CLINICAL ,HEMATOLOGICAL AND DIAGNOSTIC STUDIES OF *MYCOPLASMA WENYONII* INFECTION IN CATTLE OF BASRAH GOVERNORATE BASRAH ,IRAQ

Ali Jarad , Kamal M.Alsaad
Department of internal and preventive medicine, College of Veterinary Medicine, University of Basrah , Basrah, Iraq
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ABSTRACT

The present work were conducted on (225) local cattle breeds of both sexes , and of different ages in Basarh governorate (Basrah –Iraq). Two hundred local cattle breeds were naturally infected with. *Mycoplasma wenyonii* and (25) clinically normal cattle breeds served as controls. According to age, diseased animals were divided into four age groups (50) animals for each. Animals were found clinically infected with *Mycoplasma wenyonii* which diagnosed based on Giemsa stain blood smears and confirmed with PCR test technique. Diseased cattle show sings of partial or complete loss of appetite ,anemia with pale and / or icteric mucous membranes , decrease milk production , rapid and difficult respiration, enlargement of superficial lymph nodes , rough coat , lethargy , weight loss and edema of lower hind limbs, In addition , body temperature ,respiratory rate ,heart rate and capillary refilling time were increased statistically , compared with controls ,Furthermore statistically significant decrease ($P<0.05$) were encountered in ruminal contractions .

Results were also indicated that TRBCs, Hb, and PCV values of diseased cattle were significantly decrease than controls thereby macrocytic hypochromic type of anemia was indicated. Results were also shown a significant increase in TLC as a result of significant increase lymphocytes, *Mycoplasma wenyonii* appear coccoid or rod shape, structures, However it might found individually or in chains on the erythrocyte cell wall, Moreover diagnosis were confirmed by PCR,Since out of (96) blood samples (80) (83.3%) were found positive. Results were also revealed that animals of 2-3 years old were highly infected compared with other age groups. Changes of blood clotting factor indices were also noticed in diseased cattle compared with controls and the results showed significant decrease ($P<0.05$) in the mean values of total

thrombocytes count and Fibrinogen time ,whereas significant increase (P<0.05) were detected in thrombocytes volume, thrombocytes distribution width, prothrombin time , clotting time, and activated partial thromboplastin time. It have been concluded that *Mycoplasma wenyonii* infected local cattle breeds of Basrah Governorates and lead to substantial effect including decrease milk yield and anemia's ,which might terminated with economic losses ,Therefore all cattle reared in this area must be screened.

INTRODUCTION

Mycoplasma wenyonii (Formerly call *Eperythrozoon wenyonii)* are eperythrocytic rickettsial parasites of the family *Bartonellaceae*.(1) It also call Hemotropic Mycoplasmas or Hemoplasmas (2).

The organisms are procaryotic forms occurring out side on the surface of the red blood cells , Positive smears, stained with Giemsa-stain show a pleomorphic coccoid, rod, and ring-shaped structures might found individually or in chains on the red cell and have gram-negative staining because of the lack of a cell wall ,However none of the hemoplasmas have been cultured outside their hosts (3). Infections are common worldwide in cattle and other animals .(1,4).

The species *Mycoplasma wenyonii* cause infectious anemia in several mammalians, Their effects were vary from mild effect to death , they were reclassified as genus *Mycoplasma* depending on 16S rRNA sequences and morphologic similarities, and have been identified in different countries in the world ,their disease characterized by transient fever , anemia, lethargy , decreased milk yield, enlargement of superficial lymph nodes , anorexia, weight loss, edema of different body parts specially hind limbs ,and rough coat However, the infection might remains subclinical and / or chronic, although acute form were also suspected and diagnosed (5,6).

The organisms are found singular or in different chains on the surface of erythrocytes (7), Even though it not invade the erythrocyte ,However Groebel *et al* (8) were find out that the *Mycoplasma suis* can invade erythrocytes.

It have been documented that *Mycoplasma wenyonii* can infect different animals and shown to be transmitted by various blood sucking ecto-parasites, including ticks, flies, lice, fleas, and also mosquitoes, Moreover it have been shown that the main route of transmission of these organisms were uncharacterized, Nevertheless

mechanical and transplacental routes have been recorded(7),Furthermore *Aedes aegypt* are thought to play an important role in the transmission(9).In addition Messick, (10) was mention other route of transmission as direct contact, and vertical route and transmission via contaminated food but these routes of transmission need further investigation.

