

Diagnosis and therapeutic management of infectious kerato-conjunctivitis associated with *Mycoplasma conjunctivae* in goats

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Abstract

Infectious kerato-conjunctivitis (IKC) is a worldwide, highly contagious bacterial disease of goats caused primarily by *Mycoplasma conjunctivae*. In the present study, eye swabs were collected from goats with clinical signs like conjunctivitis, lacrimation, blepharospasm, corneal opacity and ulceration to detect *Mycoplasma conjunctivae* by Polymerase Chain Reaction (PCR). PCR was performed using primers specific to 16S rRNA gene of *Mycoplasma* spp. The amplicons obtained were then sequenced and identified as *Mycoplasma conjunctivae* by BLAST analysis. Treatment was done using Oxytetracycline @ 15 mg/kg/day intravenously and the clinical signs resolved successfully within five days of treatment.

Key words: Goat, Infectious kerato-conjunctivitis, Mycoplasma, Oxytetracycline

Highlights

- Clinical signs suggested the condition to be mycoplasmal kerato-conjunctivitis.
- Confirmation of the disease was done using PCR and nucleotide sequencing.
- Partial sequences of 16S rRNA gene obtained were subjected to homology search using BLAST technique.
- The partial sequences were identified as that of *Mycoplasma conjunctivae*.
- Systemic administration of oxytetracycline helped in resolving the clinical signs.

INTRODUCTION

Infectious kerato-conjunctivitis (IKC) in goats, caused primarily by *Mycoplasma conjunctivae* is a highly contagious disease characterised by varying degrees of blindness in infected animals (Fernandez-Aguilar *et al.*, 2017). 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). Conjunctivitis, lacrimation, blepharospasm with different stages of corneal opacity and ulceration are the usual clinical findings in infected goats (Abdullah *et al.*, 2014). Other clinical manifestations associated with IKC are mucopurulent ocular discharge, swollen red

eyes and photophobia (Browning, 2007). Stressors like dusty environmental conditions, ocular irritants, transportation and bright sunlight are a few predisposing factors for the disease (Abdullah *et al.*, 2015). Fernandez-Aguilar *et al.* (2017) found that flies serve as vectors for *Mycoplasma* spp. The condition if left untreated may progress to produce pain and worsen, leading to temporary or permanent blindness which results in inappetence and inability to locate food (Jesse *et al.*, 2017). Decreased weight gain, decreased milk production, and treatment costs associated with IKC cause an economic burden to livestock producers (Whittier *et al.*, 2009). Tentative diagnosis of IKC is usually done based on ocular and systemic signs. However, PCR,

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Table 1. Components of PCR

Sl. No.	Name of the reagent	Quantity
1	EmeraldAmp GT PCR Master Mix (2X Premix)	6.25 µL
2	Forward primer	1.00 µL
3	Reverse primer	1.00 µL
4	Nuclease free water	1.25 µL
5	Template DNA	3.00 µL
		Total = 12.5 µL

Table 2. PCR cycling conditions

Sl. No.	Name of the step	Temperature and time combination
1	Initial denaturation	94°C, 2 min
2	30 Cycles	Denaturation 94°C, 15 sec
		Annealing 59.3°C, 15 sec
		Extension 72°C, 15 sec
3	Final extension	72°C, 5 min

microbial culture and isolation can be done to confirm the disease (Kahn and Line, 2005)

MATERIALS AND METHODS

Five goats from a farm were presented to Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode with clinical signs including reddening and swelling of the eyes, mucopurulent ocular discharge, lacrimation, corneal opacity, loss of vision and inappetence. All the animals in the study group were subjected to signalment, detailed anamnesis and clinical examination. Detailed clinical examination of the eye revealed the presence of melting ulcers, corneal oedema and engorgement of stromal vessels which suggested kerato-conjunctivitis and keratomalacia (Fig. 1). Goats with severe progression of the disease also had presence of abscess in the stromal layer of the cornea (Fig. 2). Eye swabs were collected from two goats using sterile cotton swabs and DNA was extracted using Phenol-Chloroform extraction technique. Polymerase Chain Reaction (PCR) was performed using primers specific for 16S rRNA gene of *Mycoplasma* spp. (Botes *et al.*, 2005). PCR reactions were conducted in an automated thermal cycler (Eppendorf, Germany). The components of PCR mixture and the cycling conditions are given in Table 1 and 2

respectively. The amplicons obtained were then sequenced and analysed using the BLAST online tool. Treatment was carried out using Oxytetracycline @ 15 mg/kg/day intravenously. Supportive therapy included Flunixin meglumine @ 1.1 mg/kg/day intramuscularly and Chlorpheniramine maleate @ 0.5 mg/kg/day intramuscularly. The treatment was carried out for five days.

RESULTS

In the present study, the disease condition was diagnosed as mycoplasmal kerato-conjunctivitis based upon the clinical signs exhibited by the goats and identification of the pathogen using PCR and nucleotide sequencing. Two PCR amplicons of product size 270 bp was generated by the *Mycoplasma* genus specific PCR. Nucleotide sequencing of the PCR products were performed by Sanger's dideoxy method using automated DNA sequencing facility available at AgriGenome Labs Pvt. Ltd., Cochin, Kerala. Both the partial sequences of 16S rRNA gene obtained in the present study were subjected to homology search using BLAST of National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/BLAST) and identified as that of *Mycoplasma conjunctivae*. The partial sequences of 16S rRNA gene in the present study were submitted



Fig. 1. Goat presented with severe corneal edema, vascularization in peripheral area and melting areas of the cornea suggesting keratomalacia



Fig. 2. Goat presented with severe edema, vascularization and melting areas of the cornea along with stromal abscess

Table 3. NCBI- BLAST sequence analysis of *Mycoplasma* spp.

