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Molecular Identification, Histopathological, and Characterization of Pneumonic Pasteurellosis in Sheep

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Abstract | *Pasteurella multocida* is a primary pathogenic bacterium responsible for respiratory infections in sheep, posing a significant threat to animal health and productivity in livestock communities. This study aimed to isolate *P. multocida* from 54 lung samples of sheep exhibiting respiratory symptoms, collected from the Al-Qasim/Babylon region, Iraq, out of a total of 150 sheep samples. The isolation rate of *P. multocida* was found to be 36% of the total samples. Multiple diagnostic techniques were employed, including traditional culture media such as Blood agar and MacConkey agar, along with the advanced VITEK 2 system for bacterial identification and PCR molecular assay targeting the species-specific 350 bp product. The results demonstrated a high concordance between VITEK 2 and PCR methods, reinforcing the efficacy of these techniques for rapid and accurate diagnosis. Additionally, histopathological analysis and microscopic examination were performed to evaluate the tissue changes in the infected lung samples. Tissue sections were stained with HandE to highlight cellular alterations and tissue damage, and Ziehl-Nielsen staining was used to identify microbial organisms within the affected tissues. These techniques provided valuable insights into the pathogenic mechanisms of *P. multocida* infection in sheep. The antibiotic susceptibility of the isolates was also assessed, revealing resistance to several antibiotics, highlighting the challenges in managing this infection. This study recommends the use of VITEK 2 and PCR as key tools for prompt diagnosis of *P. multocida*-related respiratory diseases.

Keywords | Lung, Pneumonia, Sheep, *Pasteurella multocida*

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INTRODUCTION

Pneumonia is one of the most commonly reported diseases in sheep, and a significant proportion of studies and farmer reports attribute pneumonia as the main reason for mortality in lambs (Miao *et al*., 2023). Pneumonia is characterized by inflammation of the alveoli and interstitial lung tissue, leading to impaired gas exchange and respiratory distress (Barilli *et al*., 2022). Pneumonia in domestic animals is defined as an acute or chronic inflammatory process of the pulmonary parenchyma, primarily involving the alveolar spaces and interstitial tissues, which may occur with or without microbial infection, resulting in impaired gas exchange and respiratory dysfunction (Kleinschmidt *et al*., 2022). *P. multocida* is classified into five capsular serogroups A, B, D, E, and F based on the antigenicity of its outer capsule polysaccharide, which is a key determinant of virulence and host interactions (Smith *et al*., 2021). Capsular serogroup A of *P. multocida* is universally the most prevalent among

isolates from diverse animal hosts. It is responsible for the most severe disease outbreaks, thereby carrying significant public health, veterinary, and economic implications (Li *et al*., 2025). Sheep infected with *P. multocida* often exhibit clinical signs and gross lung lesions such as cranioventral consolidation, fibrinous exudate, and bronchopneumonia that are virtually indistinguishable from those caused by *Mannheimia haemolytica*, necessitating the use of laboratory methods (culture or PCR) for accurate diagnosis (Alemu *et al*., 2023; Ali *et al*., 2024; Al-Sailawi *et al*., 2024). The clinical presentation and gross pathological findings of pneumonic pasteurellosis in sheep, whether caused by *P. multocida* or Mannheimia haemolytica, are virtually indistinguishable, as both pathogens produce cranioventral consolidation, fibrinous exudate, and similar inflammatory infiltrates, necessitating laboratory confirmation for accurate diagnosis (Abera and Mossie, 2022). Species identification of *P. multocida* is routinely achieved using molecular techniques, such as polymerase chain reaction (PCR) targeting species-specific genes, multilocus sequence typing (MLST), and *16S rRNA* gene sequencing, to resolve phylogenetic relationships among isolates (Zhu *et al*., 2020). Recent studies have shown that genetic diversity in the *16S rRNA* gene of *P. multocida* is correlated with host specificity. For instance, a study analyzing isolates from poultry in Southwest China found that variations in the 16S rRNA sequences were associated with different host species, suggesting a role in host adaptation (Peng *et al*., 2018). Lung lesions are a hallmark of *P. multocida* infection, often resulting from the bacterium's ability to induce apoptosis in pulmonary epithelial cells. A recent study demonstrated that *P. multocida* activates the FAK-AKT-FOXO1 signaling pathway, leading to compromised pulmonary integrity, bacteremia, and a subsequent cytokine storm in animal models (Zhang *et al*., 2024). In acute infections caused by *P. multocida*, lung lesions predominantly localize to the cranioventral lobes of the lung. Gross examination reveals firm, consolidated areas with a red to gray coloration, often accompanied by fibrinous pleuritis. These lesions are indicative of acute suppurative bronchopneumonia, characterized by neutrophilic infiltration and fibrin deposition within the alveolar spaces (Praveena *et al*., 2014). In affected areas, lung tissue exhibits increased stiffness and reduced sponginess upon digital palpation, which is often attributed to fibrosis or cellular infiltration resulting from inflammation. Techniques such as shear wave elastography have been utilized to accurately assess this stiffness, revealing that diseased lung tissue is significantly less elastic than healthy tissue, making it a valuable diagnostic marker in pulmonary diseases (Petersen *et al*., 2024). The appearance of consolidated lung areas can vary, often presenting as gray or hazy regions on imaging studies, depending on the stage of inflammation and the nature of the infiltrate (Hacking *et al*., 2025). Neutrophils predominate in the exudative phase, lymphocytes increase in the early proliferative phase, and macrophages become more abundant in the late proliferative phase. This shift in cellular distribution reflects the progression of the inflammatory response in the lung (Rodionov *et al*., 2022). The study aims to isolate and identify *P. multocida* in sheep and to investigate its molecular characteristics and histological changes in the lungs of infected sheep.

