International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
NAAS Rating: 4.61
VET 2025; 10(6): 192-195

www.veterinarypaper.com
Received: 23-04-2025
Accepted: 29-05-2025

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Clinicopathological and molecular characterization of *contagious caprine* pleuropneumoniae (CCPP) in goats

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DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i6c.2329

Abstract
The present study was carried out to clinicopathological changes in goats affected with *contagious caprine* pleuropneumoniae. A Total of 751 goats from six districts (Chittoor, Nellore, Tirupati, Annamayya, Kadapa and N.T.R district) of Andhra Pradesh were examined. The main clinical signs observed in affected flocks were fever, anorexia, nasal discharge, coughing, polypnoea, and dyspnoea. In the post mortem examination gross appearance of infected lung tissues shows hepatization, various level of marbling and adhesion of lung to pleura. On histopathological examination revealed that thickening of pleura and interlobular septa with fibrous tissue separating lung into lobules. Molecular confirmation was also carried by using PCR yielding 316bp product targeting *arcD* gene.

Keywords: Pathological changes, CCPP, fibrinous pleuropneumoniae, PCR

Introduction
Mycoplasmosis is the term used to describe diseases caused by *Mycoplasma spp.* which impose serious constraints on sheep and goat production because of high mortalities, ill thrift, a substantial reduction in meat, milk, and wool yield in Africa, Europe, Middle East, Australia [1], Asia [2] and North America [3]. The indirect losses are due to the chronic nature of *Mycoplasma* infections that result in emaciation, delayed marketing, and reduced fertility[4]. Mycoplasmosis is now considered as an emerging threat and transboundary epidemiological disease posing a worldwide concern on small ruminant rearing and creating huge economic constraints for farmers and small ruminant rearing countries [3, 5-7]. Irrespective of age and sex, all goats could be affected by CCPP [5]. The important clinical signs manifested in CCPP infected animals were fever, anorexia, nasal discharge, coughing, polypnoea, and dyspnea [8]. Mortality and morbidity rates were reported to be 14-50 percent and 45-90 percent, respectively. Later, the disease was confirmed by isolation of *Mccp* [9]. Fever, dyspnoea, coughing, and frothy nasal discharge were the clinical signs observed during an outbreak of CCPP in Kerala [10]. Coughing, anorexia, labored breathing, rise in temperature up to 41ºC observed in pashmina goats of Kashmir valley [11]. Important post-mortem lesions in CCPP mentioned as extensive oedema, enlargement of interlobular septa, hepatization, and fibrinous pleurisy [12]. Lesions in CCPP were limited to the thoracic cavity and most often lungs were the only organs affected [13]. On experimental inoculation of goats with *M. capripneumoniae*, most of the goats showed pneumonia characterized by histopathological changes like mucopurulent to fibrinopurulent exudate in dilated hyperplastic bronchi and alveoli, dominated by macrophages and neutrophils and pulmonary fibrosis [14] and sometimes solely localized to the lungs with severe pleuropneumonia and partial hepatization [15]. Thickening of the interlobular septum, pleuritis, and accumulation of straw-colored pleural fluid was predominant in some of the affected animals. During an outbreak of CCPP in Kerala, extensive pleuritis with straw-colored fluid accumulation observed in the thoracic cavity [10].

Materials and Methods
In the present study a total of 33 goat lung tissues showing gross pathological lesions were collected during Post mortem examination and from Slaughter house.

The infected lung tissues were collected in Formalin and PPLO broth in sample collection vials for histopathology and molecular confirmation. The vials were brought to the laboratory over ice maintaining cold chain. Representative lungs tissue pieces were collected and fixed in 10% formal saline for histopathology. The fixed tissues were subjected to overnight washing followed by dehydration with ascending grades of alcohols. Then clearing of tissues was carried out with xylene and further embedded in paraffin and tissue blocks were prepared. 5-6 µ thickness sections were prepared with semi-automatic rotary microtome. The cut sections were spread on water bath and lifted on pre-coated Mayer's egg albumin on clean grease free glass slide and further kept overnight drying. These slides were subjected for routine Haematoxylin and Eosin staining method (H&E) [16]. Extraction of DNA from lung tissue was carried out by following method [17]. Two grams of tissue was taken from each sample and was homogenized by adding 2 ml of TE buffer in mortar and pestle. The homogenized suspension was transferred to micro centrifuge tubes and allowed to settle for 10 min. The supernatant was collected and centrifuged at 13,000 rpm for 10 min in refrigerated centrifuge (Eppendorf 5430 R). The pellet was dissolved in 100 µl of TE buffer and then boiled for 10 min. Immediately, the samples were chilled on ice. After cooling, the lysate was again centrifuged at 13,000 rpm for 2 min in a refrigerated centrifuge (Eppendorf 5430 R). Two microlitre of supernatant was used as template for PCR. Further confirmation of CCPP was carried out by PCR using primer sequences F-5'-ATC-ATT-TTTAAT-CCC-TTC-AAG-3'and R-5'-TAC-TAT-GAGTAA-TTA-TAA-TATATG-CAA-3'tageting *arcD* maintaining annealing temperature for 47ºC for 15 seconds[18] PCR positive samples were sent for sequencing (sangers dideoxy method).

