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Serological evidence of *Mycoplasma bovis* in pneumonic cattle

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Abstract

The present study was planned to investigate seropositivity for *Mycoplasma bovis* in pneumonic cattle. For this, serum samples from 41 pneumonic cattle presented to large animal clinics and ten apparently healthy cattle were investigated for antibodies to *Mycoplasma bovis* by commercial indirect ELISA kits. *Mycoplasma bovis* antibodies were detected in 21.9% (9/41) of pneumonic cattle, while all apparently healthy control cattle were seronegative, indicating an association with respiratory disease. Seropositivity was predominantly observed in chronic respiratory conditions, with 77.8% of positive animals affected by chronic active pneumonia, chronic pneumonia, or tuberculosis. *M. bovis* was consistently associated with other bacterial and viral pathogens, and only 3 of 9 seropositive cattle survived after treatment, suggesting its contribution to disease severity and poor prognosis. However, specific role of *M. bovis* as an etiological agent was difficult to assess based on the seropositivity alone but seroconversion only in affected cases suggests the role of *Mycoplasma* in pathology of the disease.

Keywords: Cattle, *Mycoplasma bovis*, pneumonia, seropositivity

Introduction

Pneumonia in cattle is a multifactorial condition that results from the interaction between infectious microorganisms, host defense mechanisms, environmental conditions, and stress [1]. Various factors such as climate and weather changes, transportation, poor ventilation, and deficiencies of vitamins and minerals can act as predisposing factors that weaken the immune system. Under these conditions, the normal flora of the respiratory tract may proliferate excessively and lead to subsequent infection of the lungs [2]. Among the infectious agents, *Mycoplasma bovis* is an important etiological agent of respiratory diseases in cattle. It commonly inhabits the upper and lower respiratory tract of healthy cattle as part of the normal microbial flora. Besides acting as a primary pathogen, *Mycoplasma* can increase the severity of respiratory disease in calves [3,4]. *Mycoplasma bovis* may also be associated with concurrent viral infections [5].

Mycoplasma bovis predominantly causes chronic pneumonia and arthritis in calves and young cattle and mastitis in adult cattle. The lung lesions are characterized by caseous and sometimes coagulative necrosis, leading to persistent infections that often respond poorly to many antibiotics [6]. Retrospective analysis has shown that the characteristic lesions were either overlooked or misattributed to other infectious agents [3] and it becomes difficult to differentiate mycoplasmal pneumonia from other bacterial pneumonia. Although, *M. bovis* can independently cause natural and experimentally induced clinical disease, but the presence of *M. bovis* does not always result in disease [6]. Although the role of *Mycoplasma bovis* in bovine pneumonia is well recognized, there are few reports from India on its occurrence in pneumonic cattle [7]. Thus, the present study was designed to investigate the seropositivity of *M. bovis* from pneumonic cattle presented to large animal clinics, GADVASU.

Materials and Methods

Forty-one cows with clinical and radiographic evidence of pneumonia were selected. The cows were subjected to blood sampling. Following proper restraint of the animal, 2 mL of blood was

aseptically collected from the jugular vein into EDTA-coated vials (Accuvette-PLUS, Quantum Biologicals Pvt. Ltd.). Additionally, 5 mL of blood was collected in serum collection tubes and centrifuged for separation of serum. The harvested serum was stored at -20°C until further use for ELISA-based detection of antibodies against *Mycoplasma bovis*. Commercial indirect ELISA kits developed by Bio-X Diagnostics®, Belgium, were used. Likewise, serum samples from the seropositive animals were also investigated for antibodies to bovine parainfluenza virus-3 (PIV-3), bovine respiratory syncytial virus (BRSV) and bovine herpes virus-1 (BoHV-1) by commercial indirect ELISA kits (Bio-X Diagnostics®). Ten apparently healthy cows were included as healthy control.

The affected cows were also subjected to tracheo-bronchial wash (TBW) sampling using the percutaneous method [8]. Diagnosis and classification of pneumonia were established on the basis of TBW cytological examination. The TBW samples from the seropositive cows were also subjected to bacterial isolation. For this, 5 mL aliquot of TBA was centrifuged at 3000 rpm for 5 min to discard the supernatant. The sediment was cultured on 5 per cent defibrinated sheep blood agar and MacConkey's lactose agar (MLA) and incubated for overnight at 37°C in aerobic conditions. All isolates were characterized and identified upto genus level using growth, staining and biochemical characteristics [9].

