Ann. For. Res. 66(1): 1410-1422, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

HEMATOLOGICAL, BIOCHEMICAL, PATHOLOGICAL, SEROLOGY AND MOLECULAR DETECTION OF *MYCOPLASMA OVIPNEUMONIAE* FROM AWASSI SHEEP IN AL-NAJAF PROVINCE, IRAQ.

Ali Hussein AL-Dujaily, Shatha Atta Abeed and Ali Mahdi Sahib
Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, University of Kufa, Iraq.
Department of Animal Production, Al-Forat Al-Awsat Technical University, Iraq
Department of Veterinary Public Health, Faculty of Veterinary Medicine, University of Kufa, Iraq.
Corresponding author: Alih.aldujaily@uokufa.edu.iq, 31003.Kin.sht@atu.edu.iq, Alim.alkaabi@uokufa.edu.iq

Abstract

Mycoplasma ovipneumoniae (M. ovipneumonea) is a major respiratory infection in sheep worldwide. The current study aims to diagnose, identify, and examine *M. ovipneumonea* in sheep using hematology, biochemistry, pathology and molecular. The current study gathered samples from Awassi sheep of both sexes and 1.5-4 years of age in the Al-Najaf slaughterhouse from January to April 2022, who were suffering from respiratory symptoms such as ocular, nasal discharge, and coughing. Before slaughter, blood samples (n=210) were obtained for hemato-biochemical and serological analysis; following slaughter, lung specimens were collected from sheep for DNA gene-based PCR analysis and the preparation of paraffin blocks for histological evaluation. Animals with pneumonic pathological changes had a range of respiratory symptoms. The appearance revealed multiple stages of pulmonary changes, including respiratory congestion, edema mixed with fibrin fluid, irregular consolidation and hemorrhagic patches on the lungs' surface, and inflammatory exudate in their airways. The infected sheep were found to be anemic and to exhibit leukocytosis on a hematological value. In compared to healthy animals, blood biochemical indicators such as cholesterol, HDL, and albumin were shown to be decreased. ALT, AST, total protein, globulin, and fibrinogen levels all increased. Histopathologically, the infected sheep had broncho interstitial pneumonia and purulent to fibrinopurulent bronchopneumonia. Serology results show that 210/123 (58.8%) confirmed the presence of *Mycoplasma spp*. *M. ovipneumoniae* DNA was extracted from lung tissue samples and replicated using genus and species-specific primers. The 16S rRNA gene of *M. ovipneumonea* was found in 123/52 (42.2%) of the PCR findings. Our findings indicated that *M. ovipneumoniae* was one of the agents involved in generating lung consolidation and pneumonia in Awassi sheep, and that PCR was more effective in identifying Mycoplasmas.

Keywords: *Mycoplasma ovipneumonia*, Hematology, biochemical, Histopathology, Serology, PCR, sheep.

1. Introduction

Mycoplasma bacteria are found in over 100 different species and impact both humans and animals (Murray *et al.*, 2020) . *M. ovipneumoniae* is a significant pathogen of respiratory disease with significant economic, veterinary, and medical importance worldwide. This respiratory and infectious disease in sheep and goats causes coughing, asthma, breathing difficulty, runny nose, progressive weight loss, and pulmonary interstitial hyperplasia inflammation (Besser *et al.*, 2017). It causes pulmonary abscess, pleurisy, and fibrinous pneumonia in sheep when exposed to severe circumstances (also known as contagious ovine pleuropneumonia) (Cassirer *et al.*, 2018).

M. ovipneumoniae generates mild and unrecognized clinical indications, and infected animals have good appetites until serious damage linked with subsequent bacterial infections. Bronchopneumonia was the most commonly identified related to *M. ovipneumoniae*, the causal agent of bronchopneumonia and bronchus epithelial cell hyperplasia (Constable *et al.*, 2017).

Significant factors such as altering macrophage activity, adhering to the ruminant's ciliated epithelium via its polysaccharide capsule, inducing the production of autoantibodies to ciliary antigens, and suppressive activity on lymphocytes all contributed to the pathogenesis of *M. ovipneumoniae* infection in sheep and other small ruminants. Furthermore, the bacteria's ability to operate as a risk factor for various diseases (Osman *et al.*, 2021).

