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Serodetection of Contagious Caprine Pleuropneumonia in Mosul City, Iraq

Mahmood T Al-lahibi and Maab I. Al-Farwachi*

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

*Corresponding author's Email: maabalfrwche@yahoo.com

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ABSTRACT

Contagious caprine pleuropneumonia (CCPP) is a severe, highly transmissible respiratory disease in goats, resulting in significant economic losses. The precise antibody detection is critical for disease surveillance and control in suspected locations. The present study aimed to detect CCPP antibodies in goat sera using indirect enzyme-linked immunosorbent assay (ELISA) and latex agglutination tests (LAT) and to compare the diagnostic performance of these two serological methods. A cross-sectional study was conducted at the University of Mosul, Veterinary Teaching Hospital, Iraq. A total of 90 serum samples were collected from unvaccinated local goats (aged 1-7 years; 30 males, 60 females) exhibiting respiratory signs, including cough, nasal discharge, and dyspnea. The serum samples were analyzed for CCPP antibodies using both an indirect ELISA and LAT. The overall seropositivity rate was 21.1% for indirect ELISA and 46.6% for LAT. Among the latex-positive samples, 16.7% were weakly positive (+), 50% moderately positive (++), and 33.3% strongly positive (+++). The seroprevalence of CCPP was significantly higher in female goats than in males, as confirmed by both ELISA and LAT. Indirect ELISA revealed seroprevalence rates of 30.0% in females compared to 3.3% in males, while LAT results indicated a seroprevalence rate of 66.7% in females compared to 6.7% in males. The highest CCPP seroprevalence was found in the 2-3-year-old age group, with rates of 89.5% by ELISA and 92.9% by LAT. The LAT results demonstrated a sensitivity of 84.21%, a specificity of 63.38%, and an overall accuracy of 67.78%. Cohen's kappa coefficient (0.33) indicated fair agreement, while McNamara's test demonstrated a significant difference between the two tests. Based on receiver operating curve analysis, the indirect ELISA demonstrated an area under the curve of 0.83, with a suitable optical density limit of 0.15, resulting in 85% sensitivity and 70% specificity. Indirect ELISA offers greater reliability in detecting CCPP antibodies compared to LAT. The variability in LAT results highlighted the need for ELISA, revealing higher seropositivity in older females, a distinct epidemiological feature of the population.

Keywords: Contagious caprine pleuropneumonia, Goat, Mycoplasma infection, Serodiagnosis

INTRODUCTION

Contagious caprine pleuropneumonia (CCPP) is caused by the F38 biotype of *Mycoplasma* (*M.*), which has recently been reclassified as *M. capricolum* subspecies *capri pneumoniae* (Ahaduzzaman, 2021; Ali et al., 2024). This specific infection is very contagious in housed goats, with high morbidity (100%), and mortality ranging from 60% to 100% (Yatoo et al., 2018; 2019; Ahmad et al., 2021). Only goats are naturally infected; clinical signs include anorexia, listlessness, pyrexia, cough, and dyspnea with accompanying grunting and wide-based forelimb stance (Elhassan and Salama, 2018; Tharwat et al., 2025). On necropsy, a fibrinous pleuritis and pleural effusion are generally found, with occasional pericarditis. The lung lobes (unilateral or bilateral) are enlarged, firm, and variegated with red, yellow, white, and gray foci (Yatoo et al., 2019; Kabir et al., 2021; Tharwat et al., 2025). While clinical signs and gross lesions may be strongly suggestive during outbreaks, they are not definitive for diagnosis and require confirmatory laboratory testing (Tharwat and Al-Sobayil, 2017). An accurate diagnostic procedure requires proper sample collection and transportation under aseptic and standardized conditions (WOAH, 2021). Isolating *M. capricolum* subspecies *capri pneumoniae* by culture and identifying it through biochemical and antigenic techniques has limitations, including low sensitivity due to contamination and cross-reactivity with other *Mycoplasma* species. Additionally, these conventional methods require specialized media, prolonged incubation, and skilled laboratory technicians, which makes them time-consuming and frequently unsuitable for controlling outbreaks (Rather et al., 2021; Khodakaram-Tafti et al., 2023).

Conventional polymerase chain reaction (conventional PCR), real-time PCR, sequencing, and isothermal methods have become routine for CCPP detection; however, their adoption is limited by technological and financial barriers (Le Grand et al., 2004; Yatoo et al., 2019).

Serology is widely used for herd surveillance and exposure detection of CCPP (Ali et al., 2024). The latex agglutination test (LAT) is rapid, simple, suitable for whole blood or serum, and recommended for diagnosis of CCPP in the field, and has a sensitivity of about 80% (March et al., 2000). Multiple reports indicate that the LAT is more sensitive

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than the complement fixation test and simpler to perform than competitive enzyme-linked immunosorbent assays (ELISA; March et al., 2000; Wambugu, 2005; Soayfane et al., 2018).

