

Isolation of *Moraxella* sp. from Cases of Keratoconjunctivitis in an Organized Sheep Farm of India

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Abstract

Keratoconjunctivitis in sheep causes severe ocular irritation thereby reducing the production performance thus causing economic loss to the farmers. Bacterial cause of keratoconjunctivitis in sheep may be due to *Listeria monocytogenes*, *Moraxella ovis*, *Mycoplasma conjunctivae*, etc. An investigation carried out at Tirupathur, Sivagangai district, Tamil Nadu, India revealed keratoconjunctivitis in sheep (population 100 animals). Isolation and identification of eye swabs from ailing animals (n = 5) showed that the bacteria was *Moraxella* sp. Polymerase chain reaction (PCR) of the isolate was positive for *Moraxella* sp. Antimicrobial sensitivity testing of the isolate showed that it was sensitive to all antibiotics used in the study. This study reports the presence of *Moraxella* sp. infection in sheep warranting further studies to know the exact status of this organism so as to prevent keratoconjunctivitis in sheep.

Keywords

Moraxella, Keratoconjunctivitis, Sheep, PCR, India

1. Introduction

Ocular infections in farm animals can cause serious economic loss to farmers [1] [2] [3]. Keratoconjunctivitis is a painful condition that can lead to temporary or permanent eye blindness in sheep. Clinical signs like serous lacrymation, increased blinking, hyperaemia, ulcers and blepharospasm which can be noticed initially in one eye and later both eyes may be affected. In the later stages the serous lacrymation may become purulent [4] [5]. Affected animals usually recover within weeks but some remain infected for longer period and this can lead

to reduced feed intake thus affecting the slaughter weight [6]. Several bacterial species namely *Listeria monocytogenes*, *Moraxella ovis*, *Mycoplasma conjunctivae*, *Chlamydophila pecorum*, *Coxiella burnetii*, etc., were isolated from sheep affected with keratoconjunctivitis [3] [5] [7]. *Moraxella bovis* was attributed as the cause of infectious keratoconjunctivitis (IBK) in cattle and later findings reported that another agent namely *M. bovoculi* was also the cause of IBK in cattle [8]. *M. bovis* was also reported from sheep but the frequency of its isolation is less [9]. Reports regarding the isolation of *Moraxella* sp. from animals in India are scarce [10] [11] [12] and very recently a report of *M. bovoculi* in cattle was recorded by Karthik *et al.* [13]. The present communication describes the isolation, biochemical and molecular characterization of *Moraxella* sp. from sheep in an organized sheep farm in India.

2. Materials and Methods

2.1. Sample Collection and Bacteriological Investigation

Investigation carried out at an organized sheep farm at Tirupathur, Sivagangai district, Tamil Nadu, India during February 2017, showed that 5 out of 100 sheep had eye infection. Affected animals were about 3 years old and the farm had mixed age group animals. On clinical examination affected sheep's had lachrymation and ulceration in one or both eyes. Eye swabs were collected aseptically without any preservatives from the affected sheep and they were transported and processed within 4 hours at the Central University Laboratory, TANUVAS, Chennai-51. Swabs were cultured on 5% sheep blood agar, MacConkey agar and nutrient agar and incubated at 37°C overnight. Biochemical tests like catalase, oxidase, indole (I), methyl red (MR), voges proskauer (VP) and citrate (C) tests were carried out as described earlier [14]. Pure isolates were subjected to antibiotic sensitivity testing (ABST) on Mueller-Hinton agar using amoxyclav, enrofloxacin, ciprofloxacin, cefixime, gentamicin, tetracycline and norfloxacin as per the standard protocol [15].

2.2. Polymerase Chain Reaction and Sequencing

DNA from isolated culture were extracted by heating a loop full of colonies in 100 µL nuclease free water at 95°C for 10 min followed by cooling at -20°C for 10 min and centrifuged at 10,000 rpm, 18°C for 10 min. Supernatant was used as the DNA for polymerase chain reaction (PCR). PCR was carried out as per Angelos & Ball [16] using published 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⁺ system. DNA of *Moraxella bovoculi* isolate from the laboratories repository was used as the positive control and non template PCR mixture was kept as the negative control during the PCR reaction. Further positive PCR product was purified using QIA quick gel extraction kit (Qiagen, USA) following manufacturer's protocol and was send for sequencing to Eurofins Genomics India, Bangalore, India. Obtained sequences were aligned and NCBI

BLAST search was used to find the matching sequences available in the data bank.

3. Results and Discussion

Two samples after overnight incubation on blood agar revealed small dry colonies showing hemolysis under the colony (**Figