
**PCR-BASED DIAGNOSIS AND THERAPY OF INFECTIOUS
KERATOCONJUNCTIVITIS ASSOCIATED WITH MYCOPLASMA SPP.
INFECTION IN JAMUNAPARI GOATS**

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ABSTRACT

The present report narrates the occurrence of Infectious kerato-conjunctivitis associated with *Mycoplasma* spp in a flock of six Jamunapari goats. The goats were presented with complaints of cough, ocular and nasal discharge since two days. Ocular examination in the affected goats revealed conjunctivitis, keratitis with cloudiness of the cornea and increased vascularity. Polymerase chain reaction of the purulent ocular discharge confirmed the presence of *Mycoplasma* spp. The affected goats were treated with inj Tylosin @10mg/kg, inj Melonex@ 0.2 mg/kg, inj Chloril, 2ml and inj Beplex forte, 2ml intramuscularly for five days. Additionally single drops each of Hypersol, Moxifloxacin, EDTA and autoserum was administered to the eyes five times daily at 10 minutes interval for

one week. Complete recovery was observed based on clinical cure by the seventh day.

Key words: Goats, Mycoplasmosis, Infectious keratoconjunctivitis, Tylosin

INTRODUCTION

Small ruminants, particularly sheep and goats play a key role in supporting the agricultural economy and food security worldwide including India through milk and meat production. According to the 20th livestock census (2019) India's goat population stands at 148.88 million, the second highest globally, following China. The low maintenance costs, low space requirement, wide range of feed, its adaptability and high economic returns makes goat farming an appealing avenue for livestock rearing. However, unscientific practices like low plane of nutrition and

lack of good hygienic practices often increase the animal's susceptibility to infectious diseases. Infectious diseases are the leading causes of morbidity and mortality in goats. Poor management practices like lack of proper ventilation, overcrowding, ammonia build up and harsh environmental conditions predispose goats to numerous infectious and contagious diseases (Chakraborty *et al.*, 2014).

Mycoplasmosis is one of the most challenging infectious diseases of small ruminants throughout the world caused by *Mycoplasma* spp (Yatoo *et al.*, 2018). *Mycoplasma* are the smallest prokaryotic organisms with in the class mollicutes distinguished by their lack of a cell wall and ability for self replication. They are extracellular parasites in mucous membranes of animals . *Mycoplasma* is an important cause of respiratory tract infections in goats though, *Mycoplasma* spp have also been identified from ocular infections in ruminants (Gupta *et al.* 2015) .Fever, anorexia, depression, cough, serous or mucoid nasal discharge, arthritis, mastitis, keratoconjunctivitis and loss of body weight are the clinical signs usually exhibited in mycoplasma infections in goats (Srivastava and Singh, 2000). Clinical signs can be variable depending on the subspecies: *M. capricolum* ssp *capripneumoniae*(Mccp)causescontagious

caprine pleuropneumonia (CCPP), *M. ovipneumoniae* leads to atypical pneumonia and *M. conjunctivae* is associated with infectious keratoconjunctivitis. Mycoplasmosis affects all ages, however, young and immune-compromised animals are more susceptible. Seasonal factors like heavy rains, cold weather and stressful events like transportation, overcrowding, underfeedingandpregnancycontributetothe infection. In addition, immunosuppression as a result of stress aggravates infection in carrier animals as it leads to shedding of organisms (OIE, 2009).

In Kerala, various authors had reported mycoplasmosis among goats showing respiratory signs (Janus *et al.*, 2009; Sumith *et al.* 2021; Borang, 2023). Mycoplasmosis is believed to be one of the most common cause of infectious keratoconjunctivitis in goats. Keratoconjunctivitis is defined as the inflammation of cornea and conjunctiva. Infectious keratoconjunctivitis (IKC) is a highly contagious ocular infection characterized by epiphora, hyperemia of the conjunctiva, blepharospasm with different stages of corneal opacity and ulceration bilateral mucopurulent ocular discharge and photophobia (Browning, 2007). *Mycoplasma conjunctivae* is considered as the primary pathogen associated with IKC in goats. (Nicholas *et al.*, 2009), but *M.*

agalactiae, *M. mycoides* LC, *M. arginini* and *Acholeplasma oculi* have also been isolated from sheep with ocular infections (Baker et al., 2001). Other organisms that are commonly associated with IKC in goats include *Chlamydophila pecorum*, *Moraxella ovis*, *Listeria monocytogenes*, *Pseudomonas spp.* and *Staphylococcus aureus* (Rhaymah et al., 2013). A definitive identification of the etiological agent is necessary for the proper therapeutic management in order to reduce the mortality among the flocks. Apart from the cultural isolation of mycoplasma, PCR has become the novel and most acceptable method for the detection of mycoplasma because of its high specificity and sensitivity.