MATERIALS AND METHODS

Collection of samples

150 sheep samples were collected from various locations in Al-Qasim city, Babil governorate, between October 2024 and March 2025. Out of these, 54 samples showed clinical signs of pneumonia, including nasal discharge, mucus, and other fluids that are commonly found in the nasal and pharyngeal passages of a sheep.

Morphological and bacteriological detection

Samples from infected cases were cultured on 3%-5% Sheep Blood Agar, MacConkey Agar, Chocolate agar, Mannitol Salt Agar, EMB Agar, and Brain Heart Infusion agar. The cultures were incubated at 37˚C for 24 hours, and the plates were subsequently examined for growth. The following characteristics were observed: no growth on MacConkey Agar. Biochemical identification was performed using Vitek technology, which showed catalase and oxidase positivity, indole production, and characteristics of non-motility and non-hemolysis on Sheep Blood Agar (SBA), as well as acid production from glucose (Moore *et al*., 1994; Atlas *et al*., 1995; Holmes, 1998).

Histopathology

Tissue samples from the lung were preserved using the standard method of staining in hematoxylin and iodine after immersion in a formaldehyde-buffered solution to facilitate sectioning into small blocks of 1 cm^3. The samples were then washed with tap water for two hours, after which they underwent routine processing procedures, including Dehydration, Clearing, Infiltration, embedding, Sectioning, Mounting, Rehydration, Staining, drying, and labeling. Dehydration was achieved through a series of alcohol concentrations, starting with 50%, 60%, 70%, 80%, 90%, and 100% alcohol for 2 hours each. Clearing was performed using Xylene twice, with a 30-minute exposure for each solution. Infiltration involved immersing tissue specimens in paraffin wax at 58–60˚C for two cycles of 2 hours each. The specimens were then embedded in paraffin wax and allowed to cool for 24 hours. The tissue was sectioned to a thickness of 5-7 µm using a rotary microtome. For mounting, a thin layer of Mayer's egg albumin was used to adhere the tissue sections to glass slides. Rehydration was done through a decreasing alcohol series starting from

100%, 95%, to 90%. Finally, the specimens were stained with Hematoxylin and Eosin (H and E) and mounted by adding DPX, followed by the placement of a cover slip over the sections (Suvarna *et al*., 2013).

Harries hematoxylin and eosin

These stains are used to demonstrate the general histological components of the tissue (Lee and Luna, 1968).

Ziehl-nielsen staining

These stains are used to demonstrate acid-fast bacilli (AFB), primarily to identify *Mycobacterium* species, including *Mycobacterium tuberculosis* (the causative agent of tuberculosis), as well as other mycobacteria, such as those responsible for leprosy. The Ziehl-Nielsen stain utilizes a strong aniline dye, carbol fuchsin, which binds to the lipid-rich cell wall of acid-fast bacteria, allowing them to retain the color even after being subjected to acid-alcohol decolorization (Azadi *et al*., 2018).

