



CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN AN ORGANISED GOAT FARM OF TAMIL NADU

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ABSTRACT

A detailed systematic approach on the disease dynamics, differential diagnosis and control measures adopted in an outbreak of contagious caprine pleuropneumonia (CCPP) in an organised goat farm located at Kattupakkam (Tamil Nadu) has been reported. The outbreak occurred during April to June 2014 among 60 newly purchased adult non-descript goats. The clinical signs were profuse bilateral mucous discharge, respiratory distress with abnormal respiratory sounds. On post-mortem gross lesions were fibrinous pleurobronchopneumonia with extensiveness of the lesions involving all lobes of either one or both lungs, thickened interlobular septae. The samples were found negative for *peste des petitis ruminants* virus by polymerase chain reaction. *Mycoplasma capricolum* subsp. *capripneumoniae* or F-38 was isolated from clinical (n=20) and necropsy samples (n=25) of goats. The characteristic restriction enzyme cleavage pattern of *M. capripneumoniae*, differentiated it from the other members of *M. mycoides* cluster. The disease outbreak of caprine mycoplasmosis was contained soon after identification of the etiological agent by adopting proper control and preventive measures.

Key words: Contagious caprine pleuropneumonia, Goat, *Mycoplasma capricolum* subsp. *capripneumoniae*

Goats are highly susceptible to respiratory diseases, which account for almost 50% mortality (Kumar et al., 2014). Irrespective of the etiology, infectious respiratory diseases contribute to 5.6% of the total diseases in small ruminants (Chakraborty et al., 2014) and are responsible for 28.7% mortality (Yatoo et al., 2018). Amongst the respiratory infections, caprine mycoplasmosis is one of the most dreadful diseases of goats (Kumar et al., 2011; Manimaran and Singh, 2017). Occurrence of mycoplasmosis in India as outbreaks or as isolated cases has been reported from different parts of the

country and continues to exist in many states. Among the *Mycoplasma* sp. infections, contagious caprine pleuropneumonia (CCPP) is of major concern and causes serious economic impact in small ruminants. Seroprevalence of CCPP to the tune of 28.68% among goats in organized farms in the northwest agroclimatic zone of Tamil Nadu has been reported (Rishikesavan et al., 2020). The reports on isolation and identification of *M. capricolum* subsp. *capripneumoniae* in goats of Tamil Nadu state are rare. The similarity in the clinical signs of CCPP and pneumonic manheimiosis and their co-localization in nearly the same endemic areas,

calls for appropriate differential diagnostic testing to accurately identify the responsible pathogen(s) (Settypalli et al., 2016). This paper reports a detailed systematic approach on the disease dynamics, differential diagnosis and the control measures adopted in an outbreak of CCPP among goats in an organised farm of Tamil Nadu.

MATERIALS AND METHODS

The study was conducted in an organised livestock farm at Postgraduate Research Institute in Animal Sciences, Kattupakkam (Tamil Nadu). The reported outbreak occurred during April to June 2014 among newly purchased 60 non-descript adult goats of both the sexes. In quarantine, one goat died on the day of arrival due to transportation stress. After the quarantine period (30 days), the goats were mixed with the existing flock of goats and reared under semi-intensive system. The newly purchased goats (60) started showing the clinical signs of illness within a week of introduction into the farm from the quarantine with profuse bilateral mucous discharge, respiratory distress with abnormal respiratory noises. A few goats died peracutely within hours of severe respiratory distress, while others died 1 to 2 days after the onset of clinical signs. Most of the ailing animals were not responsive to broad-spectrum antibiotic (strepto-penicillin) therapy.

Clinical samples (n=75) like peripheral blood smear, nasal, ocular and rectal swabs were collected from ailing animals. A detailed necropsy was performed on 59 dead animals and gross lesions were recorded. Swab samples (n=50) from heart, lung, bronchi, thoracic fluid and liver and tissue samples (n=40) from lung, liver, spleen and mesenteric lymphnode were collected aseptically. Representative samples (n=58 sets) of tissues including trachea, different lobes of lung, heart, liver, kidneys, spleen, mesenteric and mediastinal lymph nodes, abomasum and intestine were collected in 10% neutral buffered formalin. Intestinal loops were also collected in ice. Peripheral blood smears, heart blood smears and tissue impression smears from lungs, liver, spleen and lymph nodes were collected for cytological examination.