The present study documents the diagnosis and therapeutic management of infectious keratoconjunctivitis caused by *Mycoplasma* spp in a flock of goats in Wayanad district of Kerala. The authors acknowledge that a comprehensive and systematic data analysis could not be conducted due to the constraints of the rural ambulatory clinic where the case was presented. Nevertheless, this report aims to highlight the association between the observed clinical signs and *Mycoplasma* spp., providing valuable insights that may assist rural practitioners in recognizing and effectively managing similar cases. By documenting this case, the authors seek to contribute to the growing body of

knowledge on mycoplasmosis in goats, emphasizing practical approaches to diagnosis and treatment under resource-limited conditions.

CLINICAL HISTORY

A flock of six Jamunapari goats, consisting of four does and two kids of four months old were presented to Veterinary Ambulatory Clinic, Kakkavayal, Wayanad district, Kerala, India with complaints of reduced feed intake, weakness, cough, discharge from eyes and nose since two days and cloudiness in the eyes. One among the does was recently purchased. A detailed physical examination was carried out in the affected goats and the vital parameters such as rectal temperature, heart rate, respiration rate were recorded. Majority of the affected animals showed moderate systemic signs like fever, inappetence, dullness and lethargy. Cough, purulent nasal and ocular discharge was the most common clinical finding. Ocular examination revealed keratoconjunctivitis manifested by hyperemia of the conjunctiva and opacity of the cornea and increased vascularity (Fig. 1-A, B, C and Fig. 2A, B, C.). Auscultation of the lung area revealed exaggerated lung sounds.

MATERIALS AND METHODS

All applicable international, national, and/or institutional guidelines for

the care and use of animals were followed. Two millilitres of whole blood was collected from the most affected animal into ethylene diamine tetra acetic acid (EDTA) coated vial for complete blood count analysis and four millilitres blood was collected for serum biochemistry analysis. A complete blood count for analysing parameters such as total leucocyte count ($WBC-10^3/\mu L$), total erythrocyte count ($RBC-10^6/\mu L$), haemoglobin concentration (g/dL), platelet count ($10^3/\mu L$), volume of packed red cells (VPRC-per cent) were estimated using a three-part fully automated haematology analyser (Mindray BC-2800Vet) within 12 hours of collection of samples. Biochemical parameters were estimated using a semi-automatic serum biochemical analyser (MISPAVIVA 2578-10/17). Commercially available biochemical kits (Agappe diagnostics, India), of serum total protein (TP-g/dL), albumin (g/dL), creatinine (mg/dL), and aspartate amino transferase (AST-

blood count analysis revealed mild leukocytosis. All the serum biochemical parameters were within normal range.

The purulent ocular and nasal discharges of the affected animals were collected using a sterile cotton swab. The samples collected were properly labelled and transported to laboratory under a cold chain in an ice box maintained at 4°C. DNA was extracted from the nasal and ocular swabs and the isolated DNA samples were subjected to polymerase chain reaction (PCR) targeting the mycoplasma genus specific primer (Table.1). The reaction was carried out using TaKaRa EmeraldAmp GT™ PCR master mix (TaKaRa Bio Inc., USA). The PCR product was run on a 1.5% (w/v) agarose gel, stained with ethidium bromide (Himedia, Mumbai, India) and visualized under UV light. Polymerase chain reaction using purulent ocular and nasal discharge found positive for *Mycoplasma*