RESULTS

Isolation of *P. multocida*

150 samples were collected, out of which 54 samples were identified as *Pasteurella multocida*, representing 36% of the total samples. The suspected colonies exhibited growth on Sheep Blood Agar (SBA) with no apparent hemolytic activity. This indicates that the bacteria did not cause significant breakdown of red blood cells, resulting in the absence of clearing or discoloration around the colonies on the agar (Figure 1). The characteristic identification of bacterial isolates of *P. multocida* in the laboratory is as follows:

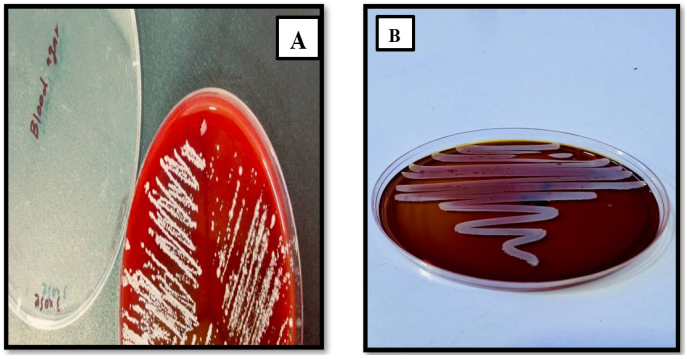

Figure 1: A, Blood Agar non-hemolysis; B, Chocolate agar.

The isolates were non-motile, meaning they lacked flagella and therefore could not move. They tested positive for catalase and oxidase, indicating the presence of specific enzymes. Additionally, they were indole-positive, which signifies the production of indole during testing. The isolates were also capable of utilizing glucose to produce acid, a characteristic frequently associated with *P. multocida*. The isolates did not grow on MacConkey agar. All isolates were positively identified as *P. multocida* using the Vitek test, which employs a commercial system that determines bacterial identification based on specific biochemical reactions. As shown in Figure 2.

bioMérieux Customer: Microbiology Chart Report Printed April 9, 2025 1:34:40 PM CDT

Patient Name: Patient ID:
Location: Physician:
Lab ID: 188 Isolate Number: 2
Organism Quantity:
Selected Organism: Pasteurella multocida

Source: Collected:

Comments:	

Identification Information	Analysis Time: 4.83 hours	Status: Final
Selected Organism	93% Probability Pasteurella multocida Bionumber: 0221714470200210	
ID Analysis Messages		

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATK	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Figure 2: The bacteria identified are *P. multocida* using the Vitek test.

Molecular Detection

However, subsequent polyclonal testing using molecular diagnostic techniques with primers confirmed the presence of *P. multocida*. The data showed that samples (100%) were positive for 16S ribosomal RNA specific for *P. multocida* (Abed *et al*., 2024). Results were significant as described in the Figure 3.

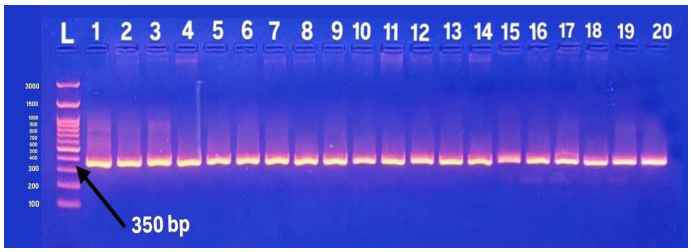

Figure 3: Molecular identification of *P. multocida isolates* with specific primers of the 16S rRNA gene, where recorded in all isolates 20/20 (100%) positive results at 350bp.

These findings highlight the importance of confirming bacterial identification through molecular techniques, such as PCR, as they provide more accurate and reliable results compared to relying solely on the Vitek test.

Histopathology

Upon gross examination of the affected tissues, they appeared inflamed and enlarged, with varying textures and appearances. Histologically, samples taken from the lungs and bronchi of infected sheep revealed red spots mixed with fibrous fluids. showed in Figures (4-10).