Different forms of ELISA, including blocking ELISA, competitive ELISA, and indirect ELISA, offer enhanced specificity and sensitivity for detecting CCPP (Sharew et al., 2005; Jean de Dieu et al., 2019; Shaheen et al., 2024). Unlike complement fixation tests, which frequently cross-react with other *Mycoplasma* species, these ELISA techniques can distinguish CCPP from other respiratory infections, offering a more specific diagnostic alternative (Peyraud et al., 2014; Khan et al., 2025). Additionally, the CCPP was isolated and detected among sheep and goats in Iraq's neighboring countries, such as Saudi Arabia (El-Deeb et al., 2017), Turkey (Özdemir et al., 2018), and Iran (Khodakaram-Tafti et al., 2023).

In Iraq, CCPP is often misdiagnosed as other forms of caprine *Mycoplasma* pneumonia, leading to its underestimation as a distinct disease. Therefore, the present study aimed to identify CCPP seropositivity in symptomatic native goats in Mosul, Iraq, and to evaluate the diagnostic accuracy of the LAT compared to indirect ELISA.

MATERIALS AND METHODS

Ethical approval

The College of Veterinary Medicine Committee at the University of Mosul granted its approval for this study (Approval number: UM.VET. 2025.038) in August 2025.

Study area

Mosul is one of the largest cities in Iraq, situated in the Northern part of the country (Figure 1), just about 400 km (250 miles) north of Baghdad on the Tigris River. The area of the city is 32,308 km². This city experiences four distinct seasons, with variable rainfall periods in autumn, winter, and early spring. The temperatures in winter potentially range from 5°C to 25°C, and in summer from 25°C to 45°C. This zone is branded by diverse districts, such as rivers, fields, pastures, and agricultural lands with biological variety (Yaqoob et al., 2023).

Figure 1. Mosul city is located in the North-west of Iraq.

Study design and animals

A cross-sectional study was conducted in August 2025 at the University of Mosul, Veterinary Teaching Hospital in Iraq. Ninety serum samples were randomly collected from local goats (aged 1-7 years) presenting with respiratory symptoms such as cough, nasal discharge, and dyspnea. The cohort comprised 30 males and 60 females, distributed across age groups (≤ 1 year: 10 goats; 2–3 years: 78 goats; and ≥ 5 years: 2 goats). Blood samples (5 mL) were collected aseptically from the jugular vein into plain vacutainer tubes. Sera were separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until further analysis. All sera were tested for antibodies against CCPP using two diagnostic methods, including commercial indirect ELISA and the LAT.

Anti-CCPP antibodies were detected using a commercially available indirect ELISA kit (ID SL0128Gt, Sunlong Biotech Co., Hangzhou, China) as the reference protocol, following the manufacturer's instructions. The optical density (OD) values were determined using an ELISA plate reader (Multiskan FC, Thermo Fisher Scientific, USA) at a wavelength of 450 nm. The effectiveness was measured by the average value of the positive control (≥ 1.00) and negative control (≤ 0.10). The significant value (CUT OFF) was determined as the average value of the negative control plus 0.15. Samples were considered negative for anti-CCPP antibodies if the optical density (OD) value was below the calculated cut-off. An OD value equal to or greater than the cut-off indicated a positive result.

Latex agglutination test

A commercial CapriLAT kit (Animal and Plant Health Agency, Surrey, UK) was used according to the manufacturer's recommendations, and positive reactions were classified into three levels, including fine agglutination (weak) as +, clear agglutination (moderate) as ++, and strong clumping as +++.

Statistical analysis

The present data was stored in a Microsoft Office Excel 2007 file. The diagnostic performance of the LAT was compared to ELISA, which was regarded as the reference standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and total accuracy were measured. Cohen's kappa coefficient (κ) was used to examine the agreement between the two tests and was interpreted as per usual standards. McNemar's test was used to determine the statistical significance differences between the two assays. Additionally, receiver operating characteristic (ROC) curve analysis was performed to determine the optimal ELISA cutoff. The distribution of positive and negative samples by age and sex was summarized using Chi-Square (χ^2), such as frequencies and percentages. Statistical significance was defined as a p-value less than 5% ($p < 0.05$). The diagnostic test evaluation was performed using MedCalc Software Ltd. (Dhamnetiya et al., 2021).

