

Molecular detection and epidemiological characterization of *Pasteurella multocida* associated with respiratory disease in sheep in Salah Alden province, Iraq

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Abstract

This study investigated the cultural, molecular, and epidemiological aspects of *Pasteurella multocida* in sheep affected with respiratory infections. Data were collected from 130 sheep that exhibited severe respiratory illness from various locations across Salah Alden province, Iraq. Nasal swabs were obtained from the sheep, cultured, identified by biochemical tests, confirmed by polymerase chain reaction (PCR), and subjected to antibiotic susceptibility testing. Seasonal patterns were analyzed, a relationship between clinical signs and infection status were established, and geographical distribution of infection sheep was assessed. The results indicated non-hemolytic colonies on blood agar that were Gram-negative and non-motile coccobacilli measuring 1-3 mm in diameter. The biochemical assays revealed positive oxidase, catalase, and indole tests, while the Simmons citrate and methyl red tests were negative, confirming the presence of *P. multocida*. The infection rate of *P. multocida* among the clinically affected sheep was 33.1% (43/130) and no significant sex difference was observed in the detection rate. Significant age-related differences were observed in the detection rate, with higher rate (62.1%) in sheep aged less than 1 year, followed by the age range of 1 to 2 years (37.8%), and the lowest (17.2%) in sheep greater than 2 years of age. The PMOut gene primers successfully amplified the 219 bp fragment from all culture positive samples, confirming the presence of *P. multocida*. The detection rate was higher in autumn (45.2%) and winter (38.7%) compared to spring (28.1%) and summer (21.4%) seasons. The main clinical signs correlating with *P. multocida* infection were fever, nasal discharge, and cough. The distribution of *P. multocida* positive cases in the Salah Alden province varied greatly among districts Tikrit revealing highest detection rate compared to Shirqat, and Tuz Khurmatu. Antibiotic susceptibility testing showed that all isolates were highly sensitive to gentamycin (100%) and completely resistant to penicillin, rifampin, and cloxacillin (100%). In conclusion, this study establishes that *P. multocida* is a major contributor to respiratory infection in sheep within the studied province with significant influence of age, season, and geography. The use of the PMOut gene for rapid and accurate identification along with epidemiological and antimicrobial testing data, provides valuable information for the development of effective disease control measures.

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1. Introduction

Pasteurellosis describes a range of diseases caused by bacteria belonging to the genus *Pasteurella*. Globally, the disease causes severe economic losses and is characterized by high mortality in sheep, goats, and cattle (Davies et al. 2003; Dziva and Mohan 2000). *Pasteurella multocida* is an opportunistic pathogen that colonizes the nasal and pharyngeal cavities of many mammals including humans. This Gram-negative, non-motile coccobacillus belonging to the family Pasteurellaceae is considered one of the main respiratory pathogens in small ruminants (Rawat et al. 2019). Because *Pasteurella*, particularly *P. multocida*, is part of the normal flora in the buccopharyngeal region, pneumonic pasteurellosis often affects debilitated, stressed, and compromised animals. The common predisposing factors are transportation stress, concurrent respiratory diseases, poor nutrition, unsanitary living conditions, and overcrowding (Piorunek et al. 2023; Yihunie et al. 2025). In the Asia-Pacific region, especially in Iraq, the disease occurs most frequently in sheep and goats during early winter or prior to the onset of the rainy season (Haji 2015).

Pasteurellosis is characterized by clinical signs including high fever (40-41°C), wet cough with foul-smelling mucus, dyspnoea, crackling sounds from the lungs, anorexia, depression, and pus-like discharge from the nose and eyes (Adegbeye et al. 2025). In addition to the direct losses due to livestock mortality, an affected livestock populations incur further additional losses associated with treatment, reduced productivity, and restrictions on trade (Mahboob et al. 2023; Sanchini, 2022). Traditionally, a variety of laboratory diagnostic methods are available and routinely used for diagnosis of pasteurellosis (Mahboob et al. 2023; Sanchini, 2022), including examination of direct blood smears, bacterial culture, biochemical tests, and serological testing. Advancements in biotechnology and the application of nucleic acid-based assays, particularly polymerase chain reaction (PCR), have successfully resulted in rapid, more accurate, and more reliable diagnosis and identification of pathogens (El-Jakee et al. 2016; Balestracci et al. 2023).