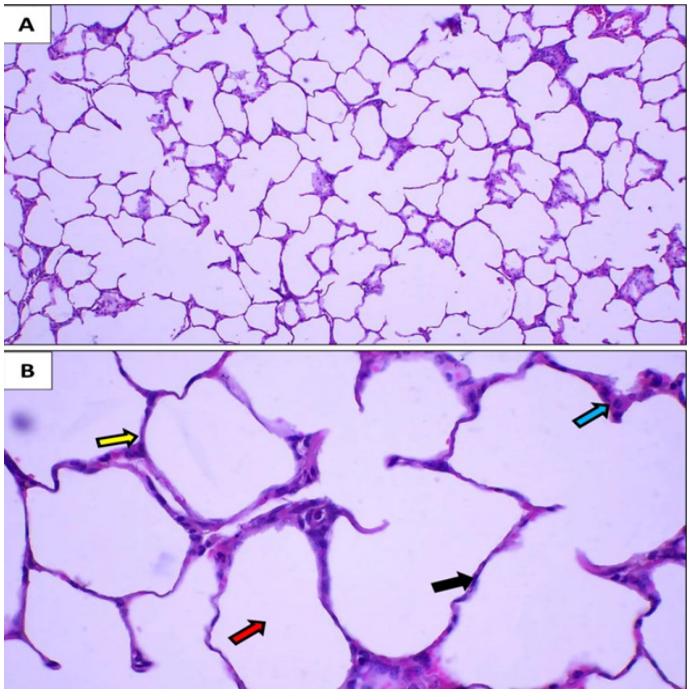

Figure 4: Photomicrograph of the lung of a control negative group sheep.
A and B: Normal histology of lung shows transparent space alveolar sac (red arrow), alveolar septum (yellow arrow), type 1 pneumocytes (black arrow), and type 2 pneumocytes (blue arrow). H and E. A: x100 and B: x400.

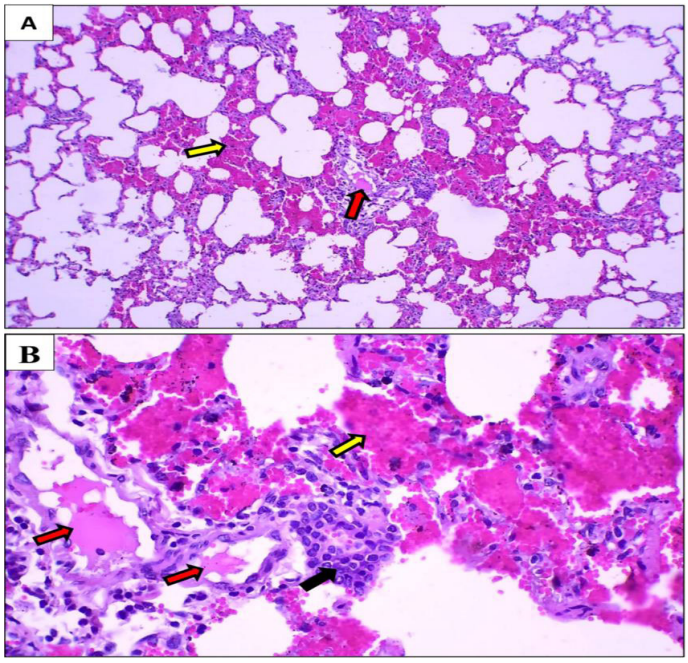

Figure 5: Photomicrograph of the lung of post-infection sheep. A and B: Destruction of the alveolar pneumocytes led to the absence of alveolar architecture, with the presence of hemorrhage (yellow arrow) due to the destruction of blood vessels in the affected area. Additionally, infiltration of inflammatory cells (black arrow) was observed in the affected area, with some alveolar cavities filled with a substantial amount of exudate fluid (red arrow). H and E. A: x100 and B: x400.

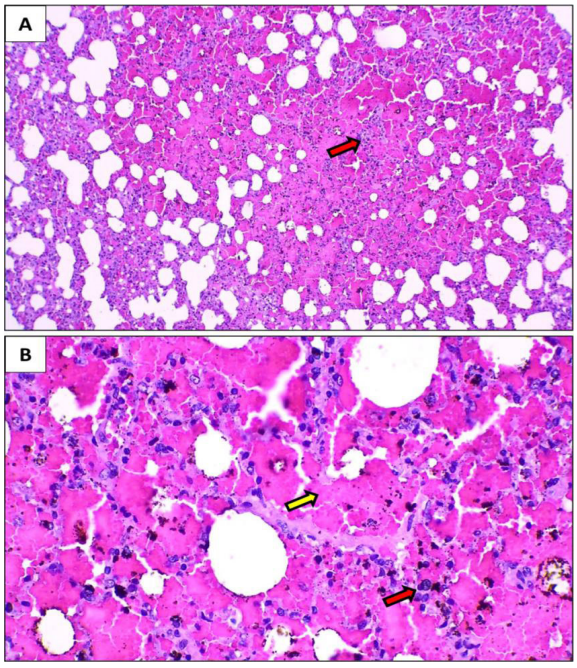

Figure 6: Photomicrograph of the lung of post-infection sheep. A and B: Destruction of the alveolar pneumocytes led to the absence of alveolar architecture, with severe hemorrhage that filled all spaces (yellow arrow) due to the destruction of blood vessels in the affected area. Additionally, infiltration of inflammatory cells was observed in the affected area; some alveolar cavities were filled with a considerable amount of exudate fluid (indicated by the red arrow). H and E. A: x100 and B: x400.

