SHORT COMMUNICATION

Molecular Detection and Phylogenetic Analysis of *Mycoplasma conjunctivae* from Ocular Infections of Goats in and around Tirupati Region

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Abstract

Mycoplasma-associated infectious keratoconjunctivitis (IKC) represents one of the common ocular infections among ruminants. *Mycoplasma conjunctivae* mainly causes infectious kerato-conjunctivitis (IKC) in goats, representing a highly transmissible condition marked by differing levels of visual impairment in affected individuals. In the current study, a total of 39 conjunctival swabs were collected from affected goats in and around the Tirupati region. Molecular detection was done by genus-specific and species-specific PCR targeting the 16S *r*RNA gene, yielding 270 bp and 748 bp products, respectively. Phylogenetic analysis of the partial 16S *r*RNA gene sequence of one of the obtained *Mycoplasma conjunctivae* isolates from goats revealed the genetic relatedness to distinct clade formed by sheep ocular swab isolate from Brazil (MK 656520.1) and clustered with a goat isolate from Kerala, India (MW425380.1).

Key words: Goats, Infectious keratoconjunctivitis, Molecular Detection, *Mycoplasma conjunctivae*, Phylogenetic analysis.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.3.34

Introduction

Mycoplasma conjunctivae mainly causes infectious kerato-conjunctivitis (IKC) in goats, representing a highly transmissible condition marked by differing levels of visual impairment in affected individuals (Fernandez *et al*., 2017). The typical clinical manifestations observed in affected goats include conjunctivitis, blepharospasm, excessive lacrimation, and varying degrees of corneal opacity and ulceration. The spread of infection is by direct contact with infected animal, ocular or nasal secretions, or by indirect contact with flies, mosquitoes, contaminated feed, and utensils (Aguilar *et al*., 2019). The incidence of *Mycoplasma* associated IKC is notably higher in goats subjected to stress conditions like introduction into a new herd, transportation, or exposure to extreme dry or cold weather conditions (Rahaman *et al*., 2018). This study reports the molecular detection and phylogenetic analysis of *Mycoplasma conjunctivae* from ocular infections of goat in and around Tirupati region of India.

Materials and Methods

A total of 200 goats were examined for occular infections like conjunctivitis, watery to purulent ocular discharges and reddening of eyes from various villages in and around Tirupati region (Andhra Pradesh, India) and 39 clinical samples were collected from August to November 2024. The conjunctival swabs were obtained from the affected goats using sterile cotton swabs. The sterile swab was gently rotated within the conjunctival sac until it became soaked with secretions. These swabs were then placed into sterile microcentrifuge tubes containing 1 mL of PPLO broth and transported to the laboratory on ice, maintaining the cold chain. Then, the samples in PPLO broth were incubated in a CO_2 incubator at 37°C maintained at 5% CO_2 for about 7-10 days.

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How to cite this article: Sreelekha, M., Vijayalakshmi, S., Nagendra Reddy, T., Sujatha, K., & Suresh, Y. (2026). Molecular Detection and Phylogenetic Analysis of *Mycoplasma conjunctivae* from Ocular Infections of Goats in and around Tirupati Region. Ind J Vet Sci and Biotech, 22(3), 171-174.

Source of support: Nil

Conflict of interest: None

Submitted 31/01/2026 **Accepted** 25/03/2026 **Published** 10/05/2026

DNA Extraction from PPLO Broth

The inoculated PPLO broth tubes were examined for colour change and turbidity. The DNA extraction was carried out as per the method described by Liu *et al.* (2001). After incubation, the clinical samples in micro-centrifuge tubes were gently vortexed to ensure the clinical material from the swab was adequately collected, and later the swab was discarded. The samples were then subjected to centrifugation for 10 min at 13,200 x g using a refrigerated centrifuge. The resultant cell pellet was washed twice with 1 mL of PBS, and subsequently, 100 µL of PBS was added to the pellet. The cell suspension was then boiled at 95°C for 10 min in a water bath and immediately subjected for snap chilling. After snap chilling the lysate was centrifuged at 13,200 x g approximately 2 min in a refrigerated centrifuge. A volume of 2 µL from the supernatant was utilized as the template for PCR.