Histopathological examination of the lungs affected by mycoplasmal pneumonia frequently reveals acute serofibrinous to fibrinonecrotic pleuropneumonia with infiltrates of serofibrinous fluid and inflammatory cells, primarily polymorphonuclear neutrophils, in the alveolar spaces, bronchioles, interstitial septae, and subpleural connective tissues (Mousa *et al.*, 2021).

M. ovipneumonia may cause infections in sheep and can be isolated from healthy sheep's lungs, trachea, nose, and eyes, as well as their respiratory tracts. (Akwuobu *et al.*, 2014). Mycoplasma infections are difficult to identify due to limitations in existing diagnostic tools and similarity in the illnesses they cause. Serology can be used as a diagnostic aid or screening tool for active mycoplasma infections, although it is not definitive in identifying active mycoplasma infections. To identify the presence of antibodies in serum, the competitive enzyme-linked immunosorbent assay (cELISA) is utilized. Antibodies are detected in serum when the host's adaptive immune system is exposed to and responds to a pathogen (Maes *et al.*, 2018).

Polymerase Chain Reaction (PCR) is a sensitive and quick diagnostic process for the early detection of Mycoplasma infection, allowing it to be diagnosed from a variety of samples. *M. ovipneumoniae* was isolated from nasal swab samples and pneumonic sheep lungs using PCR (Noll *et al.*, 2022).

Although definitive studies on *M. ovipneumoniae* in sheep with pneumonia are limited, the Iraqi province of Al-Najaf is one of the most prominent in terms of Awassi sheep rearing. The aim of the present investigation was to identify *M. ovipneumoniae* from sheep spontaneously infected with pneumonia in this area.

2. Materials and Methods

2.1. Sampling

Two hundred and ten blood samples and lungs samples were taken from Awasii sheep respiratory symptoms and revealed lesions and signs of infection in the lungs from a local slaughterhouse in Al-Najaf province, in the middle of Iraq, from January to April 2022. In addition, 30 healthy sheep served as controls. The blood samples were sent to a clinical pathology laboratory for hematological and biochemical analysis. Hematological analysis was performed using the Vet. Scan HM5 hematology equipment (ABAXIS Company). UV spectrophotometer was used to measure serum biochemical variables. The Elisa test kit (SunLong Biotech Co., LTD) was used to check for mycoplasma.

The lung specimens were kept refrigerated until they were used. The histopathological examination and PCR analysis were carried out at the Faculty of Veterinary Medicine/University of Kufa's laboratory. All samples were extracted and amplified.

2.2. Study material

Blood was collected from animals exhibiting respiratory symptoms, and before postmortem or slaughter and evisceration, the lungs were inspected in situ and any lesions detected were reported. The afflicted lungs were retrieved and extensively screened using visual examination, palpation, and bronchial tree dissection.

The serology of the serum was studied after collecting 210 blood samples and separating the serum to ensure the presence of mycoplasma, and ELISA testing indicated 123 positive samples for mycoplasma spp. After confirming the positive samples, a PCR was run on them, and the results indicated 52 positive samples (infected with *M. ovipneumoniae*).

The histology of the samples that revealed positive infection was examined using *M. ovipneumoniae* PCR. Samples of affected lungs were fixed in 10% neutral buffered formalin, regularly processed, embedded in paraffin, and five-micron sections were obtained and stained with hematoxylin and eosin (H&E) for light microscopy inspection for histopathology (Luna, 1968).

2.3 DNA extraction and Amplification

The DNA extraction was performed according to manufacturer instructions (Bioneer Company, Korea.): Lung samples were collected and prepared as described by (Santos *et al.*, 2010). They were collected in the plastic container and stored at -80°C until use. Lung tissue (25mg) was macerated in a 1.5 ml microcentrifuge tube with a pestle. To each sample, both of 200µl GST buffer solution and 20 µl of Proteinase-K were added. Samples were vortex thoroughly for 10 seconds and incubated at 60°C overnight. Dissolved samples were centrifuged at 16000 xg for 2 min, the supernatant was collected in new 1.5 ml tube, then 200 µl of GSB was added to the supernatant, again it was a vortex for 10 sec. 200µl absolute ethanol was added to the lysate sample and mixed well via vortex. All mixtures were transferred to GS columns and centrifuged at 16000 xg for 1min., then both W1(400µl) and W2 (600µl) buffers were added respectively to GS column with centrifugation. Finally, 100µl of preheated elution buffer was added to each tube to elute the purified DNA, and stored at -20°C until used.