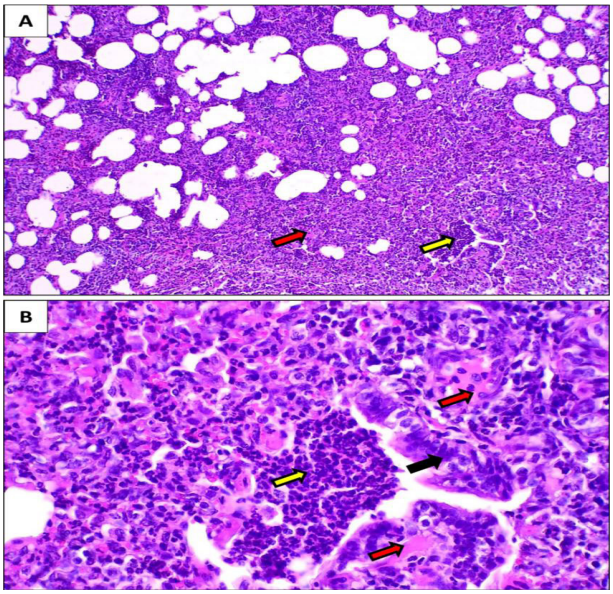

Figure 7: Photomicrograph of the lung of a post-infection sheep. A and B: Severe inflammation in the affected area led to hyperplasia of pneumocytes, with complete loss of normal pulmonary architecture (black arrow). Note that the alveolar space was utterly absent. Additionally, infiltration of inflammatory cells was observed within pneumocytes (yellow arrow). The alveolar cavity was filled with an amount of exudate fluid (red arrow). H and E. A: x100 and B: x400.

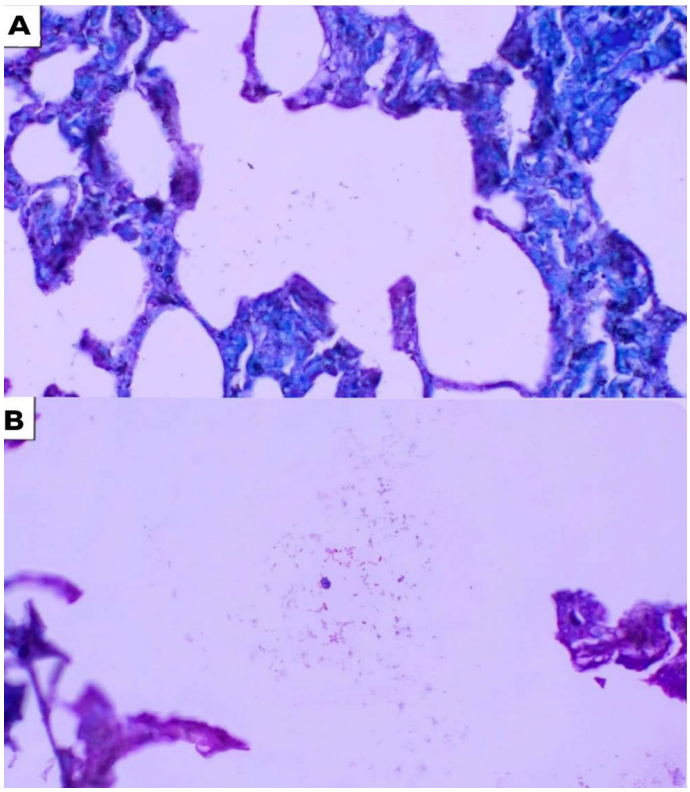

Figure 8: Photomicrograph of the lung group sheep. Small, rod-shaped, oval, or spherical bacteria (*Coccabacilli*), their cells arranged singly or in pairs, and sometimes in short chains, Zeihl Nielsen Stain. A and B: 100X.