RESULTS AND DISCUSSION

Specific antibodies against *M. capricolum* subspecies *capripneumoniae* were detected in 21.1% of serum samples using indirect ELISA and in 46.6% using the LAT (Table 1). The higher seropositivity observed with LAT might be attributed to its lower specificity and potential cross-reactivity with other *Mycoplasma* species, as previously reported in similar studies (March et al., 2000; Chandio et al., 2019). The indirect ELISA, known for its high specificity and reproducibility in CCPP serodiagnosis, produced a more conservative seropositivity result. Among latex-positive samples, 16.7% were weakly positive (+), 50% moderately positive (++), and 33.3% strongly positive (+++; Table 2). Seropositivity for CCPP was significantly higher in females than in males in both ELISA and LAT. Using indirect ELISA, seropositivity was 30.0% in females compared to 3.3% in males, by LAT 66.7% and 6.7% in females and males, respectively. These differences were statistically significant ($P = 0.0036$ for ELISA; $P < 0.0001$ for LAT). Sex-related differences in seropositivity or immune response to CCPP might be attributed to management and physiological factors. Female goats are frequently kept in herds for breeding and milk production, which increases their cumulative exposure to pathogens. Conversely, males are usually fewer and more likely to be sold or culled, which limits their duration of contact with diseased animals in the herd. Hormonal or physiological stresses associated with pregnancy and lactation might increase susceptibility or stronger antibody responses in females.

Table 1. Serological results for detecting antibodies against *Mycoplasma capricolum* subsp. *Capripneumoniae* in goats from Mosul, Iraq, 2025

Serological tests	Number of examined animals	Number of seropositive animals (%)	P-value
Indirect ELISA	90	19(21.1%)	P = 0.0003
Latex agglutination test		42(46.6%)	

Table 2. Distribution of seropositive goats according to the strength of the latex agglutination test in goats from Mosul, Iraq, 2025

Serological reactions	Number of seropositive animals	P-value
Strong	14 (33.3%)	P=0.2983
moderate	21(50%)	P=0.0661
Weak	7 (16.7%)	

Table 3. Distribution of antibodies against *Mycoplasma capricolum* subspecies *capripneumoniae* by sex and age in goats from Mosul, Iraq, 2025

Variables	Number of animals	Seropositive animals by Indirect ELISA (%)	Seropositive animals by latex agglutination test (%)
Sex			
Male	30	1(3.3)	2(6.7)
Female	60	18(30.0)	40(66.7)
P value		P = 0.0036	P < 0.0001
Age (years)			
≤ 1	10	1(5.3)	2(4.8)
2-3	78	17(89.5)	39(92.9)
≥ 5	2	1(5.3)	1(14.2)

Table 4. Diagnostic values of the latex agglutination test using indirect ELISA as a reference standard for the detection of antibodies against *Mycoplasma capricolum* subspecies *capripneumoniae* in goats from Mosul, Iraq, 2025

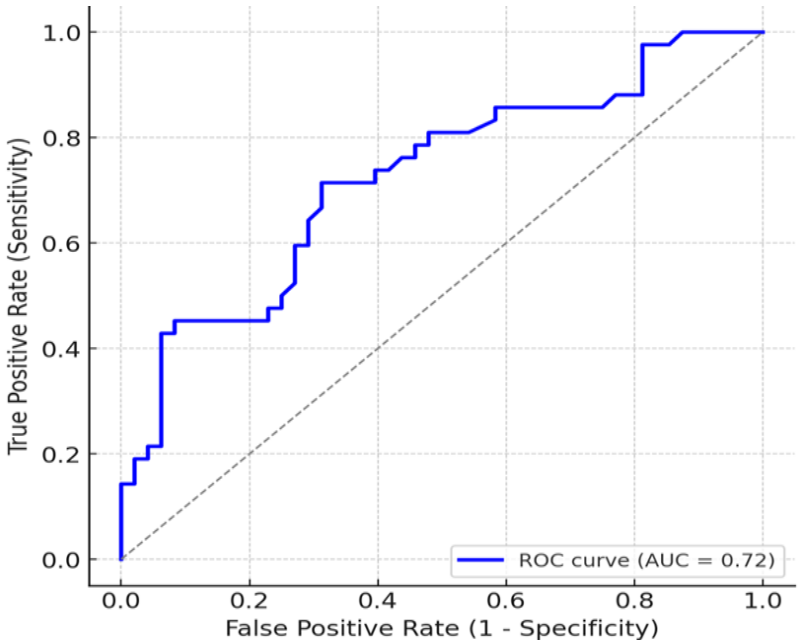
	Latex agglutination test			Total
Indirect ELISA (Reference standard)		16 (True positive)	3 (False negative)	19
		26 (False positive)	45 (False negative)	71
	Total	42	48	90
Test characteristic			Results	
Sensitivity (%)			84.21	
Specificity (%)			63.38	
Positive predictive value, PPV (%)			38.10	
Negative predictive value, NPV (%)			93.75	
Accuracy (%)			67.78	
Cohen's Kappa (κ)			0.33 (Fair agreement)	
McNemar's test (χ^2)			18.24, p < 0.0001	