Results

The main clinical signs observed in affected goat flocks were respiratory symptoms like increase in rectal temperature ranging from 41 to 43°C, dyspnea, coughing, sneezing, purulent and muco-purulent nasaldischarge (Fig 1). Other symptoms like anorexia, Frequent lying down, Open mouth breathing, Protrusion of tongue, Excessive frothy salivation also noticed.

Gross pathological lesions noticed in affected lungs were congestion, consolidation, various degrees of red to grey hepatization (Figure 2) in one or more lobes, fibrinous pleuropneumonia and low level of marbling (Figure 3). The tissues which gave positive result were further subjected to histopathological examination.

On histopathological examination revealed that thickening of pleura (Figure 4) and interlobular septa with fibrous tissue separating lung into lobules (Figure 5). Other lesions were Congested and thickened blood vessels in lungs (Figure 6). Hyperplasia of bronchiolar epithelium (Figure 7) with desquamated cells and inflammatory cells in lumen indicating bronchitis (Fig 8). Bronchiolar thickening also noticed. Alveoli filled with mononuclear cells and fibroblasts. All the samples were subjected to PCR for the confirmation of CCPP. The Positive sample yield 316bp product (Figure 9). NCBI-BLAST analysis also reveals 97.83 to 99.565% homology with *Mycoplasma capricolum* subsps *capricolum* strains.

Discussion

In the present study, a total of 751 goats from six districts (Chittoor, Nellore, Tirupati, Annamayya, Kadapa and N.T.R district) of Andhra Pradesh were examined. The affected animals showing respiratory symptoms like increase in rectal temperature ranging from41 to 43°C, dyspnea, coughing, sneezing, purulent and muco-purulent nasal discharge. Similar clinical signs were also observed in Kerala [10], in animals suspected for Mycoplasmosis which includes fever, coughing, dyspnoea and frothy nasal discharge just before death. Mondal [20] in his study at West Bengal observed that *Mycoplasma* infected animals exhibited high body temperature [19] (40-43°C), painful respiration and persistent violent cough which correlate with symptoms exhibited by the animals in our present investigation. Different scientists in their studies observed that in acute and per acute Mycoplasmal infections, animals died without any premonitory signs [5, 20, 21]. Prior to collection of lung tissues, the lungs were thoroughly examined for the presence of gross pathological lesions suspected for Mycoplasmosis. In the present study, gross pathological lesions noticed in affected lungs were congestion, consolidation, various degrees of red to grey hepatization in one or more lobes, fibrinous pleuropneumonia and low level of marbling. The cut surface of some affected lungs revealed a fine granular texture with hepatization. Some affected lungs showed frothy exudates. Similar gross pathological observations were also noticed in their studies at West Bengal, where they observed palm colored or grayish pink consolidated areas distributed throughout the apical, cardiac & anterior diaphragmatic lobes of lung with frothy exudates [22]. Similar lesions were also noticed by Kumar [6] in their studies at Gujarat, India. Abraham [10] observed extensive pleuritis with straw-colored fluid accumulation in the thoracic cavity. Arif [23] at Qatar also noticed straw colored fluid in thoracic cavity. Yu [15] from Tibet reported that lesions were localized solely to the lungs with severe partial hepatization and accumulation of straw-colored pleural fluid. Halium [24] from Egypt and Daee [25] from Iran reported grossly irregular consolidation with lobular orlobar to diffuse pattern in the cranioventral to caudal lobes of affected lungs, congestion, edema with red and gray hepatization, and different degrees of pleurisy. On histopathological examination of *Mccp* infected lung tissue revealed the thickening of pleura and interlobular septa with fibrous tissue separating lung into lobules. Congested and thickened blood vessels in lungs. Hyperplasia of bronchiolar epithelium with desquamated cells and inflammatory cells in lumen indicating bronchitis. Bronchiolar thickening also noticed. Alveoli filled with mononuclear cells and fibroblasts. Similar microscopic lesions described in *Mccp* pneumonia characterized by mucopurulent to fibrinopurulent exudate in dilated hyperplastic bronchi and alveoli, dominated by macrophages and neutrophils and pulmonary fibrosis [14]. Other lesions thickening of the interlobular septum, pleuritis noticed [15] and fibrin deposition observed by Ozdemir [26] and fibrinous bronchopneumonia identified Molecular confirmation was also carried out in the present study for the accurate result. Same confirmation was carried out by Woubait [18] in his study and PCR is preferred and quick method of diagnosis for confirmation of CCPP suggested by OIE [8].

Conclusion

The above research clearly states that for the confirmation of CCPP in goats Molecular techniques assisted with histopathology plays important role in quick diagnosis and accurate confirmation of Disease.