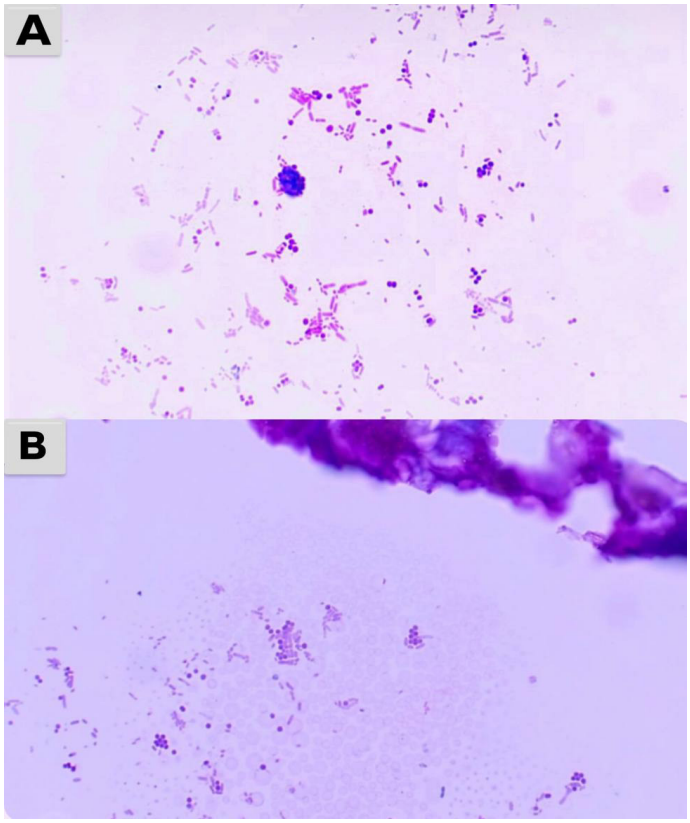

Figure 9: Photomicrograph of the lung group sheep. Small, rod-shaped, oval, or spherical bacteria (Coccabacilli), their cells arranged singly or in pairs, and sometimes in short chains. Zeihl Nielsen Stain. A and B: 400X.

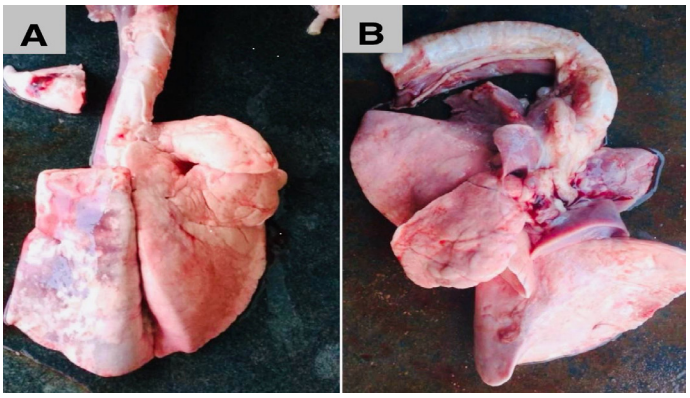

Figure 10: A: Lung infected with *P. multocida*, B: normal sheep lung.

The lung appears dark red to purple, with evident hemorrhagic and congested areas. The surface is irregular and may show signs of edema or fibrosis. These pathological changes indicate acute pneumonia caused by *P. multocida* infection. The lung appears uniformly pink with a smooth, glistening surface. There are no visible signs of inflammation, congestion, or tissue damage. The lung tissue is elastic and healthy, indicating normal respiratory function.

DISCUSSION

In this study, 150 samples were collected from sheep showing respiratory signs in the Al-Qasim, Babylon region, and *Pasteurella multocida* was isolated from 54 samples, representing 36% of the total. These findings are consistent with several previous studies conducted in various parts of the world. In the study by Ali *et al.* (2020) in Pakistan, *P. multocida* was isolated from sheep with respiratory diseases, showing isolation rates similar to those found in our study, with an isolation percentage of 36%, confirming that *P. multocida* is a common pathogen in sheep respiratory infections across different countries such as Pakistan and Iraq. Similarly, in a study by Hassan *et al.* (2019) in Iraq, *P. multocida* was isolated from sheep, with comparable isolation rates to those found in our study, highlighting the widespread presence of this pathogen in various regions of Iraq. Moreover, Mohamed *et al.* (2018) confirmed that *P. multocida* does not grow on selective media such as MacConkey and EMB, aligning with our findings, where isolates grew only on Blood agar and Chocolate agar, reinforcing the reliability and consistency of the biological characteristics of *P. multocida* across various studies. Despite the notable similarities in isolation rates, slight differences may exist between our study and others due to factors such as sample size, timing of collection, climate, and environmental conditions. The variation in isolation percentages may result from differences in sampling methods or diagnostic approaches, as well as the influence of environmental factors on the prevalence of *P. multocida*.

