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Research Article

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Development of a Latex Agglutination Test (LAT) using Whole-Cell Protein for the Detection of *Mycoplasma ovipneumoniae* Antibodies in Small Ruminants

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Abstract

Mycoplasma ovipneumoniae is a major pathogen responsible for respiratory illness in sheep and goats, particularly chronic nonprogressive pneumonia, and its presence results in substantial economic losses within the small ruminant sector. Although diagnostic methods such as culture and polymerase chain reaction (PCR) are routinely used, each approach has inherent limitations. There is no rapid, inexpensive and field-deployable serological assay available for the detection of *M. ovipneumoniae* antibodies in small ruminants. The objective of the present study was to develop a latex agglutination test (LAT) to detect *M. ovipneumoniae* antibodies in small ruminants at the field level. A latex agglutination test (LAT) was developed using whole-cell protein antigen extracted from *M. ovipneumoniae* coupled with latex beads (0.80 µm) and tested on serum samples from 33 PCR-positive and 77 PCR-negative animals. A comparison of the latex agglutination test results with those of PCR revealed 93.94% sensitivity and 93.51% specificity. The developed serological assay strongly agreed with the PCR results, with a kappa value greater than 0.81. Hence, this latex agglutination test kit offers a convenient and rapid means of diagnosing infections in field settings and is particularly useful for large-scale surveillance of infections in flocks.

Keywords: Latex agglutination test; Small ruminants; *Mycoplasma ovipneumoniae*; Antibodies; Penside diagnosis; Serology.

1. Introduction

Sheep and goats play a vital role in the livelihoods of Indian farmers by contributing substantially to meat and milk production and by exhibiting rapid growth rates. Consequently, they are considered valuable economic assets. Respiratory diseases in these animals lead to reduced weight gain, increased mortality and considerable financial losses.[1] Such infections arise from multiple interacting factors, with a wide range of etiological agents contributing to the respiratory disease complex.[2,3] Among these, mycoplasmosis, an emerging transboundary disease of sheep and goats, imposes heavy economic burdens on farmers and disrupts trade in many regions.[4-6] *Mycoplasma ovipneumoniae* is among the most important pathogens associated with chronic, nonprogressive pneumonia in small ruminants. First reported in 1963 in Scotland in sheep with pulmonary adenomatosis, the organism has since been identified in both clinically affected and apparently healthy animals.[7] Molecular detection of the organism has been reported in various parts of India: isolates from pneumonic sheep and goats in Andhra Pradesh have been confirmed using PCR on nasal swabs, while Santhiya *et al*., identified the pathogen in goats exhibiting respiratory symptoms in northern and central Kerala.[8,9] Similarly, *M. ovipneumoniae* has been detected in nasal secretions, tissues and synovial fluids of symptomatic small ruminants in the Bengaluru region of Karnataka.[10] These reports emphasize the need for accurate and prompt diagnosis to implement appropriate control measures. Although diagnostic methods such as culture, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) are widely utilized, each has inherent drawbacks. The culture is labor intensive, requires stringent growth conditions, and often fails because of the fastidious nature of *Mycoplasma* species. PCR provides superior sensitivity and specificity but is costly, depends on advanced laboratory facilities, and is impractical for large-scale screening in field settings. ELISA is more user friendly, yet its reliability may fluctuate because antigens can cross-react with related *Mycoplasma* spp. The latex agglutination test (LAT) has been explored historically as a rapid serodiagnostic tool. Kende reported that LAT performed comparably to tetrazolium reduction inhibition and complement fixation tests (CFT) for detecting *M. pneumoniae* antibodies.[11] Slavik and Switzer applied LAT to diagnose *M. hyopneumoniae* infection in pigs and observed persistent LAT antibody responses for up to 48 weeks.[12] Rurangirwa *et al*., developed a polysaccharide-based LAT for diagnosing contagious caprine pleuropneumonia (CCPP), which has a higher sensitivity than CFT does and is suitable as a pen-side test with results available in minutes.[13] Subsequent applications in various regions have demonstrated the practicality and cost-effectiveness of LAT for screening for mycoplasmal infections.[14-17] Despite these advancements, no rapid, low-cost, field-ready serological assay currently exists for detecting antibodies to *M. ovipneumoniae* in sheep and goats. LAT represents a promising alternative because of its simplicity, affordability and minimal equipment requirements. However, a dedicated LAT kit for *M. ovipneumoniae* has yet to be developed. Establishing such a test would enable quick pen-side screening, enhance surveillance programs and support timely management of respiratory diseases in small ruminant populations.