Detection of 16S *r*RNA Gene by PCR

Detection of the 16S rRNA gene of genus *Mycoplasma* by PCR was done according to the procedure described by Junqueira *et al.* (2020). Detection of the 16S rRNA gene of *Mycoplasma conjunctivae* was done by PCR according to the procedure described by Giacometti *et al.* (1999). Details of primer sequences and PCR conditions used are listed in Table 1 and 2.

Results and Discussion

Goat conjunctival swabs suspected of *Mycoplasma* were inoculated into PPLO broth. After an incubation period of 7-10 days, positive samples exhibited slight turbidity and a colour change of the broth to yellow, suggesting the multiplication of *Mycoplasma* organisms. The growth was predominantly observed at the bottom of the broth tubes, and upon slight agitation, a whirlpool like formation was noted (Fig. 1). Similar conditions for *Mycoplasma* growth were recorded in previous studies (Harasawa *et al.*, 2004; Ezzi *et al.*, 2007; Gharaibeh and Roussan, 2008). Following the incubation period, positive samples exhibited a colour change in the broth to yellow with slight turbidity.

In the present study, out of 39 goat conjunctival swabs collected, 30 DNA samples were found to be positive by PCR for genus *Mycoplasma* yielding 270 bp product (Fig. 2). Similarly, Sumit *et al.* (2022) from Kerala detected genus *Mycoplasma* from ocular swabs of infected goats. Out of 30 genus *Mycoplasma* positive samples, 18 were found to be positive for *Mycoplasma conjunctivae* yielding 748 bp product (Fig. 3). Fernandez *et al.* (2017) from Pakistan reported the occurrence of *Mycoplasma conjunctivae* in goats with conjunctivitis which is in accordance with the current study. Gulaydin *et al.* (2024) from Turkey reported the occurrence of *Mycoplasma conjunctivae* in goats with conjunctivitis. Sumit *et al.* (2022) from Kerala detected *Mycoplasma conjunctivae* in two cases out of five conjunctivitis infected goats which accounts to 80% of prevalence. For the confirmation of species *M. conjunctivae*, the PCR product from one representative sample (TPTMCON C1) was sequenced for 16S rRNA and verified by NCBI BLAST. The obtained nucleotide sequences showed 100% homology with M. conjunctivae strains. The phylogenetic analysis of present isolate segregated into distinct clade formed by sheep ocular swab isolate from Brazil (MK 656520.1) and clustered into distinct clade formed by goat isolate from Kerala, India (MW425380.1) (Fig. 4). As far our knowledge this is the first report of confirming *Mycoplasma conjunctivae* by using species specific PCR and nucleotide sequencing from conjunctival swabs of goats in Andhra Pradesh.

From the findings we conclude that goats are mostly infected with *Mycoplasma conjunctivae* associated ocular infections and as far our knowledge this is the first report of confirming *Mycoplasma conjunctivae* by using species specific PCR and nucleotide sequencing from conjunctival swabs of goats in Andhra Pradesh.

Table 1: Primers for genus *Mycoplasma* and *Mycoplasma conjunctivae*

Primers	Gene	Primer Name	Nucleotide Sequence	Amplicon Size	References
Genus *Mycoplasma*	16 S *r*RNA	GPO3	5′ GGG AGC AAA CAG GAT TAGATA CCC T 3′	270 bp	Junqueira *et al.* (2020)
		MGSO	5′ TGC ACC ATC TGT CAC TCT GTT AAC CT 3′		
Mycoplasma conjunctivae	16 S *r*RNA	MCoF1	5′ TAT CTT TAG AGT CCT CGT CTT TCAC 3′	748 bp	Giacometti *et al.* (1999)
		MCoR1	5′ CAG CGT GCA GGA TGA AAT CCC TC 3′		

Table 2: Cyclic conditions for the amplification of genus *Mycoplasma* and *M. conjunctivae*

S. No	Step	Genus *Mycoplasma*			*M. conjunctivae*		
		Temp. (°C)	Duration	Cycles	Temp. (°C)	Duration	Cycles
1	Initial Denaturation	94	4 min	1	94	5 min	1
2	Denaturation	94	30 sec	35	94	30 sec	30
3	Annealing	56	30 sec		55	30 sec	
4	Extension	72	30 sec		72	30 sec	
5	Final Extension	72	10 min	1	72	7 min	1