The disease caused by *Mycoplasma wenyonii* have been registered in Mosul ,Iraq previously (11,12) Whereas they were recorded for the first time in cattle of Basrah governorate ,Therefore the present study were aimed to investigated and study the clinical , hematological and diagnostic methods of *Mycoplasma wenyonii* infection in this area .

MATERIALS AND METHODS

The present work were conducted on (225) local cattle breeds of both sexes , and of different ages in Basarh governorate (Basrah –Iraq). Two hundred local cattle breeds were naturally infected with. *Mycoplasma wenyonii* and (25) clinically normal cattle breeds served as controls. According to age, diseased animals were divided into four age groups (50) animals for each (New born calves1-5 days old ,calves under one year old, cows of 2-3 years old and Old cows more than 5 years). Complete clinical examinations had been carried out in all infected and control cows, Moreover their fecal samples are screened for parasitic loud using the usual scientific methods.

Ten milliliter of blood were drained from each animal by jugular vein-puncture, from these (2.5) milliliter of blood mixed with EDTA used to determine Total erythrocyte count (TRBc), Hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), Thrombocytes count , mean thrombocytes volume , thrombocytes distribution width ,and total leukocytes count, (Hematology analyzer, Genex**,** USA),Furthermore differential leukocytes count were done according to Weiss and Wardrop (13) . Another (2.5) milliliter of blood mixed with Trisodium citrate (used plasma) were used to determine Fibrinogen time ,prothrombine time and activated partial thromboplastine time (Biolabo / France). Clotting time was also estimated according to Bush (14).

Blood smears had been stained with Wright and Giemsa stain for detection of *mycoplasma wenyonii* on erythrocyte surface under light microscope at

1000X,positive sample with *mycoplasma wenyonii* was recorded if one infected erythrocyte was found in 200 observed RBCs.

For the PCR assay, the blood was stored in EDTA tube until assayed.DNA was extracted from 200µl of blood using a commercial kit(Bioingentech Genomic DNA Purification Kit, BioInGentch,Chile). According to manufacture protocol of (*Mycoplasma wenyonii* Detection Kit, BioInGentch,Chile) that used for the specific amplification of a region(180-basepair) of 16S RNA gen from *Mycoplasma wenyonii*, The kit consists of the following components that are enough for amplification of genomic DNA,such as, *Mycoplasma wenyonii* Pre-mixture, PCR internal control, *Mycoplasma wenyonii* PCR Positive control, DNase/Ranse free water, PCR negative control, mineral oil solution and brig™molecular weight marker. Preparation of *Mycoplasma wenyonii* PCR mixture was done according to protocol of the same detection kit.

The amplification condition was include, one cycle Initial Denaturation at 94ċ for 2 minutes,30 cycle for denaturation at 94ċ , annealing at 57ċ and extension at 72ċ for 30 second, final extension one cycle at 72ċ for 5 minutes using an automatic cycler. Amplification products were electrophoresed on 1.5% agarose gels. Gels were stained with ethidium bromide and examined with ultraviolet illumination. Bands seen at the expected location (180- bp).

The significance of variations between infected cows and healthy animals were statistically analyzed using (SPSS) student t-test, (15).

RESULTS

Results were showed that out of 200 suspected cases of *Mycoplasma wynonii* infection (140) were detected positive by microscopical examination (Giemsa stain smears) with an infection rate of 70%.,Clinically infected animals showed different clinical manifestations which include partial or complete loss of appetite , anemia which manifested with pale and / or icteric mucous membranes which were detected on conjunctival ,nictitating and vaginal membranes, however icteration were more clear on sclera ,Furthermore decrease milk production were mentioned by the cow owners in milk producing cattle , rapid and difficult respiration, enlargement of superficial lymph nodes specially prescapuler lymph nodes ,in addition other animals were suffering from rough coat , lethargy and weight loss, However ,edema of lower hind limbs were detected in some infected animals .(Table 1).

Table 1: Clinical signs of infected cattle with Mycoplasma wenyonii

Clinical signs	Infected cattle n=140	%
Partial or complete loss of appetite	130	92.8
Anemia with pale and / or icteric mucous membranes	128	91.4
Decrease milk production	34	24.2
Rapid and difficult respiration	128	91.4
Enlargement of superficial lymph nodes	102	72.8
Rough coat	98	70
Lethargy	82	58.5
weight loss	82	58.5
Edema of lower hind limbs	34	24.2

Statistically significant increase ($p<0.05$) were encountered in body temperature, respiratory and heart rates, capillary refilling time, However ruminal contractions was decreased significantly (Table 2).

Table 2: Body temperature, respiratory and heart rate ,capillary refilling time and ruminal contractions of diseased cattle and controls.

