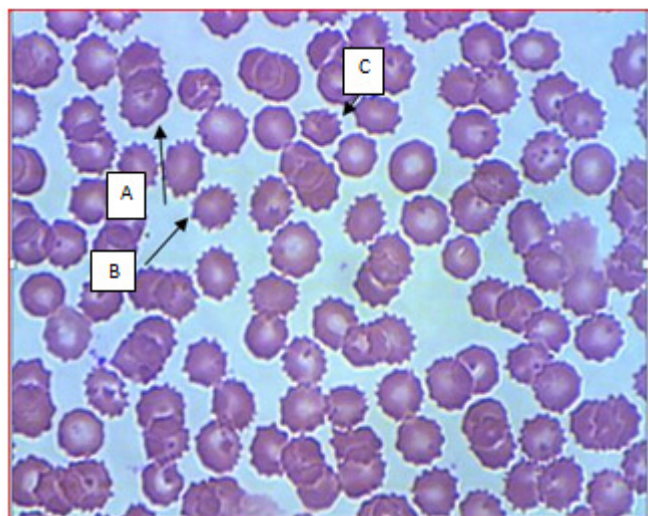


Fig. 2 : Anisocytosis (A), poikilocytosis (C) and acanthocytes (B) in dog erythrocyte.

DISCUSSION

Recently, some families have increased their desire to raise animals for different purposes, dogs were the most commonly traded in the south of Iraq in particularly in Basrah. Therefore, attention and follow-up to such cases, this is the first study to diagnose the pathogenic etiology for the first time in Iraq in general and Basra in particular. In the current study, the diagnosis of *Mycoplasma haemocanis* was based on clinical signs and blood abnormalities.

Thereby, many clinical signs that were observed on affected dogs which correspond to several researchers such as Bundza *et al* (1976). Anemia that is detected in diseased dogs, it occurs because the excessive destruction of red blood cells, it is recognized that the severity of anemia depends on the stage of parasitism, which ranges from the paleness of mucous membranes to death, so

according to the blood abnormalities, the type of animal was regenerative anemia, this is similar with these findings that mentioned by Wengi *et al* (2008) and Sykes *et al* (2004) refer that the regenerative anemia within a week of detection of organisms on the surface of RBCs during the examination of a blood smear is suggestive that this organism may be associated with hemolysis in dogs. When looking at the mucous membrane and the resulting coloration, which varies from paleness to severe yellowing according to the percentage of targeted parasitism, then if it indicates something, it indicates the excessive breakdown and microphage that occurred to the red blood cells and the appearance of the bilirubin in a way that is greater than the liver's ability to get rid of and thus its appearance on the mucous membranes as it appeared in the study (Joanne B Messick, 2003).

In current work, it was indicated that there is a clear change in the vital signs of the affected dogs in terms of increased temperature and increased rates of breathing and pulse, which occurred as a result of the increase in the level of parasitism and the destruction of many red blood cells, which instructed the body to increase the heart rate and respiratory rate to compensate for the lack of oxygen and these changes agreed with Brinson and Messick (2001).

The results of blood smears showed the presence rings or, more rarely rod and sometimes cocci that evidence of the pathogen. This result is similar to what mentioned by Fard1 *et al* (2014), where indicated that microscopic observation demonstrates bacteria in single, pairs or chain on the surface of erythrocytes, but rarely free in the plasma, which aggregate and form pinion teeth on the surface of RBCs (Hoelzle, 2008; Groebel *et al*, 2009) also mentioned these result.

Changed in the shape and size of the infected erythrocytes such as anisocytosis, poikilocytosis and acanthocytes. It proves the major effect of the causative agent on erythrocytes, causing alteration of their size and shape this results are similar to what found by Jane E Sykes *et al* (2005). Anemia which indicated in the current study could occur due to the excessive destruction of the erythrocytes with the phagocytosis of the damaged RBC done via the specific phagocytes of the reticulo endothelial system. Moreover, it is probably that *M. haemocanis* takes the benefit of the erythrocyte's metabolism, it depletes the important nutrients, as a result, reduces the age of the red blood cells, resulting in anemia during acute disease (Santos *et al*, 2011).

Additional primary virulence factors were not identified in the genome of *M. haemocanis*. The o-

sialoglycoprotein endopeptidase, related to the cleavage of glycophorin A, is conserved among hemoplasmas; the superoxide dismutase (SOD), identified in *M. haemofelis* (Santos *et al*, 2011; Barker *et al*, 2011) is also present in *M. haemocanis*, but not found in any other sequenced mycoplasma. Although, SOD may protect these bacteria from superoxide anion toxicity faced in the blood environment, it is unlikely that this enzyme plays a determinant role in the primary pathogenicity associated *M. haemocanis*.

Regarding the changes that occurred in the white blood cells, several researchers explained the reason for this and among them (Denman, 1991) was said. The microorganism trigger autoantibodies which in turn causes a malfunction in the host's immune system. Also, some genes code superantigens which is directly related to major histocompatibility complex (MHC) molecules. Thus, it stimulates large numbers of lymphocytes (lymphocytosis) (Fardl *et al*, 2014). These findings are similar to that mentioned in current study. Production of inflammatory cytokines and host immune response (activating lymphocytes) leads to symptoms such as chronic arthritis (Cole and Alkins, 1991).

In the current work, data revealed the values of clotting factors of control and diseased dogs indicated a significant decrease in the total platelets count, from another hand, an increase platelets volume, platelets distribution width, Prothrombin time and activated partial thromboplastin time in diseased dogs were compared with control, these findings were similar to that mentioned by Sudan *et al* (2012). It is also known that any bleeding process that occurs in the body must be followed by the process of clotting mechanism, as many factors participate in this process and that has a very important role, the most important of which is the vascular factor. Furthermore, the number and activation of thrombocytes also play a significant role in the process of coagulation, in which the thrombocytes aggregate and then adhere to the vessel wall causing platelets thrombus or plug (Rebar *et al*, 2005). The decrease in the number of thrombocytes observed is likely due to the decreased activity of the bone marrow and platelet wasting (Boudreaux, 2001). Furthermore, the last stage of the clotting stage is the coagulation stage, which begins with the help of several factors, the most important of which are Plasma thromboplastin antecedent factor, Hageman factor and thromboplastin component, which has an important role in converting the fibrinogen to fibrin and prothrombin into thrombin which ultimately leads to fibrin deposition inside the blood vessel. This will disturb the hemostatic mechanism enhanced by Disseminating intravascular

coagulopathy, causing micro thrombus and infarction of special organs (Bick, 2003).

CONCLUSION

It has been concluded that canine Hemomycoplasmosis have an adverse effect of dogs leads to emaciation and might be terminated with death.

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