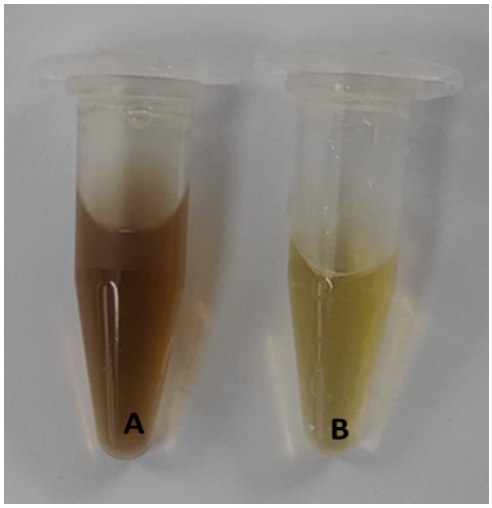

Fig. 1: Colour change in PPLO broth. (A) Uninoculated PPLO broth, (B) Colour change in PPLO broth after incubation

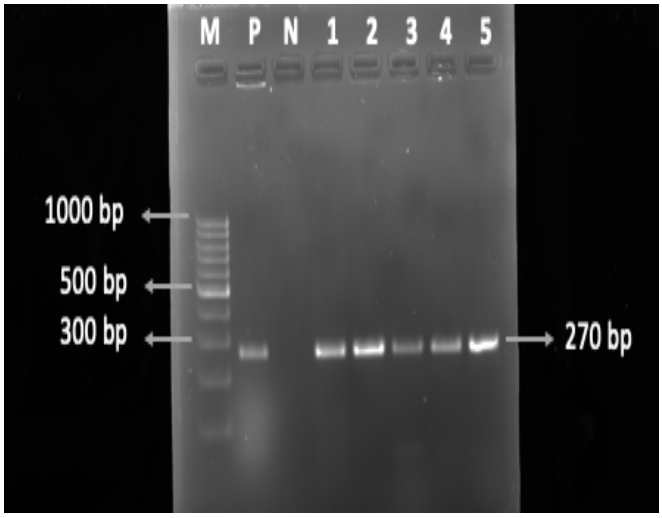

Fig. 2: Amplification of 16S rRNA gene of genus *Mycoplasma*. Lane M: Ladder (100 bp), Lane P: Positive control, Lane N: Negative control, Lane 1-5: Conjunctival swabs positive for 16S rRNA gene of genus *Mycoplasma*

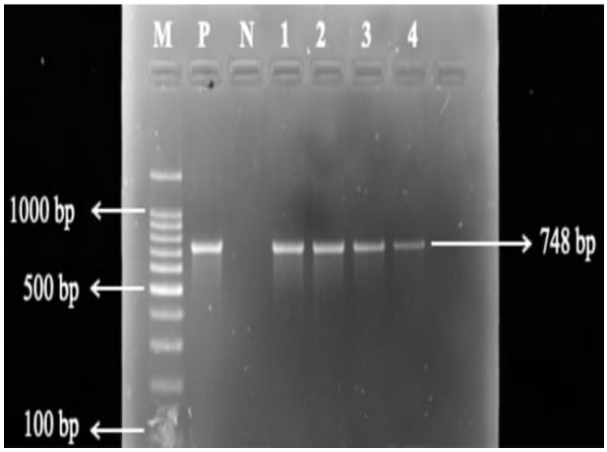

Fig. 3: Amplification of 16S rRNA gene of *Mycoplasma conjunctivae* Lane M: Ladder (100 bp), Lane P: Positive control, Lane N: Negative control, Lane 1-4: Conjunctival swabs positive for 16S rRNA gene of *M. conjunctivae* Mycoplasma

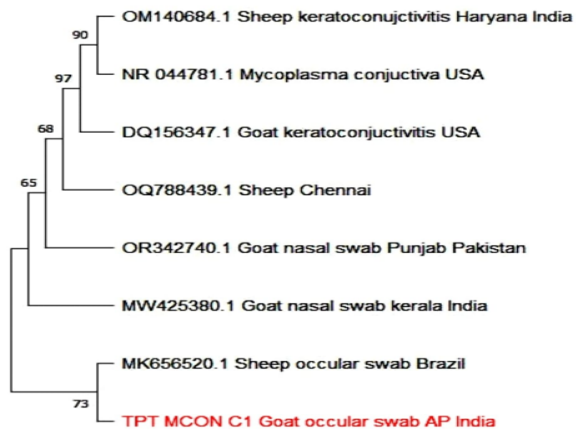

Fig. 4: Phylogenetic analysis of *Mycoplasma conjunctivae* with other reference sequences in NCBI database. Phylogenetic tree was constructed using MEGA 11 version 11.0 software by the Neighbour Joining method.