2.4. Molecular detection

The primers of LMF1 (5′-TGA ACG GAA TAT GTT AGC TT-3′) and LMR1 (5-′ GAC TTC ATC CTG CAC TCT GT -3′), were species-specific for detection of *M. ovipneumoniae* (Daee *et al.*, 2020). The temperature protocol was the same for both species as follows: after an initial denaturation step at 94°C for 3 min, 37 cycles were performed consisting of three steps: denaturation (94°C, 45 s), annealing (55°C, 30 s), and extension (72°C, 45 s) and final extension at 72°C for 5 min.

The fragments size of PCR products was 361bp for *M. ovipneumoniae*. The PCR products were run on 2% agarose gel (Biometra, Germany), containing 0.8µl ethidium bromide in TBE buffer and subjected to electrophoresis for about 2 h at 80 V. After the bonds of amplified fragments were visualized and photographed under UV transilluminator.

Statistical analysis

The data was analyzed using SPSS version 26. Least significant differences (LSD) and One-way ANOVA ($P < 0.05$) were used to detect group differences.

3. Results

3.1. Hematology findings

There were substantial variations in hematological parameters between infected and healthy animals, as shown in Table (1). There was a significant increase ($P<0.05$) in the level of Total Leucocyte Count (TLC), lymphocytes, neutrophils and monocytes between infected and non-infected sheep, with a significant decrease in hematocrit percent, hemoglobin content, RBC count in the sheep with *M. ovipneumoniae* compared with the healthy group.

Table 1: Hematological parameters in healthy and sheep infected with mycoplasma *M. ovipneumoniae*.

Parameters	Healthy sheep No. 30	Infected with *M. ovipneumoniae* No. 52
RBC	9.2-14.1 10.7±0.2A	5.6-11.9 8.9±0.1B
PCV	28-43 34.4±0.6A	15-27 24.2±0.2B
Hb	9-15 11.3±0.2A	4-8 7.1±0.08B
WBC	3900-12650 8600±516B	5150-23550 13095±346A
N	24-58 42.1±1.7B	42-88 58.4±1.0A
L	37.5-71.5 54.5±1.7A	13.5-76.5 50.7±1.3A
M	0.5-5 1.4±0.2B	0.0-12 3.5±0.1A
E	0.0-8	0.0-7

	1.6±0.3A	2.1±0.1A
platelets	163-806 364.1±28.7 A	35-630 210.2±14.1B

The differences in capital letters horizontally refer to the presence of significant value at P<0.05.

Hematological study of *M. ovipneumoniae* showed a significant reduction in RBC, Hb and PCV, resulting in anemia. Anemia may be associated with the destruction of red blood cells due to metabolites like super oxide and peroxides produced and liberated by mycoplasma organisms (Hampel *et al.*, 2014). There was a significant increase in total leukocyte count in most cases, it was due to neutrophilia that reflected acute nature of the disease and immunogenic response on the other hand, decrease in platelets count. Hematological findings were almost similar to the findings of (Shah *et al.*, 2017).

3.2. Biochemical findings

Table 2, showed an increase in the biochemical values in the infected sheep, except for high-density lipoprotein cholesterol (HDL), and cholesterol in which significant decrease was observed when compared to control group. Fibrinogen levels was considerably high in the infected sheep when compared to the healthy ones.

Table (2): Biochemical parameters in healthy and infected sheep with *M. ovipneumoniae*

Parameter	**Healthy sheep No. 30**	**Infected with *M. ovipneumoniae* No. 52**
ALT (IU/l)	20-45 30±1.0B	45-75 58±1.3 A
AST (IU/l)	54-86 67±1.8B	100-129 118±1.0A
Triglyceride (mg/dl)	30-57 42±1.5	61-95 81±1.1A
LDL (mg/dl)	45-71 60±1.3B	75-117 96±1.6A
HDL (mg/dl)	18-35 25.4±0.9A	2-16 10.2±0.6B
Cholesterol (mg/dl)	70-100 88±1.6A	30-68 43±1.4B
Total Proteins (g/l)	58-76 66±0.8B	77-92 84±0.5A
Albumen (g/l)	27-35 30±0.3A	14-26 20±0.4B
Globulin (g/l)	30-39 35±0.5B	40-61 53±0.8A

Fibrinogen g/L	2.2-4.8 3.3±0.1B	4.9-8.6 7.3±0.1A

The differences in capital letters horizontally refer to the presence of significant value at P<0.05.

Due to septicemic nature of disease caused by *M. ovipneumoniae*, different visceral organs are affected with different degree of toxicity. The liver is the most common organ targeted by this species of Mycoplasma that was evident by necrotic foci on its surface on gross examination. The hepatotoxicity ultimately increases the enzyme levels in the liver function tests. ALT and AST were increased in infected sheep rather than healthy one. This might be due to the changes in the antioxidant abilities that occurred in liver and in the phospholipid structure of the cell membrane which led to high levels of ALT and AST as markers of liver damage according to (Sadique *et al.*, 2012).

Due to the liver damage, protein synthesis is also affected, which leads to decrease in total serum protein. In the present study, there was a significant increase in total serum protein while albumin reduction significantly, this reduction may also be due to the fact that *M. ovipneumoniae*, also consumes protein for their proliferation. Of interest, serum globulins concentrations were increased in infected sheep compared to healthy ones. Globulins are serum protein produced by liver in ruminants which are produced and increased in response to acute inflammation which is the case of pneumonic sheep of this study suggesting sever oxidative stress on the liver (Kaneko, 1997).

In the current investigation, it was observed that a significant increase in the values of fibrinogen (negative acute phase protein) in Mycoplasma-infected animals when compared with healthy ones this result agreed with (El-deeb *et al.*, 2018).

3.3. Pathological findings

In the affected lungs, gross irregular consolidation with lobular or lobar to diffuse pattern was seen in the cranioventral to caudal lobes. Dark red to grey, pink, or other colors ranged in color for the combined areas (Figure 1). Numerous lesions, including rib imprints on the costal surfaces of the diaphragmatic lobes and abscesses filled with odorless pus, were observed in some afflicted lungs. These lesions included the presence of a thin coating of fibrin with a yellow tint covering the pleural surfaces.

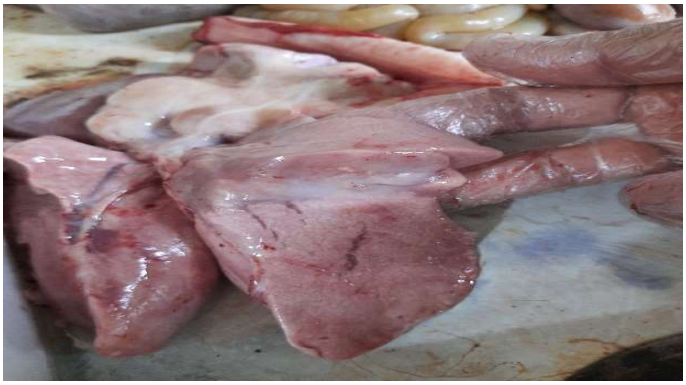

Figure (1): Lung infected with *M. ovipneumoniae*. Caseous nodules, Congestion, Ulceration, Hepatization, Marbling Appearance.

4.4. Histopathological investigation

A histopathologic examination of the affected lungs revealed purulent to fibrinopurulent broncho-pneumonia in 29 sheep (55.7%) and bronchointerstitial pneumonia in 23 sheep (44.2%). Multifocal regions of necrosis with varying quantities of fibrin, neutrophils, and macrophages were seen in the bronchioles and alveoli of patients with fibrinopurulent bronchopneumonia (Figure 2).

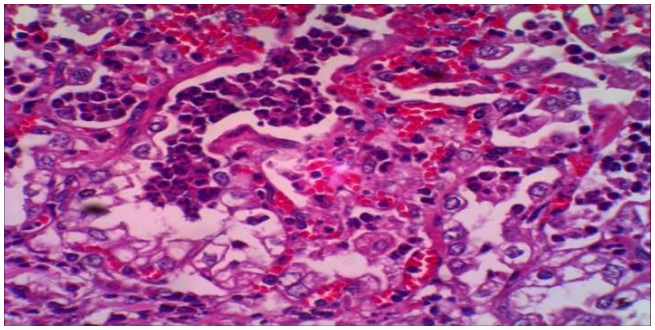

Figure 2: multifocal areas of necrosis associated with variable amounts of lymphocyte, neutrophils and macrophage in the bronchioles and alveoli, hyperplasia, inflammatory cell, and remnants of a frame of alveoli (H & E, × 40.1)

Severe hemorrhage in 47 sheep (90.3%) especially at the alveoli boundary to the point of destruction (figure 3).