Additionally, seropositivity varied by age group, with the highest percentages found in animals aged 2-3 years at 89.5% using ELISA and 92.9% using LAT. Lower seropositivity rates were observed in animals ≤ 1 year and ≥ 5 years old (Table 3). The highest seropositivity was recorded in animals aged 2-3 years, which are typically at peak productivity and most frequently in contact with the rest of the herd. Animals in this age group generally have repeated exposure to the infection, leading to higher antibody detection. In contrast, young animals (≤ 1 year) may have had limited exposure time or immature immune responses, resulting in lower positivity rates. Several factors might explain the reduced seropositivity in the oldest goats (≥ 5 years), including limited sample size, management practices that remove chronically affected animals, or natural declines in humoral immunity. The present findings aligned with previous reports suggesting that age and sex may influence exposure risk due to behavioral and physiological factors, such as prolonged herd contact and longer lifespan in breeding females (Teshome et al., 2020; Moti et al., 2024).

The LAT demonstrated a sensitivity of 84.21%, a specificity of 63.38%, and an overall accuracy of 67.78%, compared to ELISA. A sensitivity of 84.21% in the LAT test suggested the test effectively identified the majority of infected animals. McNemar's test revealed a statistically significant difference between ELISA and Latex results (Table 4). Table 4 reveals that the LAT had moderate diagnostic performance, but with a low specificity (63.38%), which resulted in a large number of false-positive results, compared to the indirect ELISA. The overall diagnosis accuracy was 67.78%, which indicated moderate agreement with the ELISA reference. The NPV was high (93.75%), whereas the PPV was low (38.1%). The low PPV (38.10%) demonstrated that many LAT-positive results were not confirmed by ELISA. On the other hand, the high NPV (93.75%) indicated that LAT was more reliable for excluding infection than confirming it. Cohen's kappa coefficient was 0.33, indicating a fair level of agreement between the LAT and ELISA. These findings confirmed that the LAT tends to overestimate seropositivity. Consequently, while the LAT is suitable for rapid field screening, ELISA remains necessary for confirmatory diagnosis and accurate serological assessment for *M. capricolum*

subsp. *capripneumoniae*. These results align with previous reports indicating that while LAT assays provide rapid and practical diagnostic advantages, they generally exhibit lower specificity and discordant results compared to ELISA, underscoring the importance of complementary testing in CCPP diagnosis (Jean de Dieu et al., 2019; Shaheen et al., 2024). Across clinical, pathological, and serological comparisons, ELISA was superior to LAT in accuracy, whereas PCR-based methods directly detect *M. capricolum* subsp. *capripneumoniae* DNA, providing a definitive diagnosis in early and subclinical infections (Tharwat and Al-Sobayil, 2017; Tharwat et al., 2025).

Receiver operating characteristic curve analysis was performed to assess the diagnostic performance of ELISA using the LAT as a reference. The area under the curve (AUC) was 0.72 (Graph 1), indicating the strong discriminative ability of ELISA, suggesting the ELISA is a reasonably accurate test for detecting CCPP, with an AUC above 0.7, typically interpreted as acceptable to strong performance in diagnostic evaluations (Aggarwal and Ranganathan, 2018). The current findings confirmed that ELISA provided reliable detection of CCPP antibodies compared to the LAT.



Graph 1. Receiver operating characteristic curve of ELISA using the latex agglutination test as the reference standard in goats

CONCLUSION

Indirect ELISA demonstrated superior reliability compared to LAT in the detection of CCPP antibodies. The greater intensity of LAT reactivity and higher seropositivity in females and goats aged 2–3 years suggest distinct epidemiological patterns within the study population. Future studies should assess the performance of LAT and ELISA in larger and more diverse goat populations, incorporating molecular diagnostic methods such as PCR for confirmation. Longitudinal antibody monitoring would help detection of infection frequency and the most effective time to conduct serological testing.

DECLARATIONS

Availability of data and materials

The data that support the findings of this study are available upon reasonable request from the corresponding author.

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Authors' contributions

Maab AL-Farwachi performed methodology, investigation, data analysis, original draft writing, statistical data, and review. Mahmood Al-lahibi provided sampling, laboratory analysis, and data analysis. The authors confirmed the final edition of the manuscript.

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Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors did not use AI for preparing any part of this study and writing this manuscript.

Availability of data and materials

The data to support the present study's findings are available upon reasonable request to the corresponding author.

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