Acknowledgement

The authors are grateful to Sri Venkateswara Veterinary University, Tirupati for providing facilities to carry out the research work.

References

Aguilar, X.F., Lopez-Olvera, J.R., Ribas, M.P., Begovoeva, M., Velarde, R., Cardells, J., & Cabezón, O. (2019). *Mycoplasma conjunctivae* in insect vectors and anatomic locations related to transmission and persistence. *Veterinary Microbiology, 228,* 7-11.

Ezzi, A., Pourbakhsh, S.A., & Bidhendi, M. (2007). Study on Mycoplasmal pneumonia at the Ziaranabbatoir. *Archives of Razi Institute, 62*(3), 161-164.

Fernandez-Aguilar, X., Cabezon, O., Granados, J.E., Frey, J., Serrano, E., Velarde, R., & Lopez-Olvera, J.R. (2017). Postepizootic persistence of asymptomatic *Mycoplasma conjunctivae* infection in Iberian ibex. *Applied and Environmental Microbiology, 83*(15), e00690-17.

Gharaibeh, S., & AlRoussan, D. (2008). The use of molecular techniques in isolation and characterization of *Mycoplasma gallisepticum* from commercial chickens in Jordan. *International Journal of Poultry Science, 7*(1), 28-35.

Giacometti, M., Nicolet, J., Johansson, K.E., Naglic, T., Degiorgis, M.P., & Frey, J. (1999). Detection and Identification of *Mycoplasma conjunctivae* in Infectious Kerato-conjunctivitis by PCR. Based on the 16S *r*RNA Gene. *Journal of Veterinary Medicine, 46*(3), 173-180.

Gulaydin, A., Gulaydin, O., Akgul, M. B., Sindak, N., & Yildirim, O. (2024). Investigation of the presence of *Chlamydia* spp., *Mycoplasma* spp. and *Moraxella ovis* in infectious keratoconjunctivitis cases in sheep and goats in Siirt province

and evaluation of clinical findings. *Polish Journal of Veterinary Sciences*, *27*(2), 183-191.

Harasawa, R., Pitcher, D.G., Ramirez, A.S., & Bradhury, J.M. (2004). A putative transposase gene in the 16S-23S rRNA intergenic spacer region of *Mycoplasma imitans*. *Microbiology, 150*(4), 1023-1029.

Junqueira, N.B., Salina, A., Oliveira, G.C., Mettifogo, E., Joaquim, S., Guimarães, F.F., & Langoni, H. (2020). Detection of clinical bovine mastitis caused by *Mycoplasma bovis* in Brazil. *Journal of Dairy Research, 87*(3), 306-308.

Liu, T., Garcia, M., Levisohn, S., Yogev, D., & Kleven, S. (2001). Molecular variability of the adhesion-encoding gene pvp A among *Mycoplasma gallisepticum* strains and its application in diagnosis. *Journal of Clinical Microbiology, 39,* 1882-1888.

Rahman, H.U., Saddique, U., Shakoor, A., Shah, M.K., Shah, S.S.A., Khan, F.A., & Rahman, S. (2018). The predominant incidence of *Mycoplasma mycoides subsp. capri* in suspected cases of contagious caprine pleuropneumonia in sheep and goats of northern Pakistan. *Pakistan Journal of Zoology, 50*(5), 1995-1998.

Sumit, K.S., Umesh, C.G., Ravishankar, C., Mathew, S., Ajithkumar, S., & Daniel, A. K. (2022). Diagnosis and therapeutic management of infectious kerato-conjunctivitis associated with *Mycoplasma* conjunctivae in goats. *Indian Journal of Animal Health, 61*(1), 147-150.