Animal No.	Sample taken for PCR	Identity (BLAST result)	NCBI Accession no.
1	Eye swab in 0.9% normal saline	<i>Mycoplasma conjunctivae</i>	MW425379
2	Eye swab in 0.9% normal saline	<i>Mycoplasma conjunctivae</i>	MW425380

in GenBank sequence database and accession numbers were obtained. The details of the samples from which the sequences were generated, the result of BLAST analysis, and the accession numbers are given in Table 3. Systemic administration of oxytetracycline helped in resolving the clinical signs after five days of treatment.

DISCUSSION

Infectious kerato-conjunctivitis caused by *Mycoplasma conjunctivae* is influenced by several factors including the virulence of the pathogenic strain involved, immune status of the predisposed animal and environmental factors (Abdullah *et al.*, 2015). Frequent occurrence of IKC can be seen when goats are exposed to stress like introduction into a new herd, transportation or relocation, and exposure to very dry or cold weather (Rahaman *et al.*, 2018). The main route of transmission from animal to animal is presumed to be by physical contact. Giacometti *et al.* (2002) studied that Muscidid flies from the genera *Musca*, *Morellia*, *Hydrotaea* and *Polietes* can possibly serve as the vectors of *M. conjunctivae*. Whittier *et al.* (2009) described four stages of IKC in ruminants based on clinical signs. The first stage initiates with increased sensitivity to light, excessive tearing and blepharospasms which later progresses to a small corneal ulcer at the centre of the cornea. In stage II as the

inflammation increases, ulcer tend to spread and the blood vessels starts to grow across the cornea to help healing. The disease progresses to stage III as the inflammation spreads into the inner parts of the eye and the ulcer covering almost the entire cornea. If the disease is left untreated the ulcer extends completely through the cornea and the iris may protrude through the ulcer at stage IV. Oxytetracycline is generally considered as the drug of choice in case of systemic therapy against IKC as it is concentrated in the corneal tissue (Abdullah *et al.*, 2015). Complete disease eradication is difficult because the causative agent is widespread and may persist in carrier animals (Rhaymah *et al.*, 2013). Good farm management, proper herd health and quarantine of new and infected animals are the key factors in preventing the spread of disease.

Conflict of interest: Authors have no conflict of interest in this study.

Author contributions: SKS: Data collection, analysis and interpretations; UCG, CR, MKM: Supervised the research work; AS, AKD: Contributed to the implementation of the research.

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REFERENCES

- Abdullah FFJ, Naidu NRG, Sadiq MA, Abba Y and Tijjani A, 2015. Prevalence of *Moraxella ovis* infection in goats under the Ladang Angkat Programme, Universiti Putra Malaysia: A cross-sectional study. IOSR J Agric Vet Sci, 8: 99-102
- Abdullah FFJ, Radzuan NS, Tijjani A, Adamu L and Abba Y, 2014. Stage II kerato-conjunctivitis in a goat: A case report. IOSR J Agric Vet Sci, 7: 16-18

- Botes A, Peyrot BM, Olivier AJ, Burger WP and Bellstedt DU, 2005. Identification of three novel mycoplasma species from ostriches in South Africa. *Vet Microbiol*, 111(3-4): 159-169, doi: 10.1016/j.vetmic.2005.10.017
- Browning LM, 2007. Kerato-conjunctivitis (Pinkeye) in goats. Alabama A & M University, Alabama Cooperative Extension System, 3: 1-2
- Fernandez-Aguilar X, Rossi L, Cabezon O, Giorgino A and Llopis IV, 2017. Infectious kerato-conjunctivitis and occurrence of *Mycoplasma conjunctivae* and *Chlamydiaceae* in small domestic ruminants from Central Karakoram, Pakistan. *Vet Rec*, 181: 237, doi: 10.1136/vr.103948
- Giacometti M, Janovsky M, Belloy L and Frey J, 2002. Infectious kerato-conjunctivitis of ibex, chamois and other *Caprinae* species. *Rev Sci Tech*, 21(2): 335-345, doi: 10.20506/rst.21.2.1338
- Jesse FFA, Chung ELT, Abba Y, Bitrus AA and Hambali IU, 2017. Clinical management of stage I pinkeye with concurrent pneumonic pasteurellosis in a goat: A case report. *J Adv Vet Anim Res*, 4(4): 390-393, doi: 10.5455/javar.2017.d228
- Kahn CM and Line S, 2005. The Merck Veterinary Manual (9th edn.). Merck & Co., Meril, USA, pp 2712
- Rahaman M, Yadav SK, Ran EA and Zaman MA, 2018. Clinical management of kerato-conjunctivitis in goat. *History*, 5(1): 6-11, doi: 10.20448/journal.504.2018.51.6.11
- Rhaymah MS, Rasheed BY and Hussain KHJ, 2013. Comparative study on the bacterial causes of ovine kerato-conjunctivitis in native breed sheep and goats. *Assiut Vet Med J*, 59(137): 77-80, doi: 10.21608/AVMJ.2013.171487
- Whittier WD, Currin N and Currin J, 2009. Pinkeye in Beef Cattle. Virginia Cooperative Extension, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University Publication, pp 400-750