To devise effective control measures against pasteurellosis, a comprehensive understanding of the epidemiology, seasonal

fluctuations, clinical associations, and antibiotic resistance profiles of *P. multocida* in goat and sheep populations is important. Therefore, this study was undertaken to provide a comprehensive and detailed evaluation of the bacteriological, molecular, and epidemiological characteristics of *P. multocida* infections in sheep, and therefore contributes to the improved disease diagnosis, treatment, and control measures in the region.

2. Materials and Methods

2.1 Study design and sample collection

A cross-sectional research study was carried out between August 2022 to August 2023, and utilized systematic random sampling technique to select 130 sheep with clinical manifestations of respiratory infection from different districts of Salah Alden province. The selected animals were classified according to sex (male/female), age (<1yr, 1-2yrs, >2yrs), season when sampling took place (spring/summer/autumn/winter), and geographical area (Tikrit, Samarra, Baiji, Shirqat, Tuz Khurmatu).

2.2 Clinical examination

Each animal underwent a thorough clinical examination such as measurement of body temperature, respiratory rate, thoracic auscultation, assessment of nasal discharge and coughing. Overall health condition assessed by numerically scoring all the clinical parameters for subsequent for correlation analysis.

2.3 Sample collection and processing

Sterile nasal swabs were collected aseptically from each animal using standard procedures. Samples were transported in Stuart's transport medium at 4°C and processed within 4 hours of collection.

2.4 Culture method

The isolation of the bacterial pathogens from the nasal cavity was done by inoculating the nasal swabs onto appropriate culture media and incubated at 37 C for 24 hours. Subsequently, the suspected plates were further cultured for an additional 24 to 48 hours under aerobic condition at 37 C on MacConkey agar to check Gram-negative contaminants and 7% sheep blood agar for isolation. The colonies displacing typical morphological features were purified and subjected to biochemical testing. The isolates exhibiting consistent biochemical profiles were considered pure culture (Said 2006).

2.5 Biochemical identification

Standard biochemical Testing of *P. multocida* consisted of a series of six standard tests (catalase, oxidase, methylred, indole, urease, and voges-Proskauer) in conjunction with the Simmon Citrate assay. All tests were performed in triplicate and interpreted according to the standard protocols.

2.6 Molecular characterization by PCR

Bacterial genomic DNA was extracted using the boil method. Briefly, three bacterial colonies were mixed with 100 µL of sterile distilled water and boiled for 15 minutes at 100 C. To 100 µL of the boiled cell mixture, 900 µL of sterile distilled water was added and vortexed for approximately 10 seconds followed by centrifugation at 12,000 x g for 10 minutes. The resulting supernatant containing genomic DNA was used as the PCR template.

PCR analysis was conducted upon the genomic DNA, using the

PMOut gene-specific primers, PM-F (5'-AGGTGAAAG AGGTTATG-3) and PM-R (5'-TTTACCTAACTCAACCAAC-3'), to yield a 219 bp PCR product (amplicon). The PCR reaction mixture of 25 µL consisted of 12.5 µL Green Master Mix, 1.5 µL PM-F, 1.5 µL PM-R, and 9.5 µL of the extracted genomic DNA template. PCR cyclic conditions were as follows: Initial denaturation at 94 C for 2 minutes; 40 cycles of 94 C for 45 seconds, 45 C for 45 seconds, and 72 C for 1 minute; and final extension at 72 C for 5 minutes according to Aziz and Lafta (2021). Both positive and negative controls were run for each run. PCR assays were performed in duplicate for each isolate.

2.7 Antimicrobial sensitivity testing

Antimicrobial susceptibility of the bacteria was tested against ten commonly used antibiotics – penicillin, erythromycin, gentamicin, aztreonam, doxycycline, rifampicin, amoxicillin-clavulanate, clindamycin, colistin sulphate, and cloxacillin, using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The assays were performed in triplicate.

2.8 Statistical analysis

SPSS software (Version 21) was used to analyze the data. The data were summarized using descriptive statistics, and correlations between categorical variables were evaluated using chi-square tests. A statistically significant P-value was defined as ≤ 0.05. To assess the relationships between a few chosen independent variables, correlation analyses were performed.

3. Results

3.1 Overall infection rate and demographic distribution

The overall detection rate of *P. multocida* infection among the infected sheep was 33.1%, which was confirmed by both culture and PCR methods (43/130). The detection rates were not statistically different between the male (34.3%) and female (32.6%) sheep. However, there were significant age related differences in the detection rates (Table 1).