Parameters	Controls n=25	Diseased cattle n=140
Body temperature C °	38.36 ± 0.29	40.5 ±2.8 **
Respiratory rate/ mint	22.72 ±6.37	81.5 ±9.2 **
Heart rate/ mint	57.6 ±3.5	121.3 ±18.6 **
Capillary refilling time / mint	1.33 ± 0.57	5.63 ± 0.82 **
Ruminal contractions / 5 mints	3.53± 0.72	1.64± 1.43 **

Values are mean ± standard error of mean. ** (P<0.05).

Mycoplasma wenyonii appears coccoid or rod shape, structures as well , However it might found individually or in chains on the erythrocyte cell wall . Figure,1and 2.

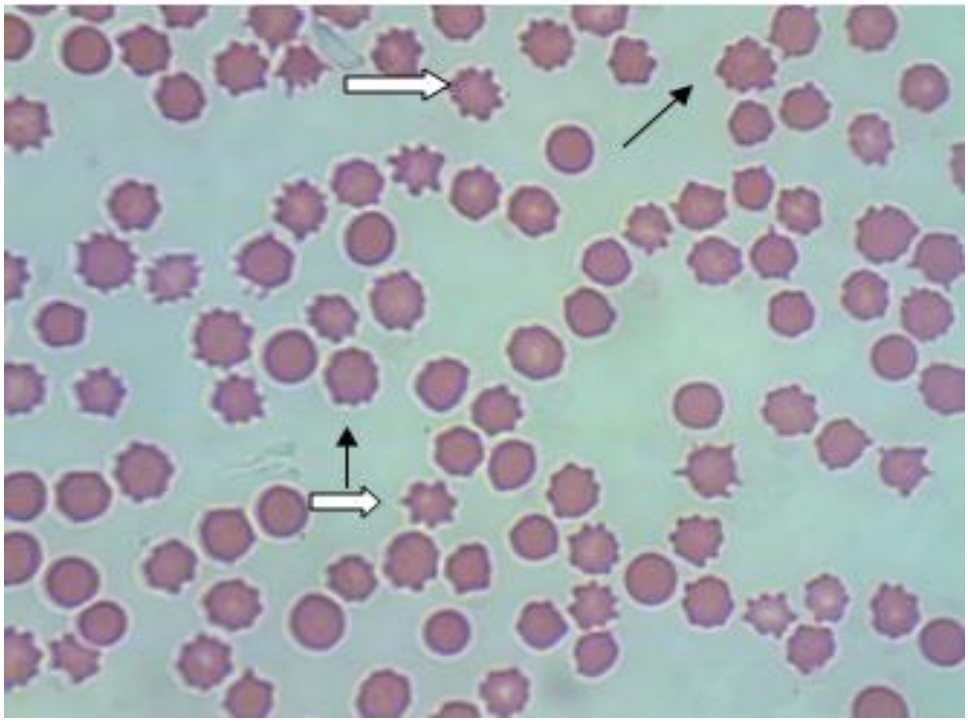

Figure 1:*Mycoplasma wenyonii*.on the erythrocyte cell wall
Giemsa stain ×1000

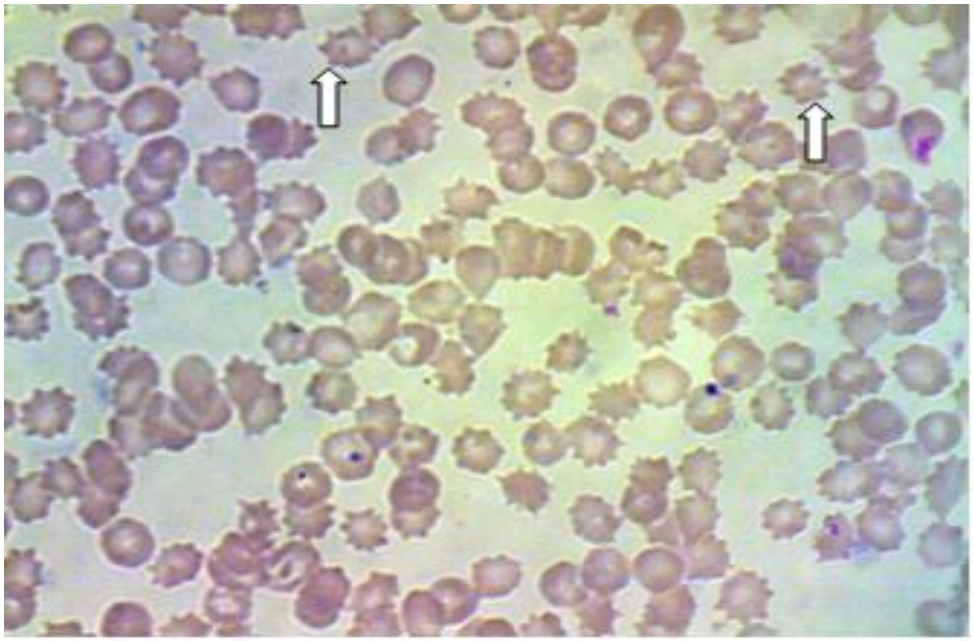

Figure: 2: Infected erythrocytes with High parasiteamia.