2. Materials and methods

2.1 Development and standardization of the Latex Agglutination Test Kit

The latex bead-sensitized whole-cell protein of *Mycoplasma ovipneumoniae* was prepared by the method outlined by Sankar *et al*., with slight modifications.[16]

2.2 Preparation of whole-cell protein

A reference isolate of *Mycoplasma ovipneumoniae,* which was isolated in our earlier study (Udhayavel *et al*.,) was initially inoculated into 2 ml of Mycoplasma experience liquid medium and incubated at 37°C for three days.[18] The inoculum was then transferred to 100 ml of medium and incubated at 37°C for an additional two days. Subculturing was subsequently performed in 500 ml of Mycoplasma Experience liquid medium, which was subsequently incubated at 37°C for three days with intermittent shaking. Afterward, the Mycoplasma cells were harvested by centrifugation at 18,000 rpm in a cooling centrifuge for one hour. Sterility checks were regularly conducted with each passage to prevent bacterial contamination. The cells were subsequently washed three times in sterile PBS, resuspended in PBS, and then sonicated with constant pulses for 30 seconds, with 30-second breaks, which was repeated 19 times. The concentration of whole-cell protein was estimated using the Bradford method.

2.3 Sensitization of latex beads with whole-cell protein

Latex beads (Sigma, 0.80 µm) were used. The latex bead suspension (10%) was washed twice by centrifugation at 6700 × g for three minutes each time in carbonate–bicarbonate buffer. The beads were then made into a 2% suspension with carbonate-bicarbonate buffer that was later mixed with an equal volume of *M. ovipneumoniae* whole-cell protein antigen (20 µg/ml) diluted in the same buffer. This mixture was incubated at 37°C for six hours with constant shaking at 250 × g. The sensitized beads were subsequently centrifuged at 6700 × g for three minutes, after which the pellet was resuspended as a 2% suspension in PBS containing 5 mg/ml bovine serum albumin (BSA; Himedia). The latex beads were then left at 37°C in a water bath overnight. Finally, the beads were centrifuged again as before, and the pellet was resuspended in PBS containing 0.5 mg/ml BSA.

2.4 Latex Agglutination Test kit

A volume of 25 µl of suspected serum was placed onto a clean glass slide. Similarly, 25 µl of the prepared sensitized latex beads coated with *Mycoplasma ovipneumoniae* whole-cell antigen was placed adjacent to the serum. The serum samples collected from the animals whose PCR results were positive and negative in our

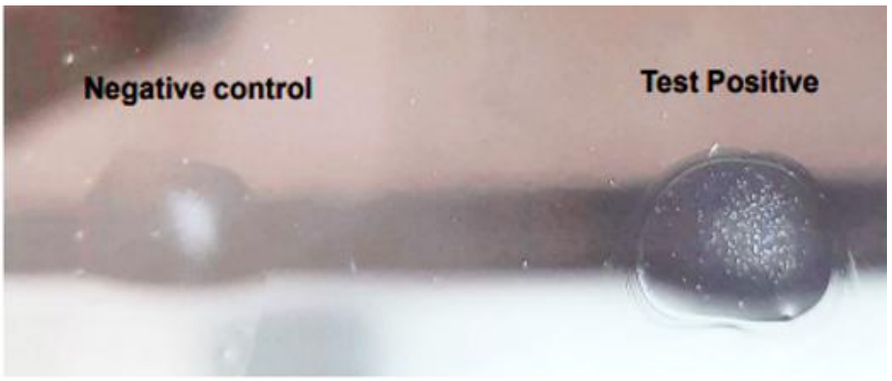


Fig. 1: Latex agglutination test for the detection of *Mycoplasma ovipneumoniae* antibodies
**Note: Agglutination with Latex beads sensitized with Mycoplasma ovipneumoniae whole-cell protein*