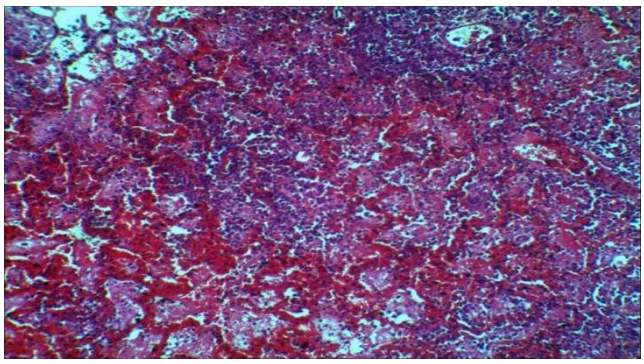

Figure 3: Severe hemorrhage especially at the alveoli boundary to the point of destruction (H & E, × 10).

Thickening of interstitial tissue between alveolus, primarily by lymphocytes and plasma cells, is associated with bronchiolar associated lymphoid tissue (BALT). Proliferation of pneumocytes were seen in a few cases, but that finding can be unrecorded in several cases that made it hard to recognize the lesion with certainty. Severe consolidation which made the interpretation of the interstitium difficult (Figure 4). In some slides, interstitial pneumonia appeared (Figure 5).

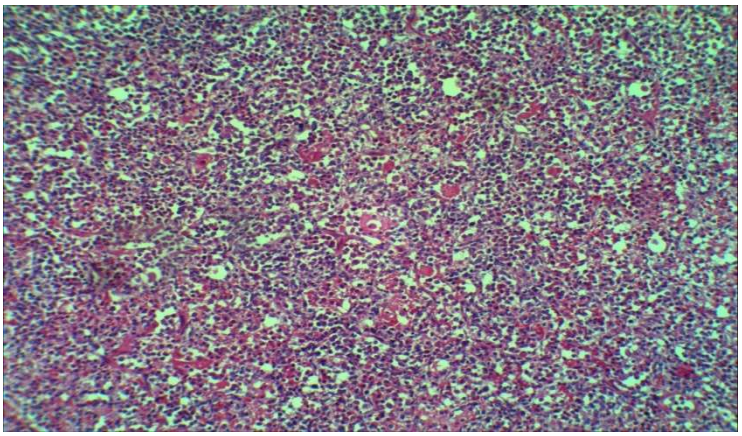

Figure 4: severe consolidation which made the interpretation of the interstitium difficult (H & E , × 10)

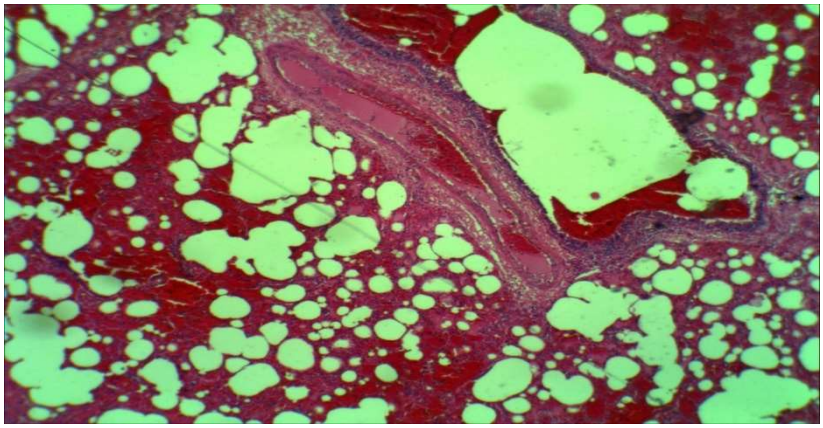

Figure 5: interstitial pneumonia appeared (H & E, × 4)

3.4. Serological findings

The percentage of Serological tests *for Mycoplasma* spp from the total samples was 210/123(58.8%) as shown in table (3).

Table (3): Results of isolation of *Mycoplasma* by Serological and PCR method from Lung samples used in the study.

