CLINICAL ,HEMATOLOGICAL AND DIAGNOSTIC STUDIES OF HEMOMYCOPLASMA INFECTION (MYCOPLASMA OVIS) IN SHEEP OF BASRAH GOVERNORATE

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(Received 17 May 2017, Accepted 4 June 2017)

Keywords: Hemomycoplasma *ovis*, Diagnosis, Acute phase response *Correspond Author e. mail:kamalsad58@yahoo.com*

ABSTRACT

Hemomycoplasmosis caused by Mycoplasma ovis was diagnosed in sheep of Basrah Governorate .The study were conducted on (225) sheep reared in different areas of Basrah governorate. Animals are of different ages and of both sexes. Twenty five clinically healthy sheep were considered as controls .Complete clinical examinations were applied to all animals, and fecal samples screened for parasitic loud. Results revealed that infected sheep show signs of anorexia, anemia with pale or icteric mucous membranes of eyes and vagina, rapid and difficult respiration, enlargement of superficial lymph nodes, rough wool coat and hemoglobinuria, Moreover milk production were decreased in lactating ewes. On clinical examinations statistically significant increase were indicated in body temperature respiratory and heart rate of diseased sheep then in controls. Diagnosis of Mycoplasma ovis were leant on examination of stained blood smears with Giemsa Since the organism appear as small coccoid or rod shape structures and it could be found as an singular or in chains on the cell membranes of infected erythrocytes of diseased animals, Furthermore the diagnosis were confirmed by indirect Elisa test. Results of hematological examinations show significant decrease in the values of total erythrocytes count ,hemoglobin concentration and packed cell volume indicating macrocytic normochromic type of anemia, However, the rate of sedimentation of red blood cells indicated significant increase in diseased sheep, Moreover, Leucocytosis due to significant increase in lymphocytes number were also registered in infected sheep then in controls, In addition evaluation of acute phase response show significant decrease in haptoglobin values and fibrinogen time in diseased sheep compared with controls. Significant decrease in total protein were detected, However

values of total and indirect bilirubin, GGT, AST, ALP, and BUN were significantly increased in diseased sheep compared with controls. It had been concluded that Hemomycoplasmosis ovis were infected sheep of Basrah governorate lead to hemolytic anemia and substantial effect might terminated with highly mortalities, Therefore all suspected sheep in the chancy area must screened for microorganism loud.

INTRODUCTION

Hemomycoplasma (Haemotrophic mycoplasmas or Epyrthrozoon) species are those microorganisms which causes infectious anemia in different mammalian and there effects were vary from mild to death (1,2,3), Haemotrophic mycoplasma are small, pleomorphic, uncultivable bacteria which parasitize the surface of red blood cells of a wide range of mammalians and can induce erythrocytes deformities and damage (4,5,6). They were reclassified as genus *Mycoplasma* depending on 16S rRNA sequences and morphologic similarities, and have been distributed and identified in different countries in the world (7). The disease manifested by high fever, anorexia, anemia, rough coat ,decreased milk production in lactating animals, enlargement of superficial lymph nodes, weakness and emaciation, Moreover hemoglobinuria were also detected specially in sheep and goats, Furthermore the classical infection are always acute although subclinical and / or chronic form were suspected and diagnosed (8). This organisms are transmitted mechanically by blood-sucking arthropods and parasitizes different domesticated animals such as cattle and buffaloes. However small ruminants, sheep ,goats and also ghazals were also infected throughout the world (5,7).

In sheep hemomycoplasmas is caused by *Mycoplasma ovis* (Formerly, *Eperythrozoon ovis*) is an uncultivated, wall-less bacterium that parasitizes the surface of sheep erythrocytes and diagnosis is made mostly by detection of organisms on erythrocytes in Romanowsky type or acridine orange-stained blood smears and also by serological methods, Furthermore parasitism of erythrocytes often occurs at high levels and parasitemia might become more than 100%, However, detection requires some times to examination of repeated blood smears (9,10). This organism has been thought to be a rickettsia because of its obligate parasitism, erythrocyte localization, small size, staining properties and transmission by arthropod vectors, Moreover the organism

have been classified in the order Rickettsiales, family Anaplasmataceae, in the genera Haemobartonella and Eperythrozoon, However ,Recently, phylogenetic analysis of 16S rRNA gene sequences has demonstrated that these wall less bacteria are not rickettsiae, but that they are actually mycoplasmas (Non hemotropic mycoplasma) (11,12,13).

Hemomycoplasmosis caused by *Mycoplasma weynioni* had been detected and registered In cattle of Mosul province (14,15) and In cattle and buffaloes of Basrah Governorate(16,17), Moreover little information had been provided on infection with *Mycoplasma ovis* in small ruminants therefore the present study designed to study the clinical ,hematological and diagnosis of these organism in sheep of Basrah governorate.

MATERIALS AND METHODS

Animals and clinical examinations:-

The study were conducted on (225) local sheep breeds reared naturally in different areas of Basrah governorate. Animals are of different ages and of both sexes. local sheep breeds show different clinical signs of high body temperature, tachycardia, difficult respiration, anorexia, anemia manifested by pale (and or) icteric mucous membranes, rough coat, and decrease milk production. Twenty five (25) clinically healthy local sheep were considered as controls.

Complete clinical examinations had been applied to all animals, and fecal samples of all animals were screened for parasitic loud using the standard coprological methods.

Collection of blood :-

Ten milliliters of blood (10 mL) were drained from each animal by jugular puncture and 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), and Total leukocytes count (TLC), (Hematology analyzer, Genex, USA), Moreover differential leukocytes count were done using Giemsa stain blood smears method according to Weiss, and Wardrop (18), Furthermore erythrocytes sedimentation rate (ESR)were also estimated according to (19).

Serological and biochemical analysis:-

Serum were obtained for evaluation of acute phase response including estimation of Haptoglobin (Haptoglobin Elisa method)(Biotechnology co -china) and Fibrinogen (Biolabo / France). Moreover , evaluation of Total bilirubine , direct and indirect

bilirubin, Total protein, Alkaline phosphatase (ALP), , Aspartate aminotrasfese (AST), Gamma glutamyltransferas (GGT) and blood urea nitrogen (BUN) have been done according to manufacture instructions of (Roche Diagnostics, Indianapolis, GMBH, Germany).

Infection with *Mycoplasma ovis*. was diagnosed on the basis of Giemsa staining blood smears and was confirmed by Indirect ELISA test (Eperythrozoon ovis ELISA kit, Biotechnology co -china)

Statistical analysis:-

Data were analyzed and the significant difference between diseased and control group were statistically indicated using (SPSS program) student t-test (20).

RESULTS

Clinically infected sheep show signs of anorexia (88.88%), anemia with pale (and or) icteric mucous membranes specially of eyes and vagina (83.55%), rapid and difficult respiration (76.88%), enlargement of superficial lymph nodes specially prescapuler lymph node(73.77%), rough wool coat of disease animals (58.66), hemoglobinuria with passing of dark color urine (41.77%), Moreover, milk production were decreased in lactating ewes (39.11%) (Table 1).

Table 1: Clinical signs of infected sheep with Haemomycoplasma (Mycoplasma ovis)

Clinical signs	Infected sheep n=225	%
Anorexia	200	88.88
Anemia with pale (and or) icteric mucous membranes	188	83.55
Rapid and difficult respiration	173	76.88
Enlargement of superficial lymph nodes	166	73.77
Rough wool coat	132	58.66
Hemoglobinuria	94	41.77
Decrease milk production in lactating ewes	88	39.11

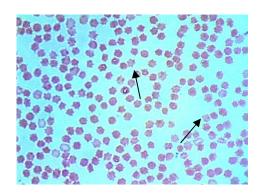
On clinical examinations of infected animals with hemoycoplasmosis statistically significant increase(p<0.05) were indicated in body temperature ,respiratory and heart rate of diseased sheep then in controls (Table 2).

Table 2: Body temperature, respiratory and heart rate of diseased sheep with Hemomycoplasma (Mycoplasma ovis) and controls.