Serodiagnosis of *Mycoplasma bovis*

The ELISA kit used microtitration plate sensitized by recombinant protein from *Mycoplasma bovis* expressed by *E. coli*. If specific anti-*Mycoplasma bovis* immunoglobulins were present in the test sera, the conjugate remained bound to the microwell that contained the recombinant *M. bovis* protein and the enzyme catalysed the transformation of the colourless conjugate into a pigmented compound.

The test sera were diluted (1:100) in dilution buffer. A 100 μl of diluted sample, positive serum and negative serum were distributed in each well. After incubation at $21 \pm 3^{\circ}\text{C}$ for an hour, the microtitre plates were rinsed thoroughly with washing solution to remove all unbound sample. This was followed by addition of 100 μl diluted conjugate – a protein G peroxidase-labelled in all wells containing samples as well as controls. Following this, the plate was incubated again at $21 \pm 3^{\circ}\text{C}$ for an hour, thoroughly rinsed and then 100 μl chromogen tetramethyl benzidine was added to each well. The plate was then incubated for 10 min at $21 \pm 3^{\circ}\text{C}$ protected from light, for development of blue color. The intensity of the resulting blue colour is proportionate to the titre of specific antibody in the sample. The reaction was stopped by addition of 50 μl Stop solution in each well after the incubation, which was marked by immediate change of blue color into yellow. The micro plate reader was run and measurements were taken at 450 nm within 10 min.

Interpretation

$$\text{Sample's Coefficient} = \frac{\text{OD sample} - \text{OD negative serum} \times 100}{\text{OD positive serum} - \text{OD negative serum}}$$

The sample was declared negative if its coefficient was < 37 per cent and positive if its coefficient was ≥ 37 per cent.

Results

Mycoplasma bovis was detected by ELISA in nine (21.9 per cent) of 41 pneumonic cows (Table 1). None of the healthy

control animals ($n=10$) was seropositive for *M. bovis*.

On evaluating the association of *M. bovis* with different forms of pneumonia, seroconversion against *Mycoplasma bovis* was detected in two cows each affected with fibrinopurulent pneumonia and chronic active pneumonia, one cow each with bronchitis and chronic pneumonia, and three cows diagnosed with tuberculosis.

Mycoplasma was invariably associated with other bacterial organisms detected during the study viz. *Pseudomonas*, *Bacillus*, *E. coli*, *Klebsiella*, *Staphylococcus* and *Pasteurella* (Table 1). The cases were followed upto 6 months and six of nine seropositive cattle collapsed due to pneumonia. The TBW sample obtained from one cow diagnosed with fibrinopurulent pneumonia showed no bacterial growth. All the seropositive cases were also positive for one or more respiratory viruses (Table 1).

Discussion

Previous studies have reported lower seroprevalence of *M. bovis* i.e. 6 to 14 per cent T_{10} . Although there are reports of seroprevalence of *M. bovis* from mastitis cows (Vishnu *et al.*, 2025), but no serological study could be traced for the seroprevalence of *M. bovis* from pneumonic cattle in India. However, there is a study [7] only 2 out of 87 bovine carcasses were reported positive for *Mycoplasma bovis* by immunohistological staining and Polymerase chain reaction. *M. bovis* is often present in the upper respiratory tract of healthy cattle [3]. Although, *M. bovis* can independently cause natural and experimentally induced clinical disease, but the presence of *M. bovis* does not always result in disease. In the present study, seropositivity for *M. bovis* was detected exclusively in pneumonic cows, suggesting the involvement of *Mycoplasma* in the pathogenesis of the condition.