Table 1. Demographic distribution of <i>P. multocida</i> infection			
Category	No. of animals tested	No. of infected animals	Infection rate (%)
Sex			
Male	35	12	34.3 ^a
Female	95	31	32.6 ^a
Age			
< 1 year	29	18	62.1 ^a
1-2 years	37	14	37.8 ^b
> 2 years	64	11	17.2 ^c
Different letters (a, b, c) indicate significant differences (P<0.05)			

3.2 Biochemical characteristics

All the 43 positive isolates revealed consistent biochemical profiles typical to *P. multocida* (Table 2).

3.3 Molecular characterization

PCR analysis successfully amplified the PMOut gene in all culture-positive samples, confirming the identity of *P. multocida* (Table 3, Fig. 1).

Table 2. Biochemical characteristics of *P. multocida* isolates (n=43)

Biochemical test	Positive (%)	Negative (%)	Typical result
Gram staining	0	100	Gram-negative
Oxidase	100	0	Positive
Catalase	100	0	Positive
Indole	100	0	Positive
Simmon citrate	0	100	Negative
Methyl red	0	100	Negative
Voges-Proskauer	0	100	Negative
Urease	4.7	95.3	Negative
Glucose fermentation	100	0	Positive
Lactose fermentation	14.0	86.0	Variable
Sucrose fermentation	9.3	90.7	Variable

Table 3. PCR results and molecular characterization

Parameter	Results
Total samples tested by PCR	43
PCR positive samples	43 (100%)
PCR negative samples	0 (0%)
Expected amplicon size (bp)	219
Observed amplicon size (bp)	219 ± 5
PCR sensitivity*	100%
PCR specificity*	100%
Optimal annealing temperature (°C)	45
PCR efficiency (%)	95.2

* Based on culture-positive samples as gold standard

3.4. Clinical signs and association with infection

Table 4 shows the frequency of clinical signs seen in both infected and non-infected sheep. The most common symptoms among infected animals were fever, nasal discharge, and cough.

3.5 Seasonal and geographical distribution

Significant seasonal variations in infection rates of *P. multocida* were observed, with higher rates during cooler months (Table 5).

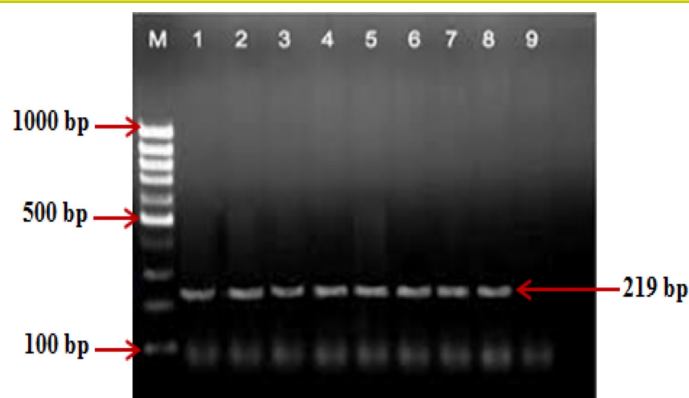


Fig. 1. *P. multocida* PMOut gene PCR products at 219 bp

Significant variations were observed in geographical distribution analysis among districts (Table 6). The Tikrit district revealed highest detection rate compared to Shirqat and Tuz Khurmatu.

3.6. Antimicrobial resistance profile

Antimicrobial sensitivity testing demonstrated variable resistance patterns among *P. multocida* isolates (Table 7).

4. Discussion

In the present study, the *P. multocida* detection rate (33.1%) is consistent with estimates published in previous studies in the comparable geographical settings. A prevalence of 32.6% in Ethiopia (Alemneh and Tewodros 2016) and 33.1% in Northwest Ethiopia (Ferede et al. 2013) has been reported. However, the overall detection rate was lower than that reported by previous studies such as 50.2% (Abera et al. 2014), 63.8% (Aschalew 1998), and 67.8% (Tesfaye 1997). Such differences in the *P. multocida* infection rates among these studies may be due to differences in sampling methods, geographic location, management practices, and other predisposing factors (Abera et al. 2014). Consistent with the study of Odugbo et al. (2006), no statistically significant differences in *P. multocida* detection rates based on sex was observed, indicating that physiological sex differences are unlikely to have any significant impact on the susceptibility of sheep to *P. multocida* when managed under similar conditions. On the other hand, Elsayed et al. (2021) reported significant differences in *P. multocida* infection rates based on the sex and attributed them to behavioral and physiological factors affecting susceptibility to *P. multocida*. Such variations suggest