Fig 1: Goat showing mucopurulent nasal discharges

Fig 2: Hepatization of goat lung tissue

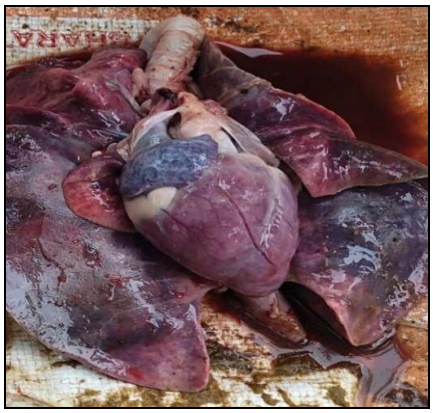

Fig 3: Marbling of affected goat lung tissue

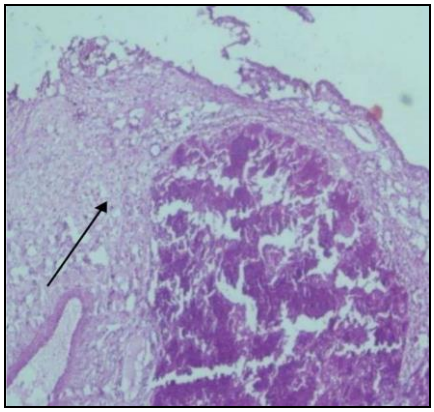

Fig 4: Thickening of pleura, H&E X100

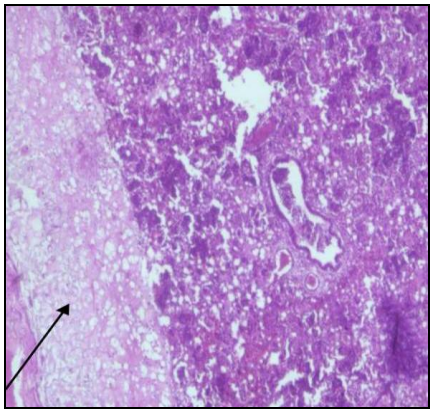

Fig 5: Thickening of interlobular septa, H&E X100

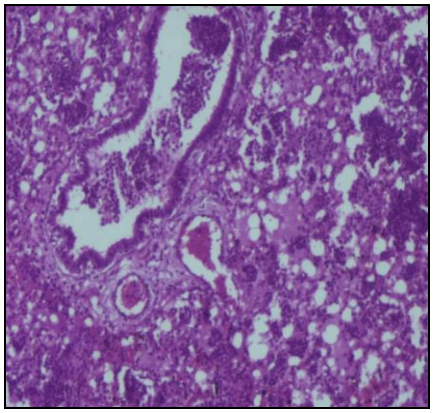

Fig 6: Congested and thickened blood vessels in lungs)

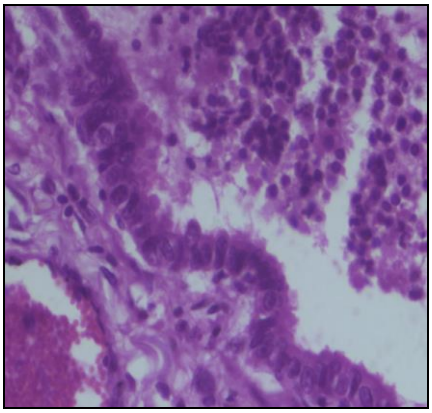

Fig 7: Hyperplasia of bronchiolar epithelium

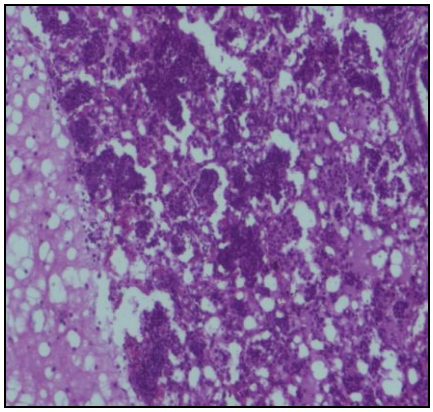

Fig 8: Desquamated cells and inflammatory cells in lumen

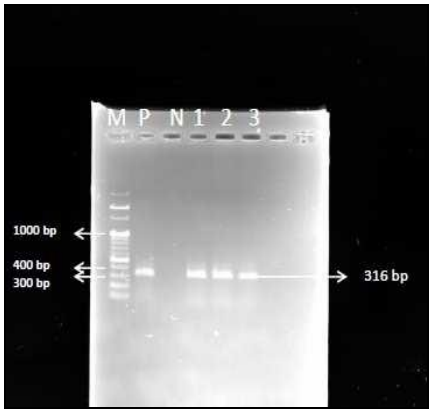

Fig 9: Amplification of *arcD* gene of CCPP

Acknowledgement
I Thank Department of Veterinary Microbiology, Department of Veterinary Pathology, college of Veterinary Science, SVVU, Tirupati for proving fund and Infrastructure for smooth completion of my research work.

Conflict of Interest
Not available

Financial Support
Not available

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How to Cite This Article
Raja D, Vijayalakshmi S, Amaravathi P. Clinicopathological and molecular characterization of *contagious caprine* pleuropneumoniae (CCPP) in goats. International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(6):192-195.