Sample collection methods can also influence the results. In our study, samples were taken specifically from sheep exhibiting clear respiratory signs, which may have resulted in higher isolation rates compared to studies using random sampling from asymptomatic animals. Studies that used random or non-symptomatic sampling may have recorded lower isolation rates compared to our study, which targeted clinically affected sheep. Environmental conditions play a crucial role in the prevalence of *P. multocida*, as regions with colder and more humid climates tend to have a higher incidence of respiratory infections, which may explain the higher isolation rates observed in our study compared to those in warmer, drier areas. Relatively low temperatures and moderate humidity characterize the Al-Qasim region, which could have contributed to the increased isolation rates recorded in this study. The VITEK 2 system successfully identified isolates within 6 hours, aligning with the results of Singh *et al.* (2021). However, contrary to Zangenah *et al.* (2012), who reported only 48.5% identification accuracy for *P. multocida*, our study demonstrated better concordance with molecular confirmation, aligning with the findings of Abubakr *et al.* (2019), who reported 100% accuracy in bovine isolates, suggesting that isolate source and database updates can influence system performance. Species-specific primers were used to detect *P. multocida* via PCR, yielding a 350-bp amplicon in all isolates. These results are consistent with those of Rahman *et al.* (2017) and Khan *et al.* (2016), who emphasized PCR as a highly accurate and confirmatory diagnostic tool, particularly when conventional methods are inconclusive. Histopathological examination of the lungs revealed significant pathological changes, ranging from mild alveolar disruption to severe destruction of pulmonary architecture, characterized by complete loss of alveolar structure, severe hemorrhage due to blood vessel destruction, heavy infiltration of inflammatory cells, alveolar cavities filled with exudate, and hyperplasia of pneumocytes. These lesions were observed in H and E-stained sections under 100X and 400X magnifications. Ziehl-Nielsen staining revealed the presence of small, rod-shaped, or coccobacillary bacteria arranged singly, in pairs, or short chains. These findings are consistent with those of Khan *et al.* (2017), who reported pulmonary hemorrhage and cellular infiltration in sheep infected with *P. multocida*, and with Yousef *et al.* (2020), who described complete alveolar architectural disruption and exudate accumulation. However, some discrepancies were noted when compared to Sabah *et al.* (2021), who observed only mild to moderate pathological changes without complete architectural loss.

CONCLUSION

This study highlights the role of *Pasteurella multocida* as a significant respiratory pathogen in sheep with a 36% isolation rate from clinically affected animals. These results are consistent with findings from previous regional and international research, indicating the widespread prevalence and pathogenic nature of this bacterium. Histopathological analysis revealed severe lung tissue damage, including alveolar destruction, hemorrhage, and inflammatory cell infiltration. The use of conventional culture methods, PCR with species-specific primers, and the VITEK 2 identification system provided a comprehensive and reliable diagnostic approach. The observed differences in isolation rates among studies may be attributed to variations in environmental, seasonal, and methodological factors. This research underlines the need for ongoing surveillance and improved control strategies to mitigate the impact of *P. multocida* infections on animal health and productivity.

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NOVELTY STATEMENT

This study presents a novel integration of molecular identification, advanced diagnostic tools (VITEK 2 and PCR), and histopathological examination for the comprehensive characterization of pneumonic pasteurellosis in sheep. It is one of the few studies in Iraq to combine these techniques, providing robust diagnostic evidence and contributing to improved understanding of Pasteurella multocida infections in livestock.

AUTHOR'S CONTRIBUTION

Baqer Hussein Jejan Al-Janabi: Collected samples, performed laboratory work, and contributed to data analysis and interpretation. Hameedah Hamzah Ajeel: Designed the study, supervised the research process, interpreted histopathological findings, and finalized the manuscript.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the conduct, interpretation, or publication of this research. This study was carried out independently as part of a postgraduate research project at the College of Veterinary Medicine, with no financial or commercial influence from any external parties.

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