earlier study (Udhayavel *et al*.,) and the antigen were mixed using a stirring rod to form a circular area approximately 1.5 cm in diameter.[18] The glass plate was gently rocked for three minutes.

2.5 Assessment of the diagnostic characteristics of the developed Latex Agglutination Test kit

A latex agglutination test was performed on serum samples collected from 33 PCR-positive and 77 PCR-negative animals. The results of the latex agglutination test were compared with those of the PCR assay to determine indices such as sensitivity, specificity and Cohen's kappa coefficient (κ) statistics, with PCR used as the gold standard. MedCalc's diagnostic test evaluation calculator was used. The strength of agreement determined by Cohen's kappa coefficient (κ) was interpreted using standard benchmarks: $\kappa \leq 0$ indicates no agreement, 0.01–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, and 0.81–1.00 indicates almost perfect agreement.

3. Results

A latex agglutination test was performed on serum samples collected from 33 *Mycoplasma ovipneumoniae*-specific PCR-positive and 77 PCR-negative animals. Among the 110 corresponding serum samples tested, 31 showed agglutination when the latex agglutination kit was used (Fig. 1). Two nasal swab samples tested positive by PCR, but the corresponding serum samples did not show agglutination in the LAT region. Similarly, five nasal swabs were negative according to PCR, but when the corresponding serum samples were tested, they were positive according to the latex agglutination test.

Therefore, among the 110 serum samples screened, 36 (32.73%) tested positive by the latex agglutination test. The results are shown in Table 1.

3.1 Assessment of the diagnostic characteristics of the developed Latex Agglutination Test kit

The sensitivity and specificity of the latex agglutination test compared with those of PCR were 93.94 and 93.51%, respectively. Kappa statistics, which were used to analyze the agreement between the two tests, revealed a score of 0.852, indicating a high level of agreement (Table 2).

4. Discussion

The plate agglutination test primarily detects animals in the early (acute) stage of the disease.[19] The plate agglutination test requires larger bacterial cells to form visible aggregates. By attaching Mycoplasma cells to carrier particles such as latex, the mass of the cells can be increased, and the specificity of the Mycoplasma surface antigens can be maintained.[20] The latex agglutination test is a simple macroagglutination test that combines sensitivity with affordability and ease of use under field conditions, eliminating the need for specialized training or equipment. In this study, a latex agglutination test (LAT) was performed using latex beads (0.8 µm diameter) coated with sonicated Mycoplasma *ovipneumoniae* whole-cell protein. Ramadass *et al*., employed latex beads with a diameter of 0.8 µm for an agglutination test and reported that the test results were influenced by the size of the latex particles.[21] In this study, carbonate–bicarbonate buffer was used to dilute the latex particles during the sensitization of the beads. This approach aligns with the findings of Ramadass *et al*., who also identified this buffer as suitable for latex bead sensitization.[14] Sankar *et al*., also developed LAT based on whole-cell protein antigens to detect *Mycoplasma gallisepticum* antibodies from chicken serum in Kerala.[16] In the current study, the results of LAT were compared with those of PCR. Among the 110 serum samples tested, 31 showed agglutination in the LAT region. Two false negative and five false positive results were obtained. Hence, the seropositivity of *M. ovipneumoniae* antibodies in the latex agglutination test was 32.73% (36/110). The sensitivity and specificity of