Blood smears and tissue impression smears were stained by Leishman-Giemsa and Gram's stains and microscopically examined for pathogens. Tissue samples in formalin were processed and stained with haematoxylin and eosin (Suvarna et al., 2018).

Swab (nasal, ocular and rectal) and tissue samples (lung, spleen and lymph node) were subjected to counter immunoelectrophoresis for initial screening of samples using hyperimmune serum raised against local isolate of PPR virus. RNA was extracted from tissue samples using TRIzol (Sigma) and cDNA was synthesized using first strand cDNA synthesis kit (Revertaid first strand cDNA synthesis kit-Thermo Fisher Scientific®) as per manufacturer's instructions. PCR was carried out targeting N gene of PPR virus as per Jaisree et al. (2017) and checked for specific amplification.

Isolation of bacteria was carried out as per the standard procedures mentioned by Quinn et al. (1994). Isolation of mycoplasma was carried out in PPLO broth and PPLO agar as mentioned by Kumar et al. (2011). DNA was extracted from tissue by using Qiagen DNA extraction kit and cultures suspected for *Mycoplasma* sp. were initially screened with *Mycoplasma* group specific primers (GPO- 1 [5'-ACTCCT ACGGGAGGCAGCAGTA-3'] and MGSO [5'-TGCACCATCTGTCACTCTGTAACT C-3']) by PCR (Kuppeveld et al., 1992). Cluster-specific PCR was carried out on DNA from 5 ml samples of undiluted and 10-fold diluted lysed cells prepared from PPLO cultures. A fragment of the 16S rRNA genes from the members of the *M. mycoides* cluster was amplified by PCR with the primers used by Ros Bascunana et al. (1994). The PCR products were then analyzed without further purification by restriction endonuclease with *Pst* I to differentiate *M. capripneumoniae* from the other species as described by Ros Bascunana et al. (1994).

RESULTS AND DISCUSSION

The outbreak was recorded during the months of summer, soon after transport and introduction of the new animals into the goat farm. Morbidity was 100%

and mortality was 50% in peracute to acute cases. As per OIE (2014) heavy rain, animal transportation over a long distance, poor climatic conditions and primary infections might predispose to CCPP outbreaks. The progression of the disease outbreak observed from peracute, acute and chronic cases was similar to that of earlier reports, wherein goats were primarily affected and CCPP lasted for about two days with high mortality while in other cases it lasted for several days (OIE, 2014). The practice of semi-intensive rearing of goats and introduction of a new flock into the farm precipitated the disease, causing high morbidity and mortality. Similarly, Yattoo et al. (2018) reported that grazing goats showed about 2 to 3-fold higher morbidity as compared to stall-fed animals and a large population was affected after the introduction of the pathogen. Abraham et al. (2015) reported 80% morbidity and 62% mortality in clinical outbreak of CCPP in a flock in Kerala.

In affected goats, the clinical signs observed were suggestive of a severe respiratory disease with fever, nasal discharge, cough, respiratory distress and grunting without mucosal lesions or diarrhoea. The manifestation of disease as peracute to acute form led to the speculation of most commonly prevalent respiratory diseases of goats like pneumonic mannheimiosis and PPR. Clinically, peracute to acute manifestations, contagious nature with severe respiratory distress, high morbidity and mortality among goats and absence of bipolar organisms in blood and tissue impression smears were highly suggestive of caprine mycoplasmosis. None of the sheep maintained in the same area were affected. The disease was also not reported in large ruminants that were maintained in the farm. Thiaucourt et al. (1996) stated that disease due to CCPP was distinguished from other goat respiratory mycoplasmoses by the characteristics like (i) it is very contagious, (ii) sheep and cattle are not affected and (iii) the clinical signs of CCPP are limited to respiratory signs. When other mycoplasmas are the cause, the pulmonary signs are concomitant with mastitis, arthritis and keratitis (Ruffin, 2001). In endemic areas, the disease must