Parameters	Controls n=25	Diseased sheep n=225
Body temperature C°	38.64 ± 0.58	41.2± 1.22**
Respiratory rate/ mint	21.54 ±6.33	$68.3 \pm 7.2**$
Heart rate/ mint	82.43 ± 5.63	132.2 ±18.27**

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

Examination of stained blood smears with Giemsa stain revealed that *Mycoplasma ovis* seems to be small cocoid or rod shape structures and it could be found as an singular or in chains on the cell membranes of infected erythrocytes of diseased animals . Fig,1 and 2.



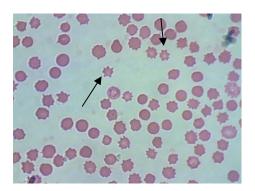


Fig 1,2: Mycoplasma ovis on erythrocyte membrane Giemsa stain ×1000

Moreover different abnormal sizes and shape were detected on microscopic examination of infected erythrocytes of diseased sheep.

Ninety six (96) of suspected infected sheep serum samples were used to confirm the diagnosis with *Mycoplama ovis* using indirect Elisa test and the results indicated that all suspected serum samples were show positive results, Table 3.

Table 3: Detection of specific antibodies against *Mycoplasam ovis* by indirect Elisa test

Animal	No.of samples	Seroposative	Seroprevalence %
Sheep	96	96	100

Results of hematological examinations show significant decrease (p<0.05) in the values of total erythrocytes count ,hemoglobin concentration and packed cell volume indicating macrocytic normochromic type of anemia comparing with controls , Furthermore the rate of erythrocytes sedimentation of red blood cells indicated significant increase (p<0.05) in diseased sheep ,Moreover ,Leucocytosis due to significant increase in lymphocytes number were also indicated in infected sheep then in controls ,Table (4) and (5).

Table 4: Blood parameters of infected sheep with Hemomycoplasma (Mycoplasma ovis) and controls

Parameters	Controls n= 25	Infected sheep n =225
$RBC \times 10^6$	8.45 ± 1.32	5.48 ± 1.76 **
Hb g/dl	10.7 ± 1.45	6.78 ± 1.82 **
PCV %	35.27 ± 3.56	22.93 ± 4.31 **
MCV / fL	32.96 ± 2.58	41.84±3.22 **
MCHC / g/dl	30.33 ± 4.24	29.56 ± 4.38
ESR mm/24hr	8.33 ± 4.32	19.45± 5.13 **

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

Table 5: Total and absolute differential leucocytes count of infected sheep with Hemomycoplasma (Mycoplasma ovis) and controls

Parameters	Controls n= 25	Infected sheep n =225
TLC ×10 ³	9.858 ± 2.45	14.504 ± 4.67 **
Nutrophiles	4122±777.38	4194.35 ± 523.22
Lymphocytes	5320 ± 521.11	9898.43± 235.14 **
Monocytes	170 ± 56.23	175 ± 42.32
Esinophiles	211± 84.21	200.35 ± 19.67
Basophiles	35 ± 7.33	37.67± 11.42

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

Evaluation of acute phase response show significant decrease (p<0.05) in the values of haptoglobin and in fibrinogen time in infected sheep with *Mycoplasma ovis* compared with controls, Table 6.

Table 6: Acute phase response of infected sheep with Hemomycoplasma (Mycoplasma ovis) and controls

Parameters	Controls n= 25	Infected sheep n =225
Haptoglobin g/dl	$0.023 \pm \ 0.012$	$0.009. \pm 0.004**$
Fibrinogen time / sec	25.91± 7.41.	$17.85 \pm 9.13**$

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

Results were also show that significance difference in different biochemical test were encountered between sheep infected with *Mycoplasam ovis* and controls since results indicated significant decrease (p<0.05) in the values of total protein ,However values of Total and indirect bilirubin, GGT, ALT, ALP, and BUN were significantly increased (p<0.05) in diseased sheep compared with controls ,Table 7.