Test type	Total No. of samples	Positive isolates for *Mycoplasma spp*	Percentage	Positive samples for *M. ovipneumoniae*	Percentage
Serological test	210	123	58.8%		
PCR test	123			52	42.2 %

3.5. Molecular Detection

Of the 123 lung samples infected with mycoplasma spp examined by PCR, 52 (42.2 %) samples were scored to be positive for the *M. ovipneumoniae* 16S rRNA (figure 6).

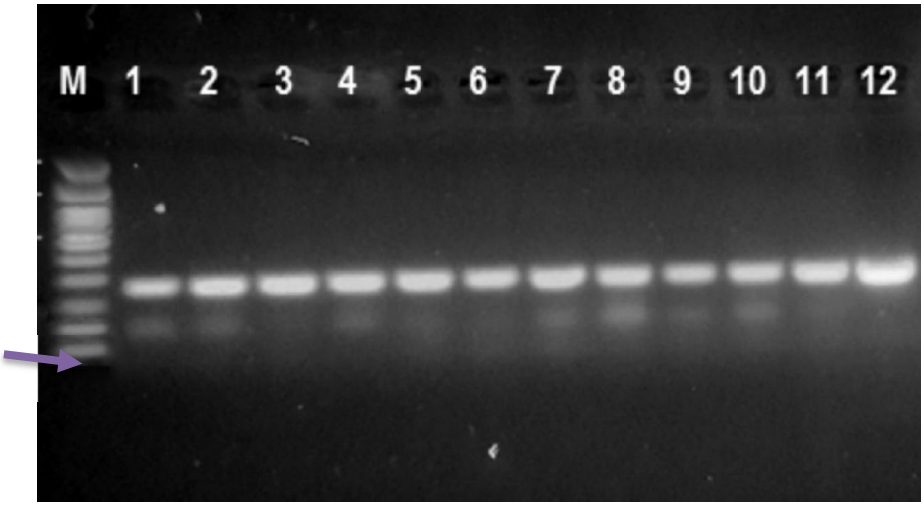

Figure. 6: Agarose gel electrophoresis image showing the PCR product of 16S rRNA gene in Mycoplasma gene of the *M. ovipneumoniae* positive isolates; M– marker (1500–100 bp); lanes 1 to 12 are positive for *M. ovipneumoniae* showing a 361 bp band.

Discussion

This study looked into the identification of *M. Ovipneumonia* in sheep in the city of Al-Najaf because this bacterium is the most commonly isolated Mycoplasma from the respiratory tract of sheep and plays a significant role in the production of respiratory diseases in sheep, which leads to economic damage in the form of declines, poor growth, and low economic returns. As a result, infected lung samples were utilized in a PCR test to diagnose this bacterium, which could then be connected to a hematological, biochemical, particular gross, and histological test (Gaeta *et al.*, 2022).

The initial suggestive diagnosis involves clinical signs from nasal discharges, coughing, and dullness of animal; these observations confirmed by post-mortem examination show the signs of inflammatory reaction from congestion collection of edematous fluid in pulmonary chest and exudate in trachea besides hemorrhagic types on the surfaces of lungs. Lungs showed pneumonic

appearance with abdomen-caudal gray hepatization, severe pulmonary edema evident by tense pulmonary capsule and separated lobules with frothy white edematous fluid oozing from bronchi at cut section and prominence interlobular septa, these findings agreed with (Daee *et al.*, 2020).

Very clear hydropericardium was recognized with a tan color fluid filling the pericardial sac and petechial to ecchymotic hemorrhages on the heart's coronary fat, followed by hematological, biochemical and serological identification then confirmed by PCR of etiological agents as has been done in other studies (Jiang *et al.*, 2017).

In the current study, 210 blood samples from sheep in Iraq's Najaf province were taken and used for genetic analysis utilizing PCR to identify Mycoplasma using primers unique to that genus (Daee *et al.*, 2020). 123 samples had positive serology results, of which 52 had PCR-proven *M. ovipneumoniae*. The outcomes demonstrated that PCR was more effective in finding Mycoplasma species, and they were consistent with those of other studies (Mousa *et al.*, 2021).

Characteristic necropsy findings can be used to make the diagnosis of mycoplasmal infection. Consolidation of the cranial lung lobes and, on occasion, the anterior edge of the caudal lobes are examples of such features. The consolidated regions have red atelectatic patches and range in color from gray to reddish-brown. On sliced surfaces, grayish-white nodules with a solid consistency can also be seen. Additionally, pleuritic symptoms might be present (Kebkiba and Antipas, 2022).

