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# Occurrence of a Mixed Mycoplasma and Moraxella Infection Associated with a Severe Eye Keratoconjunctivitis of Bovine

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Mycoplasma and Moraxella gene were both detected from conjunctival swabs taken from young dairy cattle showing symptoms consistent with keratoconjunctivitis. Based on laboratory tests and clinical observations, the first phase of the disease was likely pneumonic in nature, possibly caused by bovine respiratory syncytial virus. Bacterial cause of keratoconjunctivitis in bovine may be due to Infectious bovine rhino tracheitis (IBRT), *Moraxella bovis*, *Mycoplasma conjunctivae*, etc. An investigation carried out at R.Kunnathur, Tiruvannamalai district during the month of February 2022, Tamil Nadu, India revealed presence of Mycoplasma and Moraxella in bovine (n=100 animals). The investigation of eye swabs from affected animals showed that the presence of bacteria such as *Moraxella* spp and *Mycopalsma* spp. by genus specific polymerase chain reaction (PCR) of the isolate was positive for *Moraxella* spp. and *Mycoplasma* spp. This study reports the presence of mixed eye infection in dairy farming warranting further studies to know the exact status of this organism so as to prevent keratoconjunctivitis in bovine.

**Key words:** Moraxella, Mycoplasm, Bovine, PCR, India

Infectious bovine keratoconjunctivitis (IBK) is an important ocular disease of cattle. Clinical signs may include bilateral conjunctivitis, tearing, photophobia, and keratitis. The disease is often called "pinkeye" because of the characteristic reddening and inflammation of the conjunctiva of the eyelid and eyeball. Predisposing causes such as intense sunlight, dust, pollen or grass seeds is a factor to infection. Mixed infections are often established and act in concert or successively to result in pinkeye. Many different microorganisms have been involved in IBK, including *Moraxella* spp. and several *Mycoplasma* spp. among the mycoplasmas, *Mycoplasma bovoculi* has been widely isolated from ocular swabs of affected eyes (Barber et al., 1986 and Friis and Pedersen, 1979). It has been demonstrated experimentally that prior infection by ocular instillation with *M. bovoculi* enhances and prolongs colonization of *Moraxella bovis* and *Moraxella ovis*. In fact, because not all clinical diagnostic laboratories routinely perform isolation of *Mycoplasma* spp., the prevalence of this class of microorganisms is not known. Reports regarding the isolation of *Moraxella* spp. from animals in India are scarce (Gandhi *et al*., 2008) and very recently a report of *Morexella bovoculi* in bovine recorded and treated by Karthik *et al*.(2017). The present study describes the molecular identification of *Moraxella* spp. and *Mycoplasma* spp. from cattle in an organized dairy farm located at R. Kunnathur Tiruvannamalai district, Tamil Nadu, India.

## Materials and Methods

Investigation carried out at an organized dairy farm at Melmaruvathur, Chengalpattu district, Tamil Nadu, India during February 2022, showed that 3 out of 100 dairy animals had eye infection. Affected animals were about one calved old and the farm had mixed age group animals. On clinical examination affected animals had lacrimation and ulceration in one or both eyes. The animal has history of lumpy

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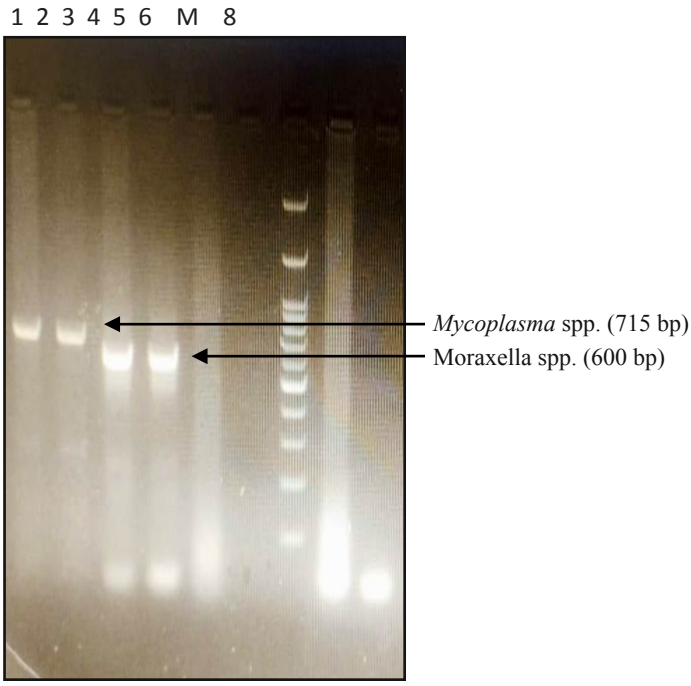


**Fig 1.** Moraxella and Mycoplasma specific pcr
Agarose gel image of genus-specific PCR for detection of Mycoplasma spp. and *Moraxella* spp.. Lanes 1 and 2—positive amplification for *Mycoplasma* spp. (715 bp). Lanes 3 and 4 positive amplification for *Morxella spp.* (600 bp) and Lane M—100 bp marker.

skin infection 15 days before. Aseptically swabs were collected from the eye lesions from the affected animals and transported and processed at the Central University Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Chennai. Swabs were inoculated on blood agar and MacConkey agar and incubated at 37˚C overnight for isolation and identification of bacteria. For confirmation of mycoplasma infection samples were inoculated into sterile glass vials containing 3.5 ml of Frey's medium and incubated at 37°C with 90 percent relative humidity until the indicator changed from red to yellow.

