

Clinical, Hematological and Serological Studies of Mycoplasma Ovipneumonia in Sheep in Basrah, Iraq

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Abstract

The study examined 876 sheep of different ages and sexes in Basrah province, Iraq, from September 2023 to March 2024. The study included 180 animals with clinical signs of pneumonia and acute vital signs. Results showed that 160 (88.88%) samples out of 180 were sero-positive for indirect ELISA. However, the age group less than 6 months exhibited a high infection rate compared with sheep more than one year old. Moreover, all infected animals showed acute clinical manifestations, including partial or complete loss of appetite, rapid and complex breathing with abnormal lung sounds (the pleural frictional sound is the common type), nasal discharge, Pleurodynia, reproductive cough, and signs of anemia. Results of the macroscopic appearance of carcasses show congestion, lung consolidation, hemorrhagic areas and spots, and hepatization. The Histopathological findings reveal a thickening of the alveolar wall, along with bleeding in the interlobular tissue and clogged blood vessels. Moreover, hyperplasia with excessive inflammatory cells in the alveolar lumen and bronchioles with necrotic areas was also indicated beside interstitial pneumonia with exudate in the bronchial lumen and hyperplasia in epithelium cells of bronchial tissues. It has been concluded that *Mycoplasma ovipneumonia* infection affected sheep hard, reflecting high morbidity and mortality rates and economic losses; therefore, a fit control measure should be planned.

Keyword: Sheep, *Mycoplasma ovipneumonia*, anemia, Basrah

Introduction

The prokaryotic microbes known as mycoplasmas are members of the phylum Mollicutes (1). Molecular evidence, morphology, genome size, diet, and ecological habitat all play a role in distinguishing these general traits. (2,3). Different species of *Mycoplasma* are involved in the pathogenesis of pneumonic syndromes in small ruminants (4). The *Mycoplasma* appears to be strictly host-specific and potentially pathogenic only within a single host species. Moreover, *Mycoplasma* causes a wide range of diseases in both animals and humans, which are commonly associated with pneumonia, arthritis, conjunctivitis, infertility and abortion (5).

Moreover, *Mycoplasma* is the predominant bacterial respiratory disorder that causes substantial health problems to livestock populations globally (6,7). Immunosuppressive stress circumstances are a common source of respiratory illnesses in ovine species, which can manifest as secondary infections or primary infections caused by bacteria and viruses (8). These respiratory illnesses are common in livestock and have a major influence on the well-being of the animals as well as a significant financial impact. (9)

Globally, *M. ovipneumoniae* is the utmost importance as a primary cause of respiratory illness in sheep (10,11). This species is a major pathogen that causes respiratory illnesses in sheep. A frequent occurrence is the co-infection of *M. ovipneumoniae* with other microorganisms, including *Pasteurella*

multocida, *Pasteurella haemolytica*, and the *Parainfluenza virus*, which worsens the severity of the disease (4). Sheep are especially vulnerable to *M. ovipneumoniae* infection, which is characterized by a fast advancement of the disease and more severe outcomes than goats. The vulnerability to *M. ovipneumoniae* infection differs among several sheep breeds (12). It was first isolated in New Zealand in 1974 and has since recovered from the respiratory tracts of healthy and symptomatic sheep worldwide (13).

Inhaled infected droplets from animals in close proximity have a significant role in the maintenance and spread of the disease throughout herds, as is the case with secretions from sick or carrier animals (14,15). Introducing sick animals into susceptible herds, excessive rainfall, animal transportation, unfavorable weather, and virulent *M. ovipneumoniae* strains are common causes of outbreaks in lambs from various flocks kept together (16,17). However, in communities where the infection persists over time, interactions between dams and lambs may play a critical role in spreading the disease (18).

Much research has gone into the processes of pathogenesis. While airway epithelial cells are the primary targets of *M. ovipneumoniae*, research has shown that these cells also play a significant role in the pathogenesis of mycoplasma infections in the lungs, in addition to serving as the initial physical barrier against environmental stresses and microbial infections (19,20).

When it comes to *M. ovipneumoniae*

infections, the initial crucial stage is the attachment of the pathogens to airway epithelial cells. This allows the bacteria to evade the host's immune responses and establish an infection (21). Recent studies have found that the metabolic products of mycoplasma cells can cause oxidative damage, cell pathological changes, and apoptosis. This occurs when a substantial amount of H₂O₂ is produced after the mycoplasma cells adhere to host epithelial cells (22,23). In certain situations, an imbalance of oxygen-free radicals can result in oxidative stress, potentially harming cells through disruptions in mitochondrial function (24,25). This can also lead to a decrease in the activity of antioxidant enzymes, such as superoxide dismutase (26). These enzymes can be triggered through the process of phosphorylation by a variety of extracellular irritants, including mitogens, growth hormones, and oxidative stress (27).

Evidence suggests that clinical indications of *M. ovipneumoniae* infection may range from moderate to severe; some animals may recover in weeks, while others may have more severe pneumonia that lasts much longer, especially during stressful situations (28). Clinical features include increased respiratory rates, abnormal respiratory sounds, coughing, nasal discharge, fever, depression of appetite and growth rate, and a drop in milk yield in lactating ewes (29).

M. ovipneumonia has been diagnosed in some countries such as Turkey and Iran (30, 31). On the other hand, the disease was diagnosed in some parts of Iraq, such as Al-Najaf province, Arbil, Al Suleimani and Mosul (32- 34). Clinical, hematological,

and serological studies were conducted in Basrah, Iraq, to investigate the clinical Mycoplasma ovipneumonia infection in sheep.

Materials and methods

The study was conducted to examine 876 local sheep breeds of different ages and of both sexes represented (20) farms located in Basrah province, which include AL-Zubair, Shatt al-Arab, Safwan, AL-Martha, AL-Qurna, AL-Nashua and others.

The study was started from September 2023 to March 2024. Only (180) local sheep breeds from the total number were showed clinical signs belonging to pneumonia with different clinical vital signs (Body temperature, respiratory, and heart rate) and divided into two groups according to their ages (less than six months and more than one year old). All clinical examinations were performed on these suspected animals. Moreover, twenty-five (25) clinically healthy local sheep breeds were served as control group.

Blood samples were taken from all suspected sheep by jugular vein puncture. Five (5 mL) of blood separated into 2.5 milliliters of blood mixed with Ethylene-Diamine Tetra Acetic acid (EDTA). TRBc is used to calculate the total erythrocyte count. Hemoglobin concentration. Packed cell volume (PCV). Mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC). Mean Corpuscular Hemoglobin (MCH). Total and absolute differential leukocyte count (TLC & DLC), Using Hematology analyzer, Genex, USA) (35). Another (2.5) milliliter of blood was used to extract serum, which was stored at -

20 until used later for sero-diagnosis using an indirect ELISA test. (*Mycoplasma ovipneumonia* indirect ELISA kit / Shanghai Ideal Medical Technology Co., Ltd). The post mortem examinations were measured spectrophotometrically at a wavelength of 450 nm, comparing it with the CUTOFF value as per manufacturer instructions.

Diseased sheep were subjected to post-mortem examination after the owner approved and according to permissible scientific laws. Nonetheless, lung tissue was examined macroscopically. All animals that were examined showed clinical signs of pneumonia. Immediately after slaughter, the lungs were grossly examined.

Histopathology examination: The bronchus and lung samples were preserved in 10% formalin for 72 hours. After passing the tissue samples through a series of graded ethyl alcohol concentrations (70–80%, 90–100%, and 100% twice), the histopathological preparation was cleared

twice with xylene. Using liquid paraffin at 56 ° C for blocking and a microtome set at 5 µm for sectioning. The next steps included staining with hematoxylin and eosin (36,37).

Statistical analysis: Using the SPSS student t-test (38), the significance of variation between infected sheep and healthy animals was statistically investigated.

Results

One hundred eighty 180 serum samples from suspected sheep from two age groups were used for indirect ELISA test to detect *M. ovipneumoneae* antibodies. The results showed that 160/180 (88.88%) samples were sero-positive.

Moreover, according to the age group, results show that the age group less than 6 months are highly infected, with an infection rate of (91.66%), in contrast, sheep more than one year old show an infection rate of (83.33%) (Table 1).

Table 1: *Mycoplasma ovipneumonia* infection rate in sheep according to age.

Age group	NO. of samples	Seropositive	%
Less than 6 months	120	110	91.66 %
More than one year	60	50	83.33 %
Total	180	160	88.88%

All infected animals that were examined showed acute clinical manifestations, including partial or complete loss of appetite (89.37%) and rapid and difficult breathing (93.85%). Occasionally, the afflicted animals exhibit symptoms such as shallow, rapid breathing and dyspnea through mouth breathing. Moreover, (80.62%) of diseased animals show abnormal lung sounds, where the pleural frictional sound is the common type of sound. Moreover, (78.12%) of diseased

sheep showed different types of nasal discharge, including mucoid and mucopurulent types. Furthermore, (54.37%), of diseased sheep showed pleurodynia (pleural pain), which was an obvious sign of diseased animals manifested with abduction elbows, depression, stiff forelimb gait, and reluctance to move or lie down with grunt sound coughing, (68.75%) of diseased animals show reproductive cough. Anemia was exhibited by (33.12%) of animals (Table 2).

Table (2): Clinical signs of infected sheep with mycoplasma ovipneumonia

Clinical sings	Infected sheep	%
	n=160	
rapid and difficult breathing	150	93.75
Partial or complete loss of appetite	143	89.37
Abnormal respiratory sound	129	80.62
Nasal discharge	125	78.12
Productive cough	110	68.75
Pleurodynia with grunt sound	87	54.37
Anemia	53	33.12

On the other hand, the diseased sheep with M ovipneumonia show a significant increase ($p \leq 0.05$) in body temperature and respiratory and heart rate when compared with the control (Table 3). Anemia indicated in diseased sheep could be an unusually accepted finding in the hematological examination, which reflected a significant decrease ($p \leq 0.05$) in values of total erythrocyte count, hemoglobin and packed cell volume in infected sheep, reflecting the Normocytic Normochromic type of anemia. Furthermore, the results showed leukocytosis due to a significant increase ($p \leq 0.05$) neutrophil (Table 4 & 5).

Macroscopic changes

The macroscopic examination of diseased sheep showed different general changes in the respiratory system, particularly in the

lung. These changes include congestion, consolidation, hemorrhagic area and spots, as well as hepatization. (fig.1)

Histopathological examination

Results indicated different histopathological changes in tissue samples taken from the diseased lung, including thickening of the alveolar wall with hemorrhage in interlobular tissue and congested blood vessels (Figure.3). Moreover, the diseased l also showed wing hyperplasia with excessive inflammatory cells in alveolar l and also showed hyperplasia with excessive inflammatory cells in alveolar lumen and bronchioles with necrotic area. Figure.4. On the other hand, some diseased lungs show interstitial pneumonia with exudate in bronchial lumen and hyperplasia in bronchial epithelium cells. Figure 5.

Table 3: Body temperature, respiratory and heart rate of diseased sheep and controls.

Clinical parameters	Controls n=25	Infected n=160
Body temperature/ C	38.6±0.57	41.6±0.88*
Respiratory rate/min	24.52±7.2	67.2±7.3*
Heart rate/min	75.4±4.62	110.2±18.25*

Mean ±standard error of mean. The significant level set as (p≤0.05)

Table 4: Blood parameters of infected sheep with *M. ovipneumonia* and controls.

Blood parameters	Controls n=25	Infected n=160
RBC ×10 ⁶	8.44±1.31	5.31±1.4*
Hb g/dl	10.8±1.44	6.79±1.74*
PCV %	34.26±3.54	22.91±3.29*
MCV (fL)	40.6±2.45	43.1±4.57
MCHC	31.5±2.66	29.6±2.11

Mean ±standard error of mean. The significant level is set as (p≤0.05)

Table (5) Total and absolute differential leukocyte count of infected sheep with *M. ovipneumonia* and controls.

Parameters	Control n=25	Infected n=160
TLC ×10 ³	9.798±2.45	14.403±4.68*
N	4223±768.29	8798±856.30*
L	5090±523.12	5253±234
M	171±57.22	176±41.66
E	210±83.2	205±20.1
B	36±7.34	39.68±12.1

Mean ±standard error of mean. The significant level set as (p≤0.05)

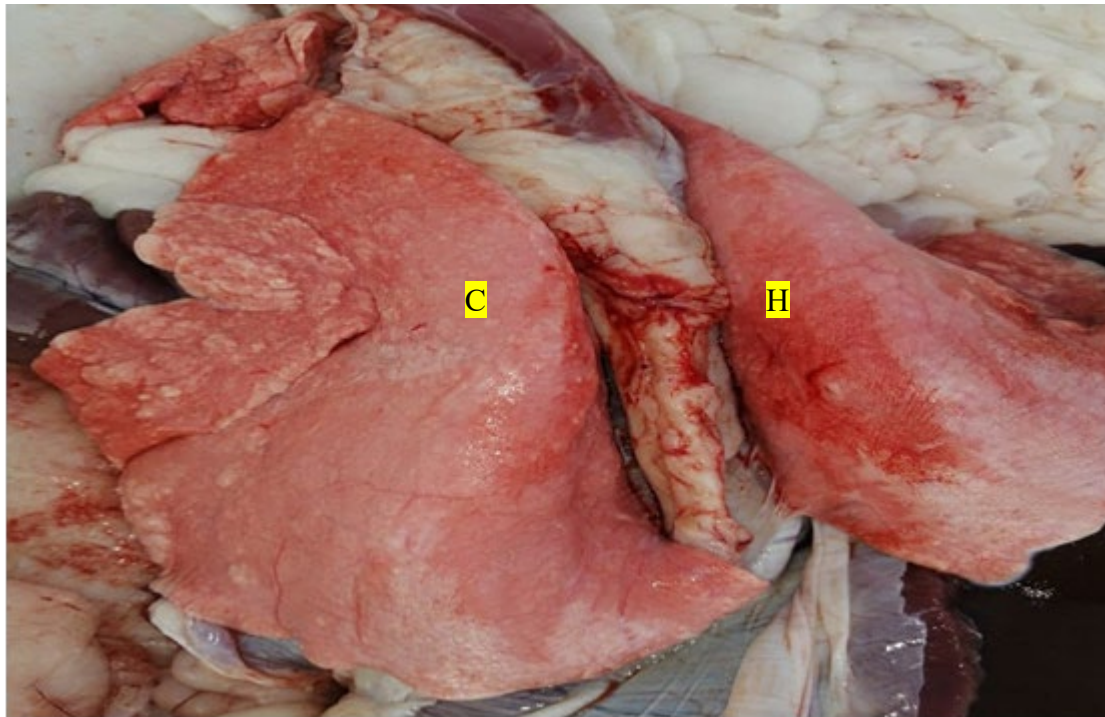


Figure.1: Lung show hemorrhagic area (H) area and consolidation(C)

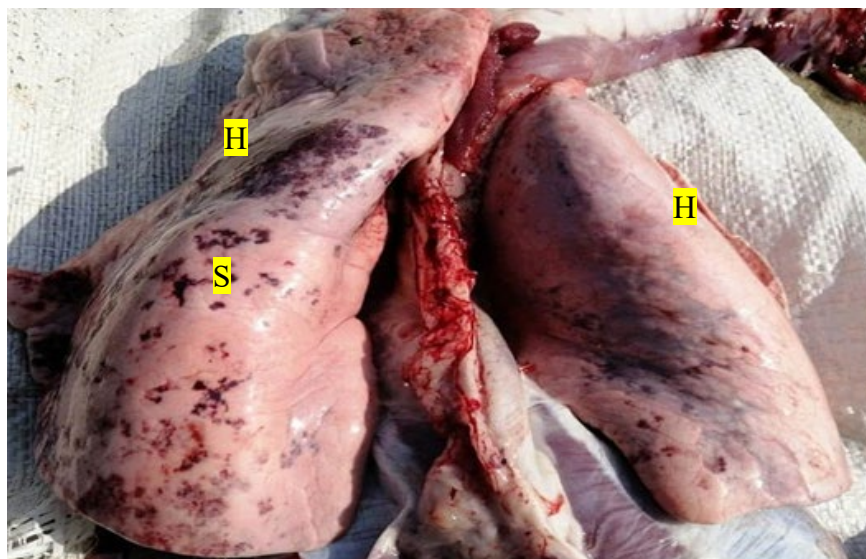


Figure 2: lung of diseased sheep shows hemorrhagic spot (S) and hemorrhagic area (H).

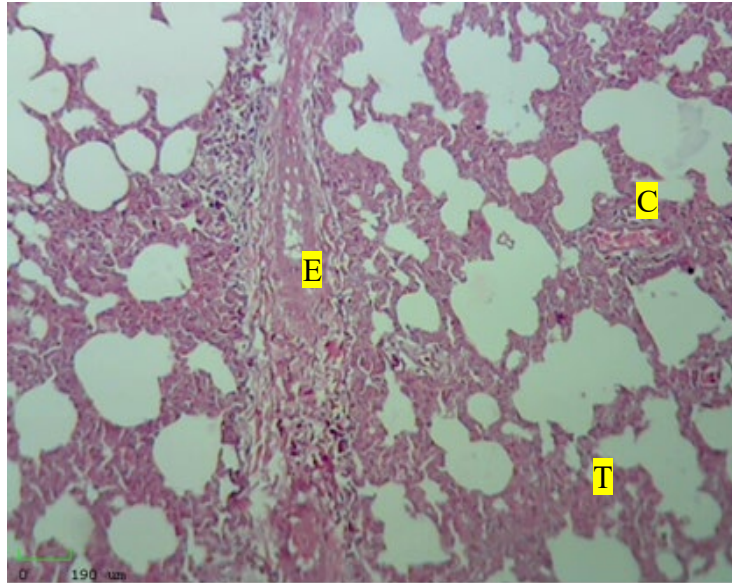


Fig.3 Section of the diseased lung with *M. ovipneumonia* infection shows thickening of the alveolar wall (T) with hemorrhage in interlobular tissue (E) and congested blood vessel (C) (H&E stain, 20x).

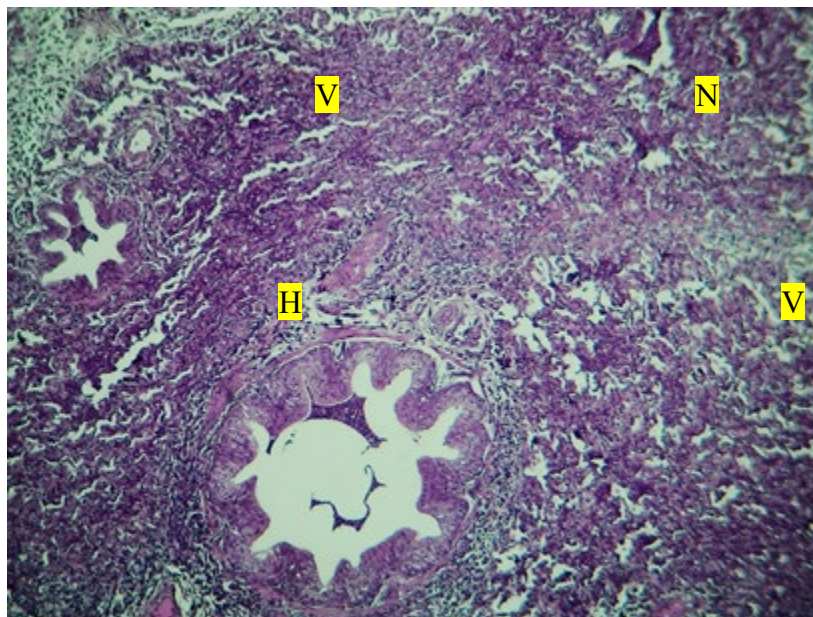


Figure 4: Section of the diseased lung with *M. ovipneumonia* infection shows hyperplasia (H) with excessive inflammatory cells in the alveolar lumen (V) and bronchioles with necrotic area (N) (H&E stain, 10x).

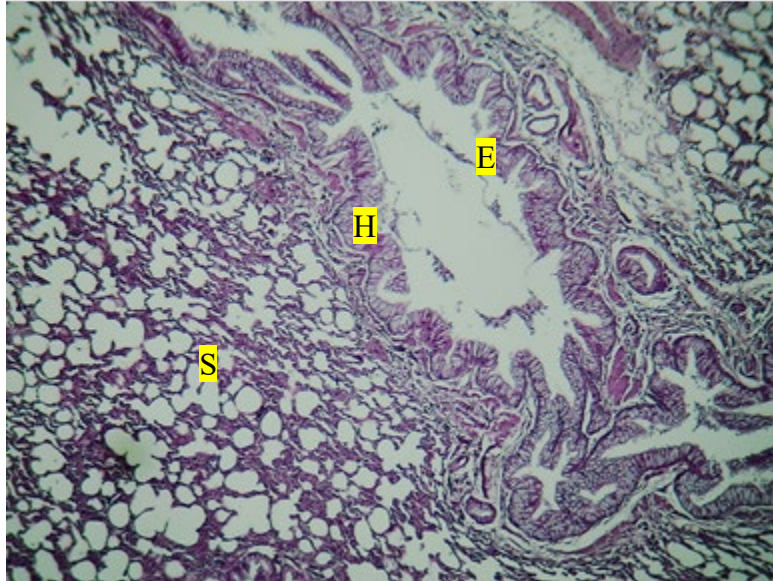


Figure 5. Section of diseased lung with *M. ovipneumonia* infection shows interstitial pneumonia (S) and exudate in bronchial lumen (E) with hyperplasia in epithelium cells of bronchial (H) (H&E stain,10x).

Discussion

This study aimed the identification of *Mycolasma Ovipneumonia* in sheep in Basrah, several studies have shown that antibodies specific to *M. ovipneumoniae* may protect by facilitating the opsonization and phagocytosis of the Mycoplasma (39). Additionally, Niang et al. (40) discovered elevated levels of *M. ovipneumoniae* antibodies in patients with clinical *M. ovipneumoniae* infection.

The current study showed an increased infection rate with *Mycoplasma ovioneumonia* in younger lambs than adult sheep.

In the present study, upon examination, infected sheep showed symptoms such as high body temperature, lack of appetite, depression, runny nose on both sides, and, in most instances, mouth breathing (41). Additionally, some of the sheep experienced

coughing, which helped to clear up their runny noses. These findings corroborate those of (42, 43), who noted that mycoplasmosis infections are characterized by high body temperature, nasal discharge ranging from watery to thick, and difficulty breathing (44). Additionally, when touched on the chest, some infected animals displayed signs of discomfort. According to (45), elevated respiratory rates are one of the clinical manifestations of mycoplasmosis in lambs, a condition that could worsen with other diseases and ultimately lead to an increased mortality rate. The lungs contain several defensive mechanisms to stop this from occurring. (46). Reproductive cough is the more common sign of respiratory tract infection, especially with mycoplasma spp. infection.

The primary methods for removing airborne particles are coughing and ciliary movement. A sticky glycoprotein-containing

mucus layer coats the airways and traps particles. After that, it goes to the pharynx to be swallowed. Additionally, mucus includes many intrinsic antimicrobial agents that work in diverse ways, such as opsonizing bacteria or preventing germs from adhering to mucosal surfaces. The pathogens can be directly damaged by some antimicrobial agents such as lacto-peroxidase or β -defensins (47).

Moreover, in the present study, there are severe systemic reactions manifested by increased body temperature, respiratory and heart rate which reflect manifested by an increase in body temperature, and respiratory and heart rate reflected in the acute crises of the disease. These findings were consistent with (48).

Hematological examinations indicate that Anaemia found in the present work occurs because of significant decrease in RBCs, Hb and PCV values which came in agreement with (32,49) and (50). Anemia may be associated with the destruction of red blood cells due to the presence of some metabolites like superoxide and peroxides produced and liberated by mycoplasma organisms (51,52).

Jones *et al* and Al-Kaissi (53,54) found the ability of *Mycoplasma ovipneumonia* to produce this effect, and this correlated with their ability to produce hydrogen peroxide, which has oxidative stress activity. Some research shows that *M. ovipneumoniae* imports glycerol and produces ROS. Glycerol import is vital for the bacteria since it provides carbon and energy. ROS like H₂O₂ produced by *M. ovipneumoniae* are pathogenic. ROS are highly reactive

chemicals that harm host cells, causing inflammation and tissue damage (55).

The red blood cell (RBC) is subjected to persistent oxidative stress. The large concentration of unsaturated fatty acid chains in the lipid bilayer makes it especially vulnerable to oxidative decay (56).

The process can significantly impair membrane fluidity, membrane potential, ion permeability, and ultimately, cell death. Additionally, it can inflict considerable damage to membrane proteins (57). Oxidative damage is linked to the first oxidation of Hb, producing methemoglobin (MetHb). Unlike normal hemoglobin, MetHb cannot bind oxygen, impeding oxygen delivery to the tissues.

Furthermore, the histopathological changes detected by light microscopic examination and were explained in the results of the current study, changes were hyperplasia of the epithelium cell of bronchioles, interstitial pneumonia and inflammatory cells (neutrophils), thickening of alveoli with hemorrhagic tissue and congested blood vessels. Furthermore, there are necrotic areas in the alveolar lumen and bronchioles, emphysema and collops, and fibrin exudate with infiltration of inflammatory cells in the alveolar lumen. These changes were consistent with the results of many researchers, such as (46, 58), who observed degeneration and deciliation of the surface epithelium of bronchiolar mucosa during the histopathological examination. This was previously described by (59), who reported that most alveoli and bronchioles were filled with a mixture of neutrophils and

macrophages, whereas other alveoli were filled with edema fluid and fibrin.

Conclusions

It had been concluded that *Mycoplasma ovipneumonia* infects the native sheep, especially lambs in Basrah Governorate, leading to substantial effects including severe respiratory distress and anemia, which might be terminated with economic losses.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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دراسة سريرية، دموية و مصلية لمفطورات ذات الرئة في الاغنام في محافظة البصرة، العراق

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الخلاصة

أجريت الدراسة لفحص (876) حيواناً من مختلف الأعمار ومن كلا الجنسين في سلالات الاغنام المحلية في البصرة، العراق. خلال الفترة ابتدأت من ايلول 2023 إلى اذار 2024. اظهرت (180) حيواناً من العدد الكلي علامات سريرية تشير إلى الالتهاب الرئوي مع تفاعلات جهازية حادة. اظهرت النتائج أن 160 عينة (88.88%) من أصل 180 عينة كانت إيجابية مصلية باستخدام تقنية الاليزا غير المباشرة، وكانت الفئة العمرية أقل من 6 أشهر ذات نسبة خمج عالية بالمقارنة مع الأغنام بعمر أكثر من سنة، فضلاً عن ذلك، أظهرت جميع الاغنام الخمجة مظاهر سريرية حادة تشمل فقدان جزئي أو كامل للشهية، وسرعة وصعوبة في التنفس مع اصوات غير طبيعية في الرئة، حيث كان صوت الاحتكاك الجنبى هو النوع الشائع، وإفرازات من الأنف، وألم الجنب، والسعال الانتاجي، بالإضافة إلى علامات فقر الدم. وأظهرت نتائج المظهر العياني لجثث الاغنام النافقة او المدبوحة احتقاناً وتصلباً في الرئتين وبقعاً نزفية بالإضافة إلى تكبد النسيج الرئوي. في حين اشارت النتائج التشريحية المرضية النسجية إلى سماكة الجدار السنخي مع وجود نزيف في الأنسجة بين القصيبات واحتقان الأوعية الدموية. كما تم ملاحظة تضخم مع غزو الخلايا الالتهابية المفرط في التجويف السنخي وكذلك القصيبات مع مناطق تنخرية بجانب الالتهاب الرئوي الخلالي مع الإفرازات في تجويف القصبات الهوائية وتضخم في الخلايا الظهارية لأنسجة القصبات الهوائية. استنتج الى أن الإصابة بالميكوبلازما المبيضية الرئوية يمكن أن تؤثر سلباً على الأغنام مما يعكس ارتفاع معدل الإصابة والوفيات مما يؤدي إلى تأثير كبير وينتهي بخسائر اقتصادية حادة، لذلك يجب التخطيط لإجراءات مكافحة وبائية مناسبة.

كلمات مفتاحية: الاغنام، مفطورات ذات الرئة، فقر الدم، البصرة.