Table 4. Clinical signs and correlation with *P. multocida* infection

Clinical signs	Infected sheep (n = 43)		Non-infected sheep (n = 87)	
	Present (%)	Absent (%)	Present (%)	Absent (%)
Fever (>39.5°C)	37 (86.0)	6 (14.0)	23 (26.4)	64 (73.6)
Nasal discharge	39 (90.7)	4 (9.3)	31 (35.6)	56 (64.4)
Cough	35 (81.4)	8 (18.6)	28 (32.2)	59 (67.8)
Dyspnea	28 (65.1)	15 (34.9)	19 (21.8)	68 (78.2)
Depression	31 (72.1)	12 (27.9)	22 (25.3)	65 (74.7)
Anorexia	26 (60.5)	17 (39.5)	18 (20.7)	69 (79.3)
Ocular discharge	22 (51.2)	21 (48.8)	14 (16.1)	73 (83.9)
Abnormal lung sounds	33 (76.7)	10 (23.3)	25 (28.7)	62 (71.3)

Table 5. Seasonal distribution of *P. multocida* infection

Season	No. of animals tested	No. of positive animals	Infection rate (%)
Spring	32	9	28.1 ^b
Summer	28	6	21.4 ^c
Autumn	31	14	45.2 ^a
Winter	39	15	38.5 ^a

Different letters indicate significant differences (P<0.05)

Table 6. Geographical distribution of *P. multocida* infection

District	No. of animals tested	No. of positive animals	Infection rate (%)
Tikrit	28	12	42.9 ^a
Samarra	26	8	30.8 ^b
Baiji	24	9	37.5 ^{ab}
Shirqat	27	7	25.9 ^b
Tuz Khurmatu	25	7	28.0 ^b

Different letters indicate significant differences (P<0.05)

Table 7. Antimicrobial resistance profile of *P. multocida* isolates (n=43)

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Penicillin	0 (0.0)	0 (0.0)	43 (100%)
Erythromycin	14 (32.6)	6 (14.0)	23 (53.4)
Gentamycin	43 (100.0)	0 (0.0)	0 (0.0)
Aztreonam	29 (67.4)	8 (18.6)	6 (14)
Doxycycline	18 (41.9)	11 (25.6)	14 (32.5)
Rifampin	0 (0.0)	2 (4.7)	41 (95.3)
Amoxicillin-Clavulanic acid	16 (37.2)	12 (27.9)	15 (34.9)
Clindamycin	31 (72.1)	7 (16.3)	5 (11.6)
Colistin sulfate	35 (81.4)	5 (11.6)	3 (7)
Cloxacillin	0 (0.0)	0 (0.0)	43 (100)

the influence of region-specific management practices and environmental conditions on the disease epidemiology.

The pattern of *P. multocida* infections in sheep relative to age indicated that young animals (<1 year) are much more susceptible than older animals. This age-associated susceptibility could be due to immature immunological capabilities and vulnerability to environmental stressors at young age (Hudson 2018; Ilgili 2025). The intermediate detection rate of *P. multocida* infections in sheep aged 1-2 years indicates the development of partial acquired immunity to the disease, whereas the animals older than two years likely established enhanced immunity from vaccination and/or naturally acquired immunity (Radostits et al. 1997). However, Benlashehr et al. (2025) reported that older sheep are more susceptible to *P. multocida* than younger sheep, which may as a result of differences in regional management practices, exposure history, and vaccination protocols. The biochemical profiles of all isolates obtained in this study were

consistent with the typical characteristics of *P. multocida*, suggesting the significant reliability of biochemical tests for preliminary identification in the laboratory. *P. multocida* positive reactions for catalase, indole, and oxidase have been reported earlier as well (Othman et al. 2014). However, variability in lactose and sucrose fermentation results indicates possible biochemical variability of *P. multocida* strains, which indicates that the presence of different biotypes and subspecies circulating within a particular geographic area.