Table 1: Comparative evaluation of the latex agglutination test with PCR

		PCR		
		Positive	Negative	Total
Latex agglutination test	Positive	31 (a)	5 (b)	36 (a+b)
	Negative	2 (c)	72 (d)	74 (c+d)
	Total	33 (a+c)	77 (b+d)	110

**Note: a - true positive, b- false positive, c- false negative, d- true negative*

Table 2: Diagnostic characteristics of the latex agglutination test

Diagnostic characteristics	Formula	Value
Sensitivity	[a/(a + c)] × 100	93.94% (95% CI: 79.82%–99.34%)
Specificity	[d/(b + d)] × 100	93.51% (95% CI: 85.50%–97.85%)
Kappa value	k= a+d-P/1-P	0.852 ± 0.054 (SE)
Statistical Significance (p-value)*	Fisher's Exact Test	p < 0.0001

**Note: An association is considered highly statistically significant when p < 0.05.*

the newly developed LAT kit were determined to be 93.94% and 93.51%, respectively. Furthermore, the calculated Cohen's kappa coefficient was 0.852, which falls within the 0.81–1.00 range. This statistically demonstrates an almost perfect agreement with the reference PCR gold standard, confirming the diagnostic robustness and reliability of the assay. This finding is in accordance with the findings of Sankar *et al*. (2013), who reported a sensitivity and specificity of 95.24% and 93.33%, respectively.[16] Hence, this agglutination-based kit offers a convenient and rapid means of diagnosing infections in field settings and is particularly useful for large-scale surveillance of infections in flocks rather than individual testing.

5. Conclusion

The latex agglutination test (LAT) developed in the present study demonstrated good diagnostic performance for the detection of *Mycoplasma ovipneumoniae* antibodies in small ruminants. The test was simple, rapid and easy to perform, with minimal technical requirements, making it suitable for routine screening of sheep and goat flocks. The satisfactory sensitivity and specificity observed in the study indicate that the LAT can serve as a useful preliminary serological screening tool for identifying animals exposed to *M. ovipneumoniae*. Its simplicity and rapid visual interpretation also offer advantages for application under field conditions, particularly in areas where sophisticated laboratory facilities are not readily available.

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CRediT Author Contribution Statement

Shanmugasundaram Udhayavel: Data curation, Formal analysis, Investigation, Writing – Original draft. **Kuppannan Sukumar:** Conceptualization, Methodology. **Kuppusamy Senthilkumar:** Writing – Review & editing. **Palani Srinivasan**: Writing – Review & editing. **Ayyasamy Elango:** Writing – Review & editing. All authors have read and approved the final version of the manuscript for publication and agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Institutional Review Board Statement

The serum samples utilized in this study were archival specimens obtained during a previous diagnostic field surveillance study [Ref. 18]. The original sampling was restricted to non-invasive nasal swabs from clinically ill field animals and post-mortem tissue from slaughterhouses, adhering to national animal welfare practices for routine veterinary diagnostics. For the current study, no new animals were handled, and the work was strictly restricted to in vitro laboratory analysis of these existing archival sera.

Informed Consent Statement

Prior verbal informed consent was obtained from the respective livestock owners during field sampling in the previous study.

Consent to Publish Statement

Not applicable.

Data Availability Statement

The datasets generated and/or analyzed during the current study that support the findings are available from the corresponding author upon reasonable request.

Conflict of Interest

Ayyasamy Elango serves as the Editorial Board Member and is a co-author of this manuscript. To ensure a rigorous and unbiased peer-review process, he was not involved in any stage start from editorial evaluation, peer review process and final publication decision. The handling of this manuscript was managed independently by another editorial board member. The other authors declare no competing interests.

Artificial Intelligence (AI) Use Disclosure

The authors declare that artificial intelligence (AI)-assisted tools were used only for language refinement, grammar improvement, and manuscript structuring purposes during the preparation of this work. All technical content, experimental implementation, results, and interpretations were independently developed and verified by the authors.

Supporting Information

Not applicable.

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