Seven of the seropositive cases {chronic active pneumonia ($n=2$), bronchitis ($n=1$), chronic pneumonia ($n=1$), tuberculosis ($n=3$)} had chronic pneumonia out of which three were confirmed for tuberculosis by culture isolation and conventional PCR (Table 1). *Mycoplasma bovis* is one of the important etiological factor for chronic bronchopneumonia with poor response to ongoing antibiotic treatment in cattle [6]. Chronic bronchopneumonia with mild to moderate caseonecrotic lesions was documented as characteristic pathological findings of *Mycoplasma bovis* infection in histopathological study [7], which is consistent with the findings of the present study. Caseonecrotic pneumonia in combination with broncho-interstitial pneumonia, suppurative bronchopneumonia, suppurative bronchitis, and bronchiolitis was reported [12] in bovine calves following experimental intratracheal inoculation of *Mycoplasma bovis*. However, establishing an etiological diagnosis, based solely on cytologic findings or pathological lesions, is challenging as pneumonic lesions caused by different etiologies often overlap. The isolation of *M. bovis* was reported along with *Mannheimia haemolytica* from the lungs of 82 per cent of feedlot calves with fibrinosuppurative pneumonia [3] which was also reported in two cows with fibrinopurulent pneumonia as a cytologic finding of TBW samples in our study (Table 1). Coinfection of *M. bovis* with *Pasteurella multocida* is commonly observed in younger calves. Arthritis is one of the major clinical manifestations associated with *Mycoplasma bovis* infection in cattle [13]. However, none of the affected cows showed arthritis in the present study.

Following treatment, survival was recorded in only 33.3% seropositive cows, suggesting its contribution to disease severity and poor prognosis. There was no bacterial growth in

TBW sample from one cow, indicating a probable infection caused solely by *Mycoplasma bovis*. Although culturing of *M. bovis* is considered the gold standard method, it was not performed in this study as it is time-consuming, requires specialized growth conditions, and can be easily overgrown by other bacteria [14]. Two cases were reported that tested positive by immunohistochemical staining and polymerase chain reaction but were negative by culture, underscoring the difficulty and limited sensitivity of culturing *M. bovis* [7].

Seropositivity for respiratory viruses alongside *Mycoplasma bovis* further supports the multifactorial etiology of bovine pneumonia. However, specific role of *M. bovis* as an etiological agent was difficult to assess on the basis of seroprevalence alone which needs isolation, molecular and histopathological studies which could not be carried out in the

present study. The role of co-pathogens restricts the confirmation of *Mycoplasma bovis* causing pathogenesis of the disease. The seropositivity for *M. bovis* could also not be associated with active infection as only individual titres were observed in the present study. Specific serum antibodies to *M. bovis* can be detected by indirect ELISA usually by 6–10 days after experimental infection [15]. Poor correlation of individual animal titers with infection in natural infections were reported, as all the diseased animals did not develop high titers [10]. Conversely, titers can remain increased for months in recovered animals. On a herd level, however, high titers are predictive of active *M. bovis* infection. Serology is therefore best applied in surveillance or as part of a biosecurity program.

Table 1: Seropositivity to *Mycoplasma bovis* (n=9)

S. No.	Type of Pneumonia	Pathogens associated with <i>M. bovis</i> infection		Clinical outcome (followed for 6 months)
		Viruses	Bacteria isolated	
1.	Fibrinopurulent pneumonia	BPIV-3, BoHV-1	<i>Pseudomonas</i>	Dead
2.	Fibrinopurulent pneumonia	BPIV-3	No Growth	Dead
3.	Bronchitis	BRSV	<i>E. coli</i>	Survived
4.	Tuberculosis	BRSV, BPIV-3	No Growth	Dead
5.	Tuberculosis	BRSV, BPIV-3	<i>Bacillus, E. coli, Klebsiella</i>	Dead
6.	Tuberculosis	BoHV-1, BRSV	<i>Staphylococcus</i>	Dead
7.	Chronic active pneumonia	BoHV-1, BRSV, BPIV-3	<i>Pasteurella</i>	Survived
8.	Chronic active pneumonia	BPIV-3	<i>Staphylococcus, Pseudomonas</i>	Survived
9.	Chronic pneumonia	BRSV, BPIV-3	<i>Pseudomonas</i>	Dead

Conclusion

Serological analysis detected *Mycoplasma bovis* antibodies in pneumonic cattle, while healthy controls were seronegative, suggesting an association with lower respiratory tract disease. Most seropositive cases exhibited chronic pulmonary lesions. Although *M. bovis* is recognized as an agent of chronic bronchopneumonia, the exact role of *Mycoplasma bovis* could not be confirmed due to the presence of co-pathogens and reliance on serology. Overall, the results indicate that *M. bovis* may contribute to bovine respiratory disease in combination with other infectious agents, highlighting the need for comprehensive diagnostic approaches for effective surveillance and control.

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Conflict of Interest

Not available

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Not available

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