PCR analysis revealed that all culture-positive samples were positive for the PMOut gene and the results are consistent with an earlier study (Dewi et al. 2021). The amplification of PMOut gene at an annealing temperature of 45 C with 95.2% efficiency indicates the reliability of PCR assay for accurate identification of *P. multocida*. Therefore, the PMOut gene based protocol can be considered a rapid and effective diagnostic tool for veterinary diagnostic laboratories. Additionally, seasonal differences in the detection rate of infection, with higher rates observed during autumn and winter, can be ascribed to the differences in the level of environmental stressors. During the fall and winter months, animals potentially experience greater respiratory stress and immune system suppression due to cooler temperatures and higher humidity (Shayegh et al. 2009) compared to the warmer months of spring and summer with comparatively better environmental conditions and availability of improved nutrition provided by pastures. This study also revealed significant geographical variation in the detection rate of *P. multocida*, with highest rate recorded in Tikrit district. These variations have been ascribed to differences in husbandry practices, animal density, biosecurity measures, and availability of veterinary services. Such differences demand region-specific disease control measures.

The strong association between clinical symptoms and the infection status, particularly fever (0.61), nasal discharge (0.58), and cough (51), indicates that these symptom can potentially be useful preliminary diagnosis. There is a need of clinical scoring system to facilitate early diagnosis and timely interventions for disease control. The present study revealed complete susceptibility of all the isolates to gentamicin indicating its use be continued as the first-line therapeutic agent against pasteurellosis. On similar lines, previous studies within the region also cite gentamicin as the first-line treatment method (Al-Othman 2019; Elshemey and Abd-Elrahman 2013). However, complete resistance was found against penicillin, rifampicin, and cloxacillin, potentially as a result of widespread and inappropriate use of beta-lactam antibiotics in the veterinary medicine. Moderate levels of resistance were observed against erythromycin (53.4%) and doxycycline (32.5%), which underscores the importance of judicious use of non-beta-lactam antibiotics. Furthermore, considerably low resistance to colistin (7%) and clindamycin (11.6%) indicates their potential as treatment options against pasteurellosis. The observed antimicrobial resistance patterns in this study are similar to those reported in other regional studies, which warrant the establishment of antimicrobial stewardship programmes (Al-Othman 2019; Elshemey and Abd-Elrahman 2013; Kalhor et al. 2010; Marru et al. 2013; Naz et al. 2012; Rasheed et al. 2024; Saleh et al. 2024; Sheet et al. 2024; Shivachandra et al. 2011).

Overall, the epidemiological findings in this study can serve as valuable information for creating a successful disease control programme. The higher susceptibility of young animals suggest the requirement of age-specific vaccination protocols and enhanced biosecurity measures in nursery areas. The observed seasonal

epidemiological trends in *P. multocida* infection can inform the timing of preventative interventions during the high-risk periods, whereas, geographical variations stress the need of region-specific vaccination or preventative programmes, taking into account the climate, management practices, and infrastructure of the region. This study's strong clinical associations support the development of standardized diagnostic methods and training programs within the veterinary profession. Despite several strengths, this study has limitations, such as its restriction to a single province and a single year and lack of genotypic study of antimicrobial resistance study. Therefore, future studies should focus on obtaining multi-year surveillance data across multiple provinces for developing a comprehensive understanding of the epidemiology of *P. multocida* infections. In addition, continued investigation into circulating serotypes and their role in vaccine development and targeted intervention strategies shall continue along with the study of genetic basis of antimicrobial resistance for more effective management of pasteurellosis in sheep.

5. Conclusions

This study demonstrates that *Pasteurella multocida* is a significant contributor to respiratory illness in sheep in Salah Alden Province, Iraq. An overall infection rate of 33.1% was observed with younger sheep and cooler months showing higher infection rates. A quick and accurate diagnostic technique for confirming the disease was PCR detection of the PMOut gene. The need for cautious antimicrobial use was highlighted by antimicrobial susceptibility testing, which showed broad resistance to several commonly used antibiotics but complete sensitivity to gentamicin. Clinical scoring systems for early diagnosis are supported by strong correlations between infection and important clinical signs, especially fever, cough, and nasal discharge. For effective pasteurellosis management, these results highlight the significance of seasonally-informed preventive measures, targeted vaccination of young animals, and the incorporation of molecular diagnostics into surveillance and control programs.

Declarations

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