also be differentiated from other conditions such as pneumonic mannheimiosis and PPR, both of them affect sheep and goats. Nevertheless, a definitive diagnosis always requires additional laboratory investigation (Ruffin, 2001). Ravishankar et al. (2011) and Abraham et al. (2015) reported similar clinical signs among goats in CCPP outbreaks. Shah et al. (2017) opined that clinical signs of CCPP typically correlate to respiratory system affection and these signs are common in susceptible herds and are independent of age and sex. Despite pathogenic mechanisms being variable, CCPP usually causes acute disease in young and immunocompromised animals especially under harsh climate, and chronic disease in healthy resistant animals. Adult male and female goats were affected. Apparently healthy carriers play a dominant role in the epidemiology of mycoplasmosis (Yattoo et al., 2018).

The pathological findings and distribution of lesions and score are summarised in Table 1 and Plates 1 to 4. Gross lesions like fibrinous pleurobronchopneumonia with extensiveness of the lesions involving all lobes of either one or both lungs, thickened interlobular septae indicated that the disease was different from that of pneumonic pasteurellosis or PPR, wherein acute fibrinous pleurobronchopneumonia with bilateral cranioventral lung involvement would be predominant. The lesions were consistent with the findings of CCPP among goats as reported by earlier workers (Mondal et al., 2004; Hussain et al., 2012; Abraham et al., 2015). Unilateral fibrinous pleuro-bronchopneumonia is the most common lesion of CCPP (OIE, 2014). However in this outbreak, involvement of both lungs with thickened interlobular septa was observed in acute cases and severe fibrosis of the lung with encapsulation of necrotic areas similar to sequestrum was observed in chronic cases. The wide range of histological lesions presented in the lungs from peracute to chronic cases with serofibrinous pleuro-bronchopneumonia, prominent peribronchiolar lymphoid tissue cuffing were consistent with histological findings previously reported in CCPP (Hussain et al., 2012; Sadique et al., 2012).

Table 1. Distribution of lesions in the lung lobes

Stage	%	Unilateral lesion	Bilateral lesion	Distribution of lesions in lung lobes					Salient lesion
				Right			Left		
				C	M	D	C	D	
Peracute	17.2 (10/58)	70.0 (7/10)	30.0 (3/10)	+++	++	+	++	+	Enlargement of lungs, fibrinous pleuro-bronchopneumonia
Acute	34.5 (20/58)	75.0 (15/20) 71.4	25.0 (5/20)	+++	++	+++	++	+++	Fibrinous pleuro-bronchopneumonia, concentric layers of grey and red hepatization, thickening of interlobular septae
Chronic	48.3 (28/58)	(20/28)	28.6 (8/28)	+	+	+++	+	+++	Adhesions of visceral pleura to thoracic wall, sequestra-like lesion

C-Cranial; M-Middle; D- Diaphragmatic; + 25%, ++ 40%, +++ 75% indicates distribution of lesions in lungs