DNA from organism were extracted by heating methods followed by cooling at −20˚C for 10 min and centrifuged at 10,000 rpm, 18˚C for 10 min and used as the DNA for polymerase chain reaction (PCR). PCR test was performed using the primers targeting the 16S-23S intergenic spacer region and products were run on 2% agarose gel and results were documented using Bio Rad Gel Doc XR+ systemas per Angelos & Ball. The positive cultures of *Mycoplasma* spp.

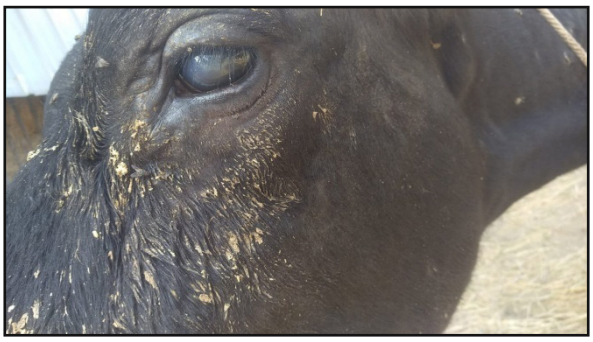

**Fig 2.** Cow noticed with keratoconjunctivitis symptoms

were tested by Polymerase Chain Reaction (PCR) for genus-specific identifications of mycoplasma employing the primers as described by Lauerman (1998) and PCR test was performed.

## Results and Discussion

Samples were streaked in blood agar plate and incubated overnight revealed complete hemolytic small colonies from eye swabs. Microscopic examination of the culture revealed gram negative coccoid organisms. Biochemical tests like catalase and oxidase yielded positive results. Moraxella infection confirmation by PCR using 16S–23S intergenic spacer region seems to be better option for accurate identification of the pathogen. In this study, PCR reaction yielded amplicon of expected product size of 600 bp (Fig.1). Samples developed yellow colour change of Frey's broth after 3 to 4 days of incubation for confirmation mycoplasma infection which is in accordance with the observation of Khalifa *et al.* (2013).PCR test, cultured samples showed 715 bp product size with genus specific primers of mycoplasma.

Keratoconjunctivitis is a serious problem of the eye noticed in several animal species and it can cause economic loss to the farmer. Causative agent of this condition is many and among infectious agents in bovine bacterial origin namely *Moraxella bovoculi, Moraxellabovis*, *Moraxellabovis* and*Mycoplasma bovis* are predominant (Jeyabal *et al.*,2013). Many cases of keratoconjunctivitis remain unnoticed therefore the exact incidence of this condition is not understood. This investigation reports the PCR based confirmation of *Moraxella* spp. and *Mycoplasma* spp. infection from dairy animals. Though *M. bovis* is the predominant organism of IBK in cattle and

frequency *M. bovoculiand Mycoplasma* spp. of mixed infection from is less. *Moraxella*spp. has been documented by several incidences in India (Karthik *et al*., 2017) irritants like dust, wind, cold climate, infection with bovine herpes virus 1 or Thelazia spp.

Factors like age group, high fly population, UV irradiation and vitamin A deficiency and climate condition can predispose IBK infection in cattle Angelos (2010b). In the present investigation above two years of age were affected. Though recovery was noted in infected animals, few animals had recurrent infection which might be due to high fly population density. It was earlier reported that house fly and face fly play role as vectors in the transmission of IBK (Abdullah *et al.,* 2015). Affected animals were treated with injection of Enrofloxacin, Tylosin and anti-inflammatory injections like flunixmeglumaine for one week resulted in improvement of eye lesions and slowing by about 20 days animals were recovered and also advised for fly control.

## Conclusion

Current investigation reports the presence of *Moraxella* spp. and *Mycoplasma* spp. in bovine causing keratoconjunctivitis. Hygienic farm practices can minimize vector control thereby can prevent vector transmission of *Moraxella* sp. and other infection like LSD and IBRT which predispose the occurrence of mycoplasma infection. Further studies need to be carried out to know the virulence factors of *Moraxella* sp. and *Mycoplasma* spp. causing keratoconjunctivitis.

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