Wright stain ×1000

Moreover diagnosis of *Mycoplasma wenyonii* were confirmed by PCR.Figure 3 .Since out of (96) blood samples (80) (83.3%) were found positive by this technique.

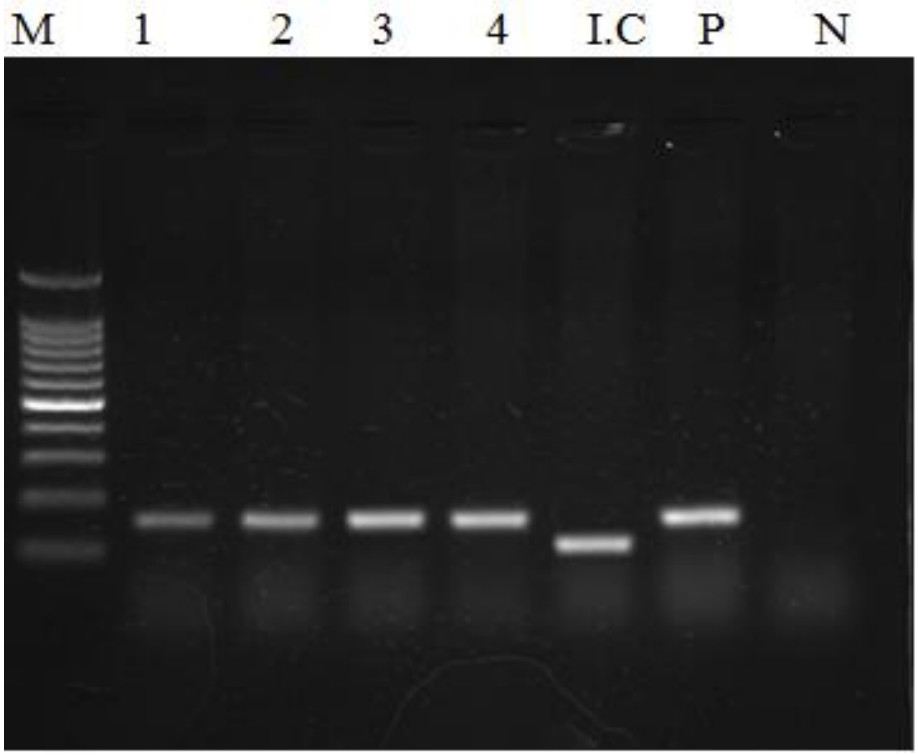

Figure:3 amplification of Mycoplasma wenyonii genomic DNA by PCR

Lane M: BrigTM Molecular Weight Marker (Bioingentech Ltd.)

Lane 1,2,3 and 4: *Mycoplasma wenyonii* Positive samples(180-bp

Lane I.C.: Internal control(140-bp)

Lane P: Positive control(180-bp)

Lane N: Negative control

Nevertheless different abnormal shaped and size erythrocytes were also indicated such as Acanthocytosis , Anisocytosis (macrocytosis and microcytosis) and Hypochromasia were detected in blood smears of infected cows. Figure 4 and 5