Table 7:-Biochemical parameters of infected sheep with Hemomycoplasma (Mycoplasma ovis) and controls

Controls Parameters Infected sheep n = 25n = 225Total protein (g/dl) 7.12 ± 1.34 5.42±1.39** Total bilirubin (mg/dl) 0.48 ± 0.13 $0.82\pm0.24**$ Direct bilirubin (mg/dl) 0.32 ± 0.18 0.31 ± 0.11 0.16 ± 0.11 $0.51 \pm 0.21**$ Indirect bilirubin (mg/dl) GGT (U/L) 80.34 ± 2.78 110.43± 12.23** AST (U/L) 22.56 ± 1.78 $53.85 \pm 3.54 **$ ALP (U/L) 119.35 ± 4.89 $249.34 \pm 12.56**$ $55.32 \pm 7.51**$ BUN (U/L) 14.88 ± 2.67

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

DISCUSSION

Little information had been provided on hemomycoplasmosis caused by *Mycoplasma ovis* at Basrah governorate, since the disease were registered previously in cattle and buffaloes (16,17), Moreover it have been also diagnosed in other parts of Iraq (14,15).

Hemomycoplasma infect different domestic animal species where cattle, buffaloes, camels and swine are always susceptible, Furthermore the organism could also infect dogs, cats and small ruminants such as sheep, goats and ghazals (2,3). The organism

Hemomycoplasma were classified in the order Rickettsiales based on morphology and its response to antibiotic therapy, However, molecular studies based on the 16S rRNA gene have shown that the genera Haemobartonella and Eperythrozoon are more closely related to the mycoplasmas, leading to the re-classification within the genus Mycoplasma (5,9). In the current study diseased sheep show different clinical signs most of them were belong to and mentioned by others(3,4,7), Where the increase of body temperature of diseased sheep reflect the acute feature of the disease since it indicate the liberation of endogenous pyrogens of the causative agents and due to cellular lyses stimulating thermoregulatory centers of the hypothalamus in the brain, Moreover the severity of fever might depend on the severity of causative microorganism, the type of lesion and the form of the disease status (22). Increase respiratory and heart rate might reflect the systemic reaction occur due to the acute crises of the disease and the anemic pattern caused by the disease since rapid respiration were affected diseased sheep might occur because of anemic hypoxia as a result of decrease erythrocytes count and hemoglobin concentration which affected the oxygen transmitted to different tissues of the body, Therefore failure of tissues to receive an adequate supply of oxygen will occur, and increase abdominal type of respiration of diseased sheep were detected clinically, (23,24).

Pale (and or) icteric mucus membranes exhibited the development of anemic phase and reduction of blood indices concentration which were due to destruction and removal of infected erythrocytes by the reticuloendothelial system ,Moreover , icteric mucus membranes which were also detected in some diseased animals reflected the progressive anemia and increase libration of indirect bilirubine, which might developed in advance stages of ovine hemomycoplasmosis (25,26). Sings of hemoglobinurea were considered as unusual clinical manifestation although it were mention by (9,27) in diseased sheep , since hemoglobinurea reflected the intravascular hemolysis of erythrocytes in infected animals as the destruction of RBCs might occur due to increase phagocytic activities of the reticuloendothelial system beside increase the fragility mechanism of the infected red blood cells due to conception of the important menial components of the cell wall by the microorganism(23),The destructed erythrocytes were released its hemoglobin materials which in turn passed through the urinary tract (the kidney) and discolored the urine to brownish or dark coffee like color (22).

Enlargement of superficial lymph nodes which were mentioned in the current study might occur because of the hyperplasia of the lymphoid tissue due to proliferation of the microorganism inside this type of tissue terminating as development of inflammatory reactions of the affected node (80), Furthermore, McGavin, and Zachary[28], were added that multiplication of cells within the node, including lymphocytes, plasma cells, and even monocytes, as well as the draining of an antigens as infection will lead to palpable enragement of the affected lymph node.

Examinations of Giemsa stained blood smears of infected sheep revealed that *Mycoplasma ovis* are small coccoid or rod shape structures and it could be found as an singular or in chains on the cell membranes of infected RBCs of diseased animals, same result were mentioned by others, (4), Moreover, The period of high parasitemia may lasts for more than five days, However then the organism might become less frequent since anemia were developed (5).

Infected erythrocytes are removed from the circulatory system by the spleen, Since its believed that the organism was make an alteration of the diseased erythrocytes membrane, exposing new antigenic determinants and stimulating the development of ant-erythrocyte antibodies, Moreover, the severity and duration of the anemia varies between animals but commonly lasts from more than one month or might be more, Furthermore during the stage of recovery there may be further cycles of parasitemia and anemia which might become less severe, However, death which will follow occur mostly due to anemic hypoxia (6,13,29).

Results indirect Elisa test revealed that all infected sheep Hemomycoplasmosis were seropositive to specific antibodies detected, similar results were also recorded by (22,25) whom indicated that indirect Elisa test might be an alternative for increased and sensitive detection of acute and also latent Mycoplasma ovis infections. Since in the indirect Elisa test, the sample antibody is sandwiched between the antigen coated on the plate and an enzyme-labeled anti-species globulin conjugate, beside that the addition of an enzyme substrate-chromogen reagent causes color to develop, this color is directly proportional to the amount of bound sample antibody thereby the more antibody present in the sample, the stronger the color development in the test wells, thus, this format of indirect Elisa is suitable for determining total antibody level in samples, Moreover, Novacco, (30) added that Elisa using recombinant antigens which were developed as a more specific method for the serodiagnosis of Hemomycoplasmosis, Furthermore, Constable, (3), refers that the disease were more pathogenic and more common and distributed in endemic countries.

Results of hematological examination indicated anemia of diseased sheep compared with controls, Since the indicated anemia had been occur because of significant decrease in values of total erythrocytes count hemoglobin concentration and packed cell volume ,Furthermore in the current study macrocytic normochromic type of anemia were encountered, same results were also documented by (24,26), Where the hemolysis caused by hemomycoplasma infections is typically intravascular and results in regenerative type of anemia with erythrocytes agglutination may be present, In addition the increase in mean corpuscular volume (MCV) shows the appearance of immature red blood cells and is the index of regenerative anemia (3,23), Furthermore, Neimark, (9) added that hemolytic anemia that diagnosed is often transient and recurrent as well as being regenerative, characterized by reticulocytosis, polychromasia, macrocytosis with basophilic stippling, and Howell-Jolly bodies and the clinical syndrome is usually more severe in young, splenectomized, and immunocompromised animals, In contrast results were disagreed with (16,17) whom recorded macrocytic hypochromic type of anemia specially in cattle and buffaloes infected with Mycoplasma wynionii.

An increase in sedimentation rate of erythrocyte values was in agreement with (18,19) whose refers to the correlation between the sedimentation of RBCs and the intensity of anemia, where the increase settling of RBCs will take place when anemia are more intense and severe.

Increase in total leucocytes counts (Leucocytosis) and increase in lymphocytes number (lymphocytosis) which were indicated in the present study, might indicated increase in immune system capability and stimulation of bone marrow ,Since Leucocytosis can be a reaction to various infectious, inflammatory, and, in certain instances, up normal physiologic processes such as stress, or even unusual exercise, This reaction is mediated by several molecules, which are released or up regulated in response to stimulatory events that include growth or survival factors (cytokines) ,Since the peripheral leucocytes count is determined by several mechanisms, including the size of precursor and storage pool of myeloid and lymphoid cells ,the rate of release of the cells from the storage pool in the bone marrow, the rate of marginating cells out of blood vessels into the tissues, and finally the rate of consumption of the cells in the tissues (31) .The pronounced lymphocytosis induced

by infectious organism may be caused by stimulation for increased new cell production of lymphoid system and bone marrow as immune response against the *Mycoplasma ovis* or their toxins, Moreover lymphocytosis specially in Hemomycoplasmosis was also reported by (16,23), whose stated that significant increase in lymphocytes count were encountered and marked during the formation of antibodies in response to antigen during infection.

Depression of acute phase response were indicated in the current study reflected by significant decrease in both haptoglobin and fibrinogen values which were mentioned also by (32,33), As haptoglobin, binds free hemoglobin released from erythrocytes with high affinity, Therefore deterring its oxidative activity (34,35). Haptoglobin levels will be decreased in hemolytic anemia in the process of binding hemoglobin, because haptoglobin sequesters the iron within hemoglobin, preventing iron- utilizing organism from benefiting from hemolysis (36,37), Moreover ,Korman (38), added that a significant difference in the concentration of this biomarker in Hemomycoplasma infection were a trend for haptoglobin concentrations to decrease might be because circulating haptoglobin complexes with hemoglobin causing transient decreases in haptoglobin during hemolysis In addition Hypofibrinogenemia, were also indicated in this study ,where Fibrinogen is an acute phase reactant protein and the liver increases production of this protein in response to inflammatory cytokines, However, It is considered as a moderate, and might be delayed marker for some inflammatory processes, Since, concentrations may increase within 24-48 hours of an inflammatory stimulus and moderate increases are seen and values can remain increased for a while after resolution of the inflammation (39). Low fibringen values were indicating with early liver failure or insufficiency when hepatic functions reduced, congenital afibrinigenemia, hyper fibrinogenolysis decrease synthesis of fibrinogen and increase consumption of fibrinogen when disseminating intravascular coaggulopathy processes had been started (40).

Significant difference had been encountered in biochemical analysis of diseased sheep with Hemomycoplasmsis compared with controls ,where Hypoprotenemia are indicating in infected sheep compared with controls which were also mentioned by (41,42) ,whose stated that decrease protein levels during those types of infection may occur due to digestive disturbances and severe limitation of protein intake in the diet, destruction of proteins due to fever as macrophages activated in the liver and spleen secrete tissue necrotic factor (TNF-alpha) into the bloodstream resulting in

hypoproteinemia, Moreover decrees production and synthesis from liver specially albumin and when more plasma proteins loss in urine due to renal disease and nephritic syndrome.

Hyperbilirubinemia due significant increase into indirect bilirubin were also indicated in the current study which might resulted from excessive destruction of erythrocytes and the indirect hepatocellular damage, Furthermore (43,44) were added that measurement of indirect bilirubin might be helpful in measurement the increased breakdown of hemoglobin as in hemolytic anemia which will increase the production of unconjugated bilirubin, which is presented to the liver in excess, and can result in increased total bilirubine in blood.

The enzyme γ-glutamyl transferase (GGT) cleaves terminal glutamyl groups from amino acids and transfers them to another peptide or to an amino acid, It is important in glutathione metabolism, amino acid absorption and protection against oxidant injury (45), Although GGT is found in many tissues, the main source of serum activity is the liver (primarily biliary epithelium), Thereby, GGT is used mainly as a sensitive indicator of cholestasis (46), Significant increase of GGT had been encountered in diseased sheep with hemomycoplasmosis which have been thought that the increase values reflecting secondary biliary hyperplasia or induction of synthesis. Values of AST were also increase significantly in diseased sheep which were agreed with (39,45) whose stated that damage to the skeletal or heart muscles, hepatic tissues and erythrocytes may resulted in considerable increase in the level of due to the fact that bulk of those tissues throughout the body could be considered as an ample reservoir of enzymes liable to be released and detected during pathological situation, In addition Intravascular or in vitro hemolysis or leakage from cells can cause erroneously high activity of the enzyme which present in erythrocytes as well (47).

It have been documented that increases in serum ALP activity are usually due to the liver problems or hepatobiliary disease, bone and corticosteroid-inducible isoforms, Therefore it were attribute that high ALP activity due to cholestasis, and increased osteoblastic activity (not osteolysis), Increased ALP in serum or plasma due to increases in other isoforms are rare, (48), Moreover ALP were also elevated in disorders of the skeletal system that involve osteoblast hyperactivity and bone remodeling, A considerable rise in alkaline phosphatase activity caused by increased osteoblast activity following accelerated bone growth is sometimes seen young

animals as well as less indicative in cases of hyperparathyroidism, rickets and osteomalacia, fractures, and malignant tumors (49) .Increase level of BUN may indicated indirect damage of renal tissue, or blockage of the normal flow of urine and the presences of globis catabolites librated from hemoglobin lysis by reticulo-endothelial system through the process of erythrophagocytosis (50,51) ,Increase values of BUN were detected in animals with Hemomycoplasmosis and same data were mentioned by(22,52) .

دراسه سريرية، دمية وتشخيصية لخمج (Mycoplasma ovis) في ضأن محافظة البصرة

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الخلاصة

شخص خمج المايكوبلازما الدموية في ضأن محافظة البصرة،اد شملت الدراسة فحص (833) حيواناً مثلت ستة عشر قطيعاً من الضأن من كلا الجنسين وبأعمار مختلفة والتي ترعى طبيعياً في محافظة البصرة منها (225) حيواناً اظهرت علامات سريرية مختلفة تمثلت بأرتفاع درجات حرارة الجسم وتزايد معدلات ضربات القلب وترداد التنفس ، قلة الشهية وفقر الدم اختير (25) حيواناً من الضان المحلى السليم سريرياً كمجموعة سيطرة بطبقت جميع الفحوصات السريرية على حيوانات الدراسة كما تم فحص البراز بالطرائق المختبرية النموذجية اظهرت الضأن المصابة علامات فقدان الشهية ،فقر الدم مع شحوب او اصفرار الاغشية المخاطية المبطنة للعين والمهبل ،صعوبة وتسارع التنفس ،تضخم العقد اللمفية السطحية وبخاصة العقد اللمفية امام لوح الكتف ، البيلة الهيموكلوبينية مع تصبغ البول بلون داكن فضلاً عن ذلك فقد تناقص انتاج الحليب في النعاج المنتجة له اظهر الفحص السريري تزايد معنوي في معدلات درجات حرارة الجسم ،ضربات القلب وترداد التنفس في الحيوانات الخجمة بالمقارنة مع السليمة اعتمد تشخيص العامل المسبب Mycoplasma ovis على فحص المسحات الدموية المصبوغة بصبغة كمزا ،اذ لوحظ المسبب بشكل كروي او عصوي صغير متواجد بشكل منفرد او بهيئة سلاسل على جدار كريات الدم الحمر للحيوانات الخمجة ،كما تم تأكيد الخمج باستخدام فحص الاليزا غير المباشر أظهرت نتائج الفحوصات الدموية تناقص معنوي في معدلات العدد الكلي لكريات الدم الحمر ، معدلات خضاب الدم وحجم خلايا الدم الحمر المرصوصة في الحيوانات الخمجة بالمقارنة مع السليمة ،اذ كان فقر الدم من النوع ذي الكريات كبيرة الحجم سوية الصباغ ،فضلا عن ذلك فقد ارتفعت معنوياً معدلات سرعة تثقل كريات الدم الحمر في الحيوانات الخمجة بالمقارنة مع السليمة ، كما لوحظ ارتفاع معنوي لمعدلات العدد الكلى لخلايا الدم البيض بسبب الزيادة المعنوية لمعدلات الخلايا اللمفية في الحيوانات الخمجة بالمقارنة مع السليمة اظهرت نتائج الدراسة ايضاً تناقص معنوى في معدلات الهابتوكلوبين ومنشىء الليفين في الحيوانات الخمجة بالمقارنة مع السليمة في حين كان هناك فروقات معنوية واضحة في معدلات الفحوصات الكيموحيوية في الحيوانات الخمجة بالمقارنة مع السليمة اذ لوحظ من خلال نتائج الدراسة تناقص معدلات

البروتين الكلي معنوياً وعلى العكس من ذلك فقد ارتفعت معدلات الصفراوين الكلي وغير المباشر وخمائر الاسبارتيت ناقلة الامين AST و الفوسفاتاز القاعدي ALP والكاما ناقلة الامين AST ومعدلات اليوريا نتروجين الدم في الحيوانات الخمجة بالمقارنة مع السليمة .

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