Plate 1. Fibrin clot in lumen of trachea



Plate 2. Serofibrinous exudate in thoracic cavity and on the lungs

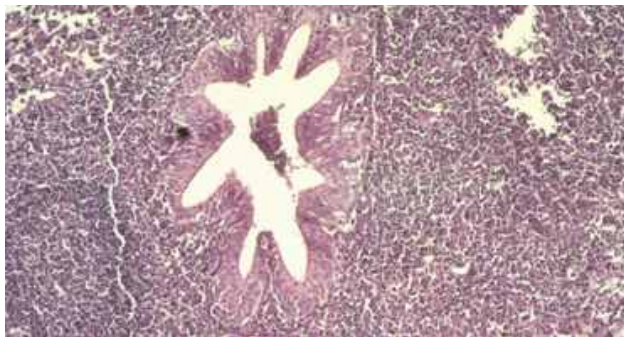


Plate 3. Dense cuffing of mature lymphocytes around the bronchioles H&E x100

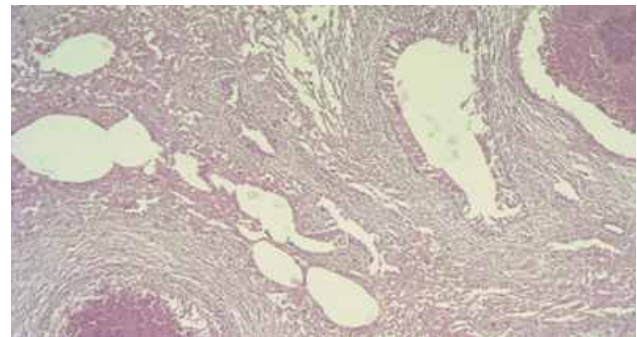


Plate 4. Multifocal necrotic areas with fibrosis in lung H&E x100

The samples tested were found negative for PPR virus by PCR. Except in PPLO broth and PPLO agar no bacterial growth was noticed in other plates or broths. PPLO agar plates were viewed under stereomicroscope for the presence of fried egg appearance of colonies (Plate 5) which is said to be characteristic to *Mycoplasma* sp. Similar isolation studies for *Mycoplasma* were reported by Nicholas (2002) and Kumar et al. (2011). Yatoo et al. (2019)

isolated, identified and molecularly characterised *Mycoplasma* isolates from goats of Gujarat state and reported *M. mycoides* subsp. *capri*, *M. agalactiae*, *M. capricolum* subsp. *capricolum* (Mcc) as the main isolates. In the present outbreak, *M. capricolum* subsp. *capripneumoniae* or (F-38) was isolated from clinical and necropsy samples of goats. On PCR, 83.3% (25/30) positivity was detected with *Mycoplasma* group specific primers GPO-1 and

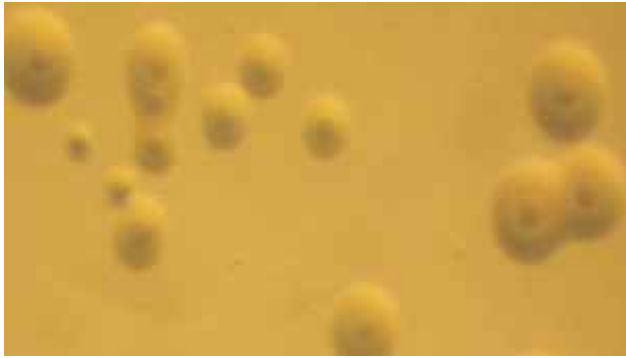


Plate 5. Unstained fried egg appearance of colonies of Mccp on PPLO agar 40x

MGSO that yielded a 715 bp fragment (Plate 6). Similar amplification of 715 bp product was observed by Mounier et al. (2019) on isolation and molecular characterisation of *Mycoplasma* spp. among sheep and goats in Egypt. By REA, the presence of three bands, the uncleaved 548-bp fragment (Plate 7) and the two cleavage products of 420 and 128 bp (Plate 8) indicated the presence of *M. capripneumoniae* genome in the sample (Ros Bascunana et al., 1994). The uncleaved DNA fragment of 548 bp originates from the *rrnB* operon of *M. capripneumoniae*, which lacks the restriction site for *Pst* I in the *M. mycoides* cluster. PCR-REA system has earlier been shown to differentiate *M. capripneumoniae* from other mycoplasmas described from goats (Bolske et al., 1996). The characteristic restriction enzyme cleavage pattern of *M. capripneumoniae*, differentiating it from the other members of *M. mycoides* cluster, is based on a single nucleotide position. These results confirm the assumption that this nucleotide substitution in *M. capripneumoniae* is unique within the *M. mycoides* cluster. *M. capripneumoniae*, which showed an atypical REA pattern, also possesses this unique nucleotide substitution. It was the two cleaved fragments from the other operon that appeared as comparatively weak bands in the REA. This could be explained by the presence of another nucleotide substitution located in the target region of the reverse primer (MmR) in the *rrnA* operon, an A instead of a G at position 1259 (F38 numbering), which probably caused a less efficient amplification of the segment. Similar findings were also made by Saeed and Osman (2018) on laboratory diagnosis of CCPP in Saudi Arabia.