Fig. 1A

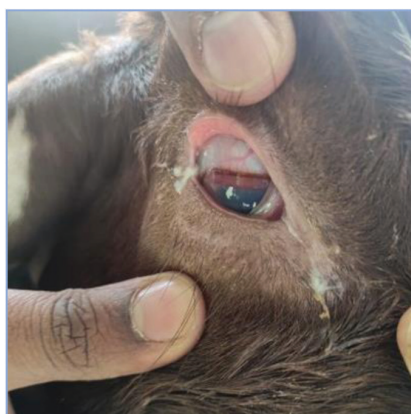


Fig.1. B

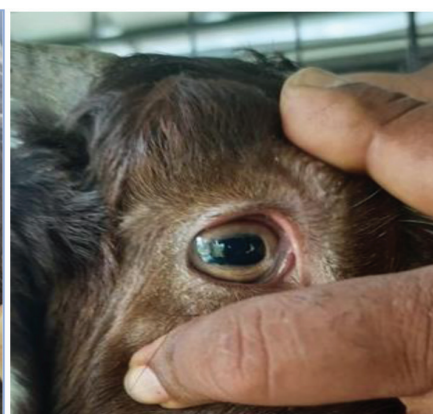


Fig. 1. C

Fig. 1. A. Increased vascularity in the left eye along with mucopurulent discharge, day 1, **Fig.1. B,** day 2, **Fig. 1. C.** day 3.



Fig. 2. A

Fig. 2. B

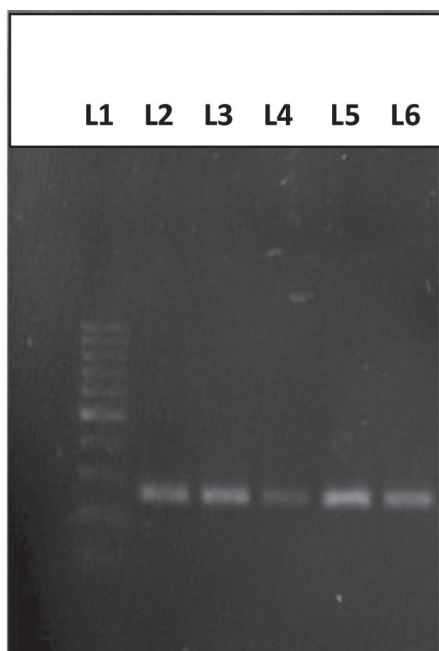
Fig. 2.C.

Figure. 2. Corneal opacity in the right eye 2.A. Day1, 2. B. Day2, 2.C. Day3.

Table 1. Details of genus specific primer and thermocyclic conditions used for the detection of *Mycoplasma* sp

Primer : <i>16S rRNA</i> Primer sequence: F:TGGGGAGCAAACAGGATTAGATACC R:TGCACCATCTGTCACTCTTGTTAACCTC	Initial denaturation	94°C, 2 min
	Denaturation	94°C, 15 sec
	Annealing	59.3°C, 15 sec
	Extension	72°C, 15 sec
	No. of cycles	30
	Final extension	72°C, 5 min

spp. Positive amplicons of *16S rRNA* of mycoplasma was detected at 270 bp (Botes *et al.*, 2005). Fig. 3



RESULTS

Based on the clinical examination and PCR the condition was diagnosed as infectious keratoconjunctivitis associated with *Mycoplasma* spp. All the affected goats were treated with Inj.Tylosin @ 10 mg/kg once daily intramuscularly for 5 days. Supportive treatments; inj meloxicam @ 0.2 mg/kg once daily IM, chlorpheniramine maleate @ 0.5 mg/kg once daily IM and B-complex injections were given as per

Fig. 3. Agar gel electrophoresis of *16SrRNA* gene with an amplicon size of 270 bp
 Lane L1: DNA marker 100 bp
 Lane L2: Positive control
 Lane L3, L4, L5, L6: Samples

the requirement. In addition, ophthalmic medications like hypersol, moxifloxacin, EDTA drops and auto-serum (five times daily at 10 minutes interval) were administered to the eyes for 7 days. The goats showed reduced respiratory signs after the first day of treatment. There was remarkable improvement in the clinical signs after three days of treatment and the goats showed complete recovery after seven days (Fig. 1.C, 2. C). Subsequent follow-up confirmed that all treated goats remained clinically healthy, with no recurrence of respiratory or ocular symptoms.