As illustrated in Fig. 1 anatomic lesions of the lung from sheep were detected. Macroscopic pathological abnormalities in sheep's lungs were substantially more severe inflammatory lesions in lungs, including large-scale red and gray hepatization and bulging nodules. These results agreement with (Li *et al.*, 2021).

Histopathologic features are those of an interstitial, cuffing-type pneumonia, with nodular lymphoid hyperplasia and mononuclear lymphocytic cuffing around bronchioles and blood vessels. Exudate, composed mainly of macrophages and a few neutrophils, is observed within the alveoli (Mohammed *et al.*, 2022).

A characteristic feature of *Mycoplasma spp*. infections is the presence of nodular hyaline “scars” in the bronchial walls. In present study, however, these necropsy findings were present in only 60% of cases, and the remaining 40% of cases did not exhibit these pathologic features, as a result, our study's findings were consistent with (Carvallo and Stevenson, 2022).

The current study's discovery of *M. ovipneumonia* in the pneumonic lungs of sheep from the Al-Najaf province is suggestive of a risk to our herd of animals. To battle *M. Ovipneumonia*, however, across the entirety of Al-Najaf is urgently necessary in order to supply animal fodder for our expanding population and earn foreign currency from the leather industry due to our porous borders. Because *M. Ovipneumonia* poses such a serious danger to the livestock industry, it is crucial to combat this disease.

In conclusion, the findings of this study concluded that *M. ovipneumoniae* was the agent capable of causing pneumonia and lung consolidation in sheep, and that PCR was more effective than other techniques in identifying mycoplasmas.

ACKNOWLEDGEMENTS
The authors thank faculty of the Veterinary Medicines/ University of Kufa, Iraq for their Moral support.

CONFLICTS OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION
All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING
None.

DATA AVAILABILITY
All datasets created or investigated during this study are involved in the manuscript and/or the Supplementary Files.

ETHICS STATEMENT
This article does not contain any studies with human participants or animals performed by any of the authors.

References

Akwuobu, C. A.; Ayling, R. D.; Chah, K. F. and Oboegbulem, S. I. (2014). Studies into the prevalence of Mycoplasma species in small ruminants in Benue State, North-central Nigeria. *Trop. Anim. Health Prod.*, *46*(6), 1087–1092.

Besser, T. E.; Cassirer, E. F.; Potter, K. A. and Foreyt, W. J. (2017). Exposure of bighorn sheep to domestic goats colonized with Mycoplasma ovipneumoniae induces sub-lethal pneumonia. *PLoS One*, *12*(6), e0178707.

Carvallo, F. R. and Stevenson, V. B. (2022). Interstitial pneumonia and diffuse alveolar damage in domestic animals. *Vet. Pathol.*, 03009858221082228.

Cassirer, E. F.; Manlove, K. R.; Almberg, E. S.; Kamath, P. L.; Cox, M.; Wolff, P.; … Harris, R. B. (2018). Pneumonia in bighorn sheep: Risk and resilience. *J. Wildl. Manage.*, *82*(1), 32–45.

Constable, P. D.; Hinchcliff, K. W.; Done, S. H. and Grünberg, W. (2017). Veterinary Medicine 11th ed., 965-969. Elsevier Ltd, St. Louis, MI, USA.

Daee, A. A.; Khodakaram-Tafti, A.; Derakhshandeh, A. and Seyedin, M. (2020). Identification of Mycoplasma ovipneumoniae and Mycoplasma arginini in sheep with pneumonia in North East of Iran. *Iran. J. Vet. Res.*, *21*(1), 15.

EL-DEEB, W.; ALMUJALLI, A. A.; ELJALII, I. and ELMOSLEMANY, A. (2018). Clinical and biochemical studies on contagious caprine pleuropneumonia with special reference to lipoproteins profile and fibrinogen levels. *Lucr. Ştiinţifice-Medicină Vet. Univ. Ştiinţe Agric. Şi Med. Vet. Ion Ionescu La Brad" Iaşi*, *61*(1), 31–38.

Gaeta, N. C.; de Sá Guimarães, A. M.; Timenetsky, J.; Clouser, S.; Gregory, L. and Ganda, E. (2022). The first Mycoplasma ovipneumoniae recovered from a sheep with respiratory disease in Brazil–draft genome and genomic analysis. *Vet. Res. Commun.*, 1–8.

Hampel, J. A.; Spath, S. N.; Bergin, I. L.; Lim, A.; Bolin, S. R. and Dyson, M. C. (2014). Prevalence and diagnosis of hemotrophic mycoplasma infection in research sheep and its effects on hematology variables and erythrocyte membrane fragility. *Comp. Med.*, *64*(6), 478–485.

Jiang, Z.; Song, F.; Li, Y.; Xue, D.; Deng, G.; Li, M.; … Wang, Y. (2017). Capsular polysaccharide is a main component of Mycoplasma ovipneumoniae in the pathogen-induced toll-like receptor-mediated inflammatory responses in sheep airway epithelial cells. *Mediators Inflamm.*, *2017*.

Kaneko, J. J. (1997). Serum proteins and the dysproteinemias. In *Clinical biochemistry of domestic animals* (pp. 117–138). Elsevier.

Kebkiba, B. and Antipas, B.-B. B. (2022). Respiratory pathologies in small ruminants and factors contributing to their outbreak. *GSC Adv. Res. Rev.*, *10*(1), 156–165.

Li, Z.; Du, Z.; Li, J. and Sun, Y. (2021). Comparative analysis on lung transcriptome of Mycoplasma ovipneumoniae (Mo)-infected Bashbay sheep and argali hybrid sheep. *BMC Vet. Res.*, *17*(1), 1–11.

Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology.

Maes, D.; Sibila, M.; Kuhnert, P.; Segalés, J.; Haesebrouck, F. and Pieters, M. (2018). Update on Mycoplasma hyopneumoniae infections in pigs: knowledge gaps for improved disease control. *Transbound. Emerg. Dis.*, *65*, 110–124.

Mohammed, Z. M. A.; Ibrahim, W. M. and Abdalla, I. O. (2022). Pneumonia in Slaughtered Sheep in Libya: Gross and Histopathological Findings. *Eur. J. Vet. Med.*, *2*(1), 4–9.

Mousa, W. S.; Zaghawa, A. A.; Elsify, A. M.; Nayel, M. A.; Ibrahim, Z. H.; Al-Kheraije, K. A.; … Salama, A. A. (2021). Clinical, histopathological, and molecular characterization of Mycoplasma species in sheep and goats in Egypt. *Vet. World*, *14*(9), 2561.

Murray, P. R.; Rosenthal, K. S. and Pfaller, M. A. (2020). Medical Microbiology E-Book. Elsevier Health Sciences.

Noll, L. W.; Highland, M. A.; Hamill, V. A.; Tsui, W. N. T.; Porter, E. P.; Lu, N.; … Grossman, P. C. (2022). Development of a real-time PCR assay for detection and differentiation of Mycoplasma ovipneumoniae and a novel respiratory-associated Mycoplasma species in domestic sheep and goats. *Transbound. Emerg. Dis.*

Osman, M. M.; Abu Elmakarem Mohamed, M.; N Deif, H. and M Osman, K. (2021). Virulence Traits and Antimicrobial Sensitivity Testing of Untyped Mycoplasma species Recovered from Sheep and Goat in Egypt. *J. Appl. Vet. Sci.*, *6*(4), 39–45.

Sadique, U.; Chaudhry, Z. I.; Younus, M.; Anjum, A. A.; Idrees, M.; Qureshi, M. S.; … Subtain, S. M. (2012). Clinico-pathological study of contagious caprine pleuropneumonia (CCPP) in small ruminants. *J. Anim. Plant Sci.*, *22*, 45–50.

Santos, E. M.; Paula, J. F.; Motta, P. M.; Heinemann, M. B.; Leite, R. C.; Haddad, J. P.; … Reis, J. K. (2010). Comparison of three methods of DNA extraction from peripheral blood mononuclear cells and lung fragments of equines. *Genet. Mol. Res. GMR*, *9*(3), 1591–1598.

Shah, S. S. A.; Sadique, U.; Hassan, Z. U.; Ahmad, S.; Khan, H.; Shah, M. K.; … ur Rahman, H. (2017). Clinico-pathological profile and frequency of Mycoplasma mycoides subsp. capri infection in goats in northern zone of Khyber-Pakhtunkhwa, Pakistan. *Veterinaria*, *66*(2), 72–77.