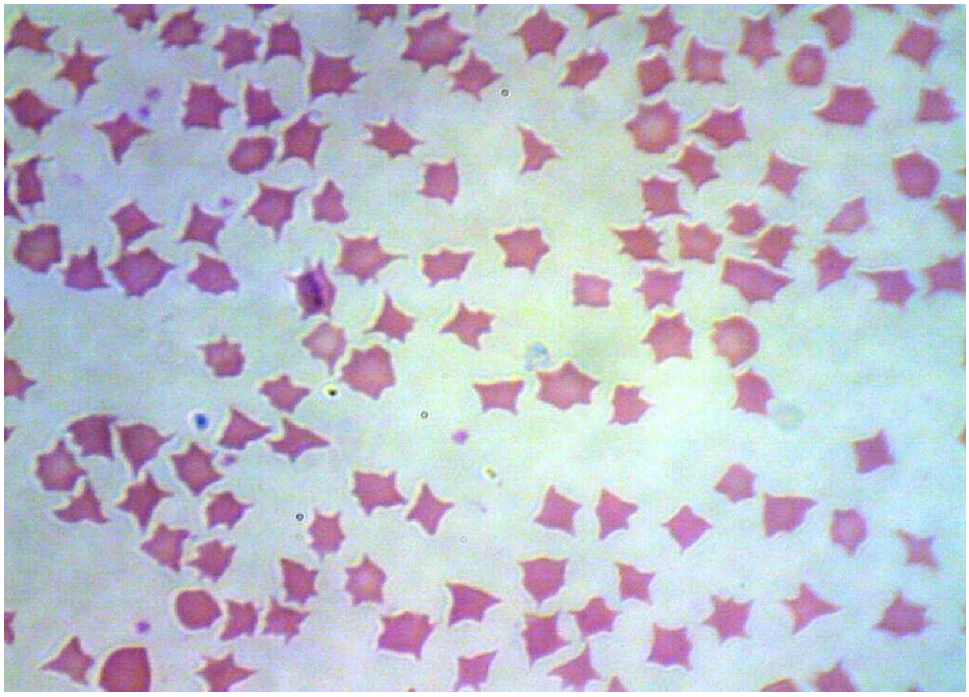

Figure 4:Acanthocytes in blood smear of infected cow .Giemsa stain ×1000

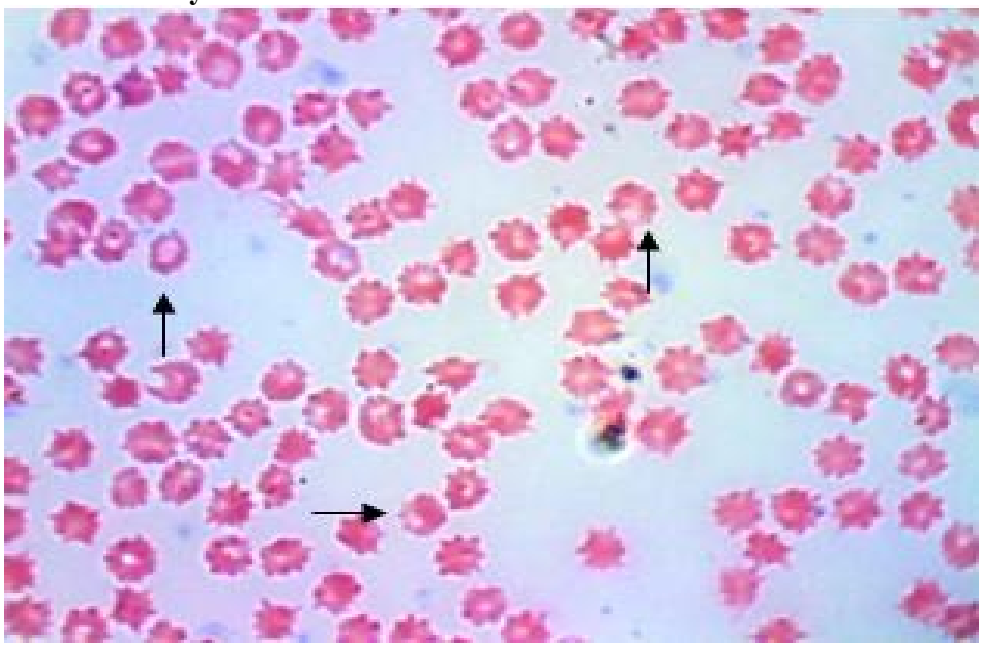

Figure: 5 Infected erythrocytes of cattle with Hypochromasia Giemsa stain ×1000

Results were also revealed that animals of 2-3 years old were highly infected compared with other age groups. Table .3

Table .3 :Percent of Infection rate according to age group

Age group	No. diseased animals	% Infection rate
New born calves 1-5 days old	30	21.4
Calves under one year old	28	20
Cow of 2-3 years old	46	32.8
Old cows more than 5 years	36	25.7
Total	140	70

Data related to hematological parameters indicated significant decrease, ($P<0.05$) in Total erythrocytes (TRBCs), hemoglobin concentration (Hb) and packed cell volume (PCV) , since it reflected Macrocytic Hypochromic type of anemia Moreover ,results were also indicated significant increase ($p<0.05$) in total leukocytes count which were due to significant increase ($p<0.05$) lymphocytes (Table 3 and 4).

Table 3: Blood parameters of diseased cattle and controls

Parameters	Controls n=25	Diseased cattle n=140
RBC $\times 10^6$	7.55± 1.46	4.72 ± 1.65 **
Hb g/dl	13.2 ± 1.83	7.33 ± 2.21 **
PCV %	35.7 ± 6.72	23.46±7.91 **
MCV /fl	45.2 ± 5.43	50.21± 5.72 **
MCHC/dl	36.9 ± 7.63	31.24 ± 7.24 **

Values are mean ± standard error of mean. ** (P<0.05).

Table 4:Total and absolute differential leukocytes count of diseased cattle and controls.

Parameters	Controls n=25	Diseased Cattle n=140
TLC $\times 10^3$	9.83 ± 6.22	15.63 ± 7.53 **
Lymphocytes	4353 ± 255.12	8798 ± 735.83 **
Nutrophiles	4382 ± 521.32	4326.265 ± 536.11
Monocytes	549 ± 363	557 ± 315
Eosinophiles	389 ± 21	391 ± 23
Basophiles	81 ± 63	81 ± 66

Values are mean ± standard error of mean. ** (P<0.05).

Changes of blood clotting factors indices were also noticed in diseased cattle with *Mycoplasma wenyonii* infection compared with controls and the results showed significant decrease ($P<0.05$) in the mean values of total Thrombocytes count and Fibrinogen time, whereas significant increase ($P<0.05$) were detected in

Thrombocytes volume, Thrombocytes distribution width, clotting time, prothrombin time and activated partial thromboplastin time. Table (5).

Table 5:Indices of clotting factors in diseased cattle and controls.

Parameters	Controls n=25	Diseased Cattle n=140
Total Thrombocytes count $\times 10^3$	569.533 ± 76.744	332.233 ± 62.53**
Thrombocytes volume /fl	11.535 ± 6.141	16.289 ± 1.922 **
Thrombocytes distribution width / %	14.653 ± 1.864	24.292 ± 5.788 **
Fibrinogen time / sec	20.54± 2.27	13.26 ± 5.21**
Clotting time / mint	3.563 ± 1.723	5.234 ± 2.755 **
Prothrombin time /sec	14.276 ± 2.551	31.423 ± 3.622 **
Activated partial thromboplastin time /sec	52.534±6.443	69.455± 13.663 **

Values are mean ± standard error of mean , (P<0.05).**

DISCUSSION

There were no scientific document clarify the registration of *Mycoplasma wenyonii* in Basrah governorate and other south parts of Iraq in cattle , Nevertheless bovine clinical infection hade been documented and seen in Mosul ,North of Iraq , (11 and 12) whom mentioned that , It seems these concurrent infections are in animals imported from the neighboring countries such as Turkey, Saudi Arabia and may be Iran .

Hemoplasmas , occurs in most domesticated animals such as cattle , Buffaloes ,sheep, Swine, llamas, dogs and cats and has greater clinical occurrence in those animals ,However latent Hemoplasmas might also affected mules, deer, elk and goats since the organisms mostly appear to be species specific.(16).Those organisms were incriminated and involved to rickettsial (Mycoplasma) parasite of the mammalian erythrocytic cell membrane worldwide causing a febrile and haemolytic clinical disease in a different livestock, especially food animals (17).

In the current study infected animals show different clinical manifestations which were agreed with those mentioned by (1,2 and 3), since difficult and rapid respiration which have been detected in diseased animals were due to Anemic hypoxia, as decrease erythrocytes count and hemoglobin concentration were affected the oxygen transmitted to body tissues, thereby failure of tissues to receive an adequate supply of oxygen will occur, and panting with Dyspnea of diseased animals were detected clinically, (16). The presences of pale mucus membranes will exhibited the development of anemia and reduction of blood indices concentration was due to destruction and removal of parasisetized erythrocytes by the reticulo-endothelial

system ,Whereas icteric mucus membranes which were also seen reflected the progressive anemia and bilirubinemia, developed in advance diseased animals (18).

Lethargy which had been shown by diseased cattle might occur due too decrease muscle mass confirmed by decrease values of serum creatinine, presumably associated with the poor body condition(19).