DISCUSSION

Mycoplasma spp causes contagious caprine pleuropneumonia, keratoconjunctivitis, arthritis, mastitis and pneumonia in goats (Yatoo *et al.*, 2018). Common mycoplasmal pathogens causing respiratory tract infections in goats are *M. capricolum* ssp *capripneumoniae* (Mccp), *M. mycoides* sps *capri*, *M. ovipneumoniae* and *M. arginini* (Udhayavel *et al.*, 2024) , *M. agalactiae* (Reji *et al.*, 2018) . whereas keratoconjunctivitis, one of the important infectious ocular disease is mainly caused by *M. conjunctivae*. Different mycoplasma species also produce clinical signs similar to other members making clinical diagnosis further complicated. Some species are peculiar for certain clinical signs but cannot be considered confirmatory (Kumar *et al.*,

2015). So molecular diagnostic methods are necessary for the confirmatory diagnosis of mycoplasmosis.

Etiological agent can be detected by PCR from eye swab samples obtained from the animals clinically diagnosed with keratoconjunctivitis (Gulaydin *et al.*, 2024) . In the present study, the condition was diagnosed as mycoplasmal keratoconjunctivitis based on clinical signs and PCR. Positive amplicons of genus specific *16SrRNA* of mycoplasma was detected at 270 bp by PCR. This was in agreement with Botes *et al.* (2005) who standardised the PCR targeting the *16SrRNA* for detection of mycoplasma in goats. In our study, mycoplasma identification only upto the genus level was done. In an early study, Sumith *et al.* (2022) carried out genus level identification of mycoplasma targeting the *16SrRNA* in a goat diagnosed with keratoconjunctivitis and BLAST analysis revealed it as *M. conjunctivae*. Similarly Hadani *et al.*, (2013) also identified *M. conjunctivae* in goats diagnosed with keratoconjunctivitis targeting the species specific primer. Considering the similarity in clinical signs, *Mycoplasma* could be the etiological agent in the present study also. There exits a research gap in the present study since the species level identification was not carried out. Early diagnosis and treatment is very important in reducing

the severity of the disease and to prevent mortality in the flock. Antibiotics like tetracyclines, fluoroquinolones, macrolides and aminoglycosides are effective for mycoplasmosis in goats (Parray *et al.*, 2022). However the recovered animals can act as potential carriers and complete elimination of mycoplasma is very difficult because of their ability to form biofilms and wide dissemination within the host animal (McAuliffe *et al.*, 2006). Macrolides especially tylosin are considered to be the drug of choice of mycoplasmosis. Tylosin is a bacteriostatic macrolide antibiotic that binds to the 50s ribosomal subunit inhibiting bacterial protein synthesis. In the present study, affected animals were successfully treated with tylosin @ 10 mg/kg body weight intramuscularly once daily for five days which was in accordance with Borang *et al.*, (2023). In contrast to the present study, Sumith *et al.* (2022) successfully treated a goat diagnosed with keratoconjunctivitis caused by *M. conjunctivae* using oxytetracycline at 15 mg per kg/day intravenously for 5 days along with supportive therapy such as flunixin meglumine at 1.1 mg per kg/day intramuscularly and chlorpheniramine maleate @ 0.5 mg/kg/day intramuscularly. Proper farm and herd health management practices and quarantine of new and infected animals are essential in preventing the spread of disease in the flock. It is hoped

that this report will aid rural practitioners in recognizing and managing similar cases more effectively, thereby improving outcomes in small ruminant health.

A follow-up with the flock revealed that all treated goats remained healthy and free of clinical signs several weeks after completing the treatment protocol. This outcome reinforces the effectiveness of the therapeutic approach employed in managing the infection and highlights the potential for favorable long-term outcomes when *Mycoplasma spp.* infections are promptly diagnosed and appropriately treated.

CONCLUSION

The present case highlights the occurrence of infectious keratoconjunctivitis caused by *Mycoplasma spp.* in a flock of Jamunapari goats, emphasizing its potential association with respiratory and ocular conditions. Rapid diagnosis using polymerase chain reaction (PCR) and a targeted therapeutic regimen, including systemic and ocular treatments, led to effective resolution of clinical signs and complete recovery within seven days. This case underscores the importance of accurate etiological identification and prompt intervention in managing mycoplasmosis in goats, thereby preventing the spread of infection within the flock.

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