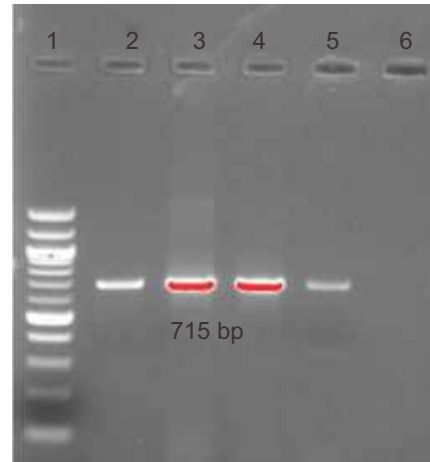


Plate 6. *Mycoplasma* group specific PCR. Lane 1-100 bp marker; Lane 2- positive control; Lanes 3 to 5- clinical samples; Lane 6- negative control

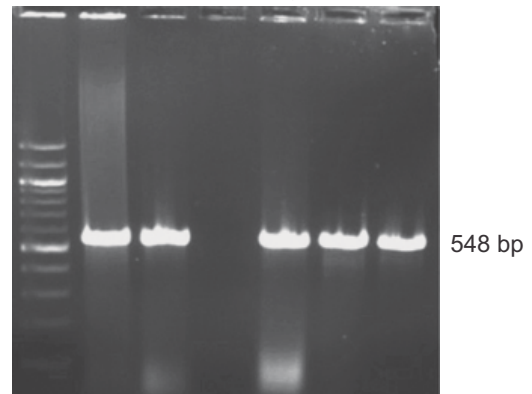


Plate 7. *Mycoplasma* cluster-specific PCR. Lane 1-100 bp marker, Lane 2 – positive control, Lane 4 negative control, Lanes 3, 5 to 7- clinical samples

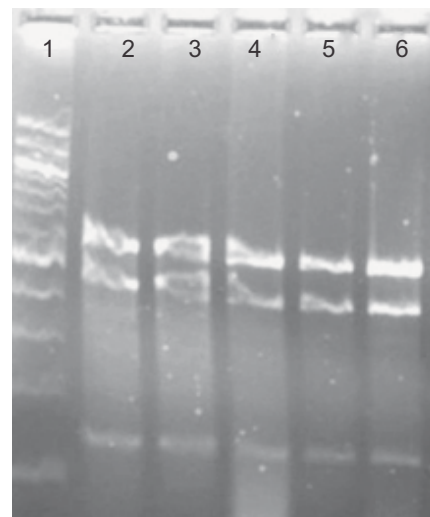


Plate 8. Restriction enzyme digestion of PCR products with *Pst*I enzyme. Lane 1-100 bp marker; Lane 2 to 6- *M. capripneumoniae*

The outbreak of caprine mycoplasmosis was contained soon after identification of the etiological agent by adopting proper control and preventive measures viz., segregation of ailing goats, stall-feeding and administration of Tylosin (@ 20mg/kg body weight intramuscularly) daily for 6 days along with anti-inflammatory drugs, vitamins and mineral supplements. Goats that were seriously ill were sacrificed to prevent the spread by carrier status. Disinfection of infected premises was carried out and strict biosecurity measures were adopted in the farm. Dead carcasses were necropsied and disposed safely.

The clinical outbreak of CCPP among goats in an organised farm in Tamil Nadu and its diagnosis emphasized the need for differentials from common respiratory diseases of goats. To confirm the causative agent as *Mycoplasma* sp. Isolation studies and identification of *Mycoplasma* sp. was carried out from clinical specimens by molecular methods which complemented the conventional culture methods.

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