It have been proved that following experimental infection there is a variable prepatent period, usually last for 1-3 weeks, which is followed by a period of intense parasitemia, Ring form, coccoid and rod-shaped organisms are evident in stained blood smears, Moreover , The organism is epicellular, infecting the surface and periphery of erythrocytes, Nevertheless it were also could found free in the plasma in blood examinations.(7). On the other hand there is a profound hypoglycemia during the parasitemic phase which is believed to be due to direct consumption of glucose by the parasite.(20). The period of intense parasitemia lasts for a period of 5-10 days following which visible organisms in the blood become much less frequent and anemia develops(21). Parasitized erythrocytes are removed from the circulation by the spleen , It is believed that the parasite alters the erythrocyte membrane, exposing new antigenic determinants and stimulating the development of antierythrocyte antibodies, Moreover, the severity and duration of the anemia varies between individuals but commonly lasts from 1-2 months , In addition during recovery stage there may be further cycles of parasitemia and anemia which might become less severe However, death which will follow occur due to anemic hypoxia (22).

Some diseased animals were also show edema of lower limbs and this were also mention by (11). As well the development of edema always need a change in one or more of a forces in a direction that might supported an increase in net filtration, However This can be produced by an increase in capillary hydrostatic pressure, capillary permeability, or interstitial usual venous pressure, or by a reduction in the plasma oncotic pressure, Furthermore, edema it also can be induced by obstruction of lymphatic tissue parts , since the fluid that is normally filtered is not returned to the systemic circulation.(23).Moreover Diskin *et al* (24) were also added that increased venous pressures due to central or regional venous obstruction or to an expansion in plasma volume are transmitted to the capillary bed, thereby increasing hydrostatic pressure and predisposing to edema , However hypoprotenemia which may expected to occur were also play good role .

Enlargement of superficial lymph node were mention in the current study as one of the clinical manifestations encountered by diseased animals , it have been reported that some plasma and cells in the interstitial space, along with certain cellular material, antigens, and foreign particles enter lymphatic vessels, becoming lymphatic fluid However, Lymph nodes filter the lymphatic fluid on its way to the central venous circulation, removing cells and other material, Moreover The filtering process also presents antigens to the lymphocytes contained within the nodes, Furthermore, the immune response from these lymphocytes involves cellular proliferation, which can cause the nodes to enlarge (lymphadenopathy),(25), In addition Smith (19) , added that pathogenic microorganisms carried in the lymphatic fluid can directly infect the nodes, causing lymphadenitis .

Increase body temperature ,respiratory and heart rate were also mentioned by Adresi and saki (1) which reflected the acute phase of the disease, However decrease ruminal contractions reflected the atony of ruminal smooth muscles which mostly reflected by lack of ruminal fibers followed by anorexia (16).

Results were also indicated increase capillary refilling time in diseased cattle compared with controls .It have been documented that the capillary refilling time is a quick test done used to monitor some disease problems such as dehydration ,shock ,peripheral vascular disease and hypothermia, thereby prolong time of the test might reflected the less amount of blood flow reaches to tissues which were indicated in infected animals of current study (21).

Mycoplasma wenyonii appears coccoid or rod shape, structures however it might found individually or in chains on the red blood cells, same results were also mention by others (7) ,However ,Sudan *et al* (17) added that microscopic examination of Giemsa stained blood smear evidenced characteristic light pinkish to blue stained cocci (0.5–1.0 μm diameter) and/or short rod (1–3 μm longer) shaped rickettsial pathogens nesting in the depressions on the periphery of erythrocyte cell membrane as well as extra cellular free rickettsial bodies in the plasma, some studies have also favored the use of PCR as an aid in diagnosing bovine hemoplasmosis(6) , PCR amplification can be perform directly from whole blood for detecting blood organisms, since this test were detected the organism even in very small amounts, as the method allows direct detection of pathogens in a blood sample (26). In this study, (96) cows blood samples were used to molecular analysis to confirm the presence of *Mycoplasma wenyonii*, Therefore infected animals gave a strong bands on this

technique, Moreover, this finding is agreed with the results that obtained by others(27).

High infection rate were indicated in age group of 2-3 years old ,since this result were consistent with (3 and 16).

Anemia which were indicated in the present work occur because of significant decrease in values of TRBc, Hb and PCV ,Moreover Macrocytic hypochromic type were indicated ,same results were also documented by (18), Moreover Radostitis *et al*(16) were also mention that the hemolysis caused by hemoplasma infections is typically extra vascular and results in regenerative anemia with erythrocyte agglutination may be present, In addition the increase in MCV shows the appearance of immature red blood cells and is the index of regenerative anemia (17).Increase in total leukocytes counts and lymphocytosis might indicated increase in immune system capability (cellular immune excess) which were agreed with (18 and 28).

Little document had been mention the relation of hemoplasma infection and the effect on clotting factors indices, Nevertheless in infected animals thrombocytopaenia might occurs regularly in acute stages of the disease although the reduction of platelets count does not always result in marked hemorrhages, even though the cause of decrease platelets count is not completely clear , However megakaryocytes lysis and reduced production of thrombocytes by megakaryocytes, with increased consumption of platelets in the periphery, and defects of its functions might all been suggested as factors predispose to decline its levels (19).Moreover hemorrhagic diathesis were only indicated when platelets count are too low (29).

In the present study clotting factors indices were indicated clear disturbances in clotting system of diseased animal with imbalanced regulation may lead to hyper coagulation and / or hypo coagulation which might indicated the initiation of disseminated intravascular coagulation (30).

دراسة سريرية ،دمية وتشخيصية لخمج الابقار بالمايكوبلازما الدموية في محافظة البصرة، العراق

علي جراد ، كمال الدين مهلهل السعد

فرع الطب الباطني والوقائي ،كلية الطب البيطري ،جامعة البصرة
البصرة ،العراق

الخلاصة

تم في هذا البحث دراسة وتشخيص مرض المايكوبلازما الدموية في الابقار المحلية ،اذ شملت الدراسة فحص مئتان وخمس وعشرون من الابقار المحلية ومن كلا الجنسين وبحسب الفئات العمرية قسمت الحيوانات إلى اربعة مجاميع (خمسون حيواناً) لكل مجموعة (عجول حديثة الولادة بعمر 1-5 يوم ،عجول بعمر اقل من سنة ، ابقار بعمر 2-3 سنة وابقار بعمر اكثر من خمسة سنوات) في محافظة البصرة، البصرة – العراق . حيوانات الدراسة كانت خمجه سريريا بالنوع *Mycoplasma wenyonii* والتي شخصت بالاعتماد على فحص المسحات الدموية المصبوغة بصبغة كمزا وتم تأكيد التشخيص باستخدام فحص البلمرة المتسلسل كما تم فحص خمس وعشرون بقرة محلية سوية سريرياً عدت كمجوعة سيطرة.اظهرت الحيوانات المريضة علامات سريرية تمثلت بفقدان الشهية ،شحوب الاغشية المخاطية وبخاصة المبطنة للعين او المهبل ،انخفاض انتاج الحليب ،تزايد ترداد التنفس وصعوبتة ،تضخم العقد اللمفية السطحية، خشونة الجلد،الخمود،وذمة الاطراف الخلفية مع فقدان الوزن . فضلا عن ذلك فقد ارتفعت درجات حرارة الجسم ،ومعدلات ترداد التنفس وضربات القلب وزمن رجوع الدم في الاوعية الدموية وبشكل معنوي في الحيوانات الخمجة بالمقارنة مع حيوانات مجموعة السيطرة ، كما لوحظ تناقص في معدلات تقلصات الكرش . لوحظ النوع *Mycoplasma wenyonii* في المسحات الدموية بشكله المكور والعصوي متطفلا على جدار كريات الدم الحمر ومتجمعاً بشكل منفرد أو بهيئة سلاسل مفردة، كما اكد فحص البلمرة المتسلسل إن (80.3%) من الحالات المفحوصة كانت موجبة للفحص، وقد سجلت النتائج ان اعلى نسبة خمج سجلت في الابقار بعمر 2-3 سنة بالمقارنة مع الفئات العمرية الاخرى .ازدادت وبشكل معنوي معدلات العدد الكلي لكريات الدم الحمر، تركيز خضاب الدم وحجم خلايا الدم المرصوصة في الحيوانات المريضة بالمقارنة مع حيوانات مجموعة السيطرة ، اذا سجل فقر الدم من النوع ذي الكريات كبيرة الحجم سوية الصباغ ، كما اتضح من نتائج الدراسة حدوث زيادة معنوية في العدد الكلي لخلايا الدم البيض بسبب تزايد الخلايا اللمفية معنوياً . كما سجلت نتائج الدراسة حدوث الاختلاف في عوامل تخثر الدم إذ لوحظ تناقص معنوي في معدلات العدد الكلي للصفيحات الدموية ،وقت منشيء الليفين في حين سجل تزايد في معدلات حجم الصفيحات الدموية ومعدل انتشارها ،زمن تخثر الدم ، زمن سابق الخثرين وزمن حرك الخثرين الجزئي بالمقارنة مع مجموعة حيوانات السيطرة . استنتج من هذه الدراسة ان الابقار المحلية تصاب بالنوع *Mycoplasma wenyonii* مما يؤدي الى تأثيرات جانبية كبيرة قد تنتهي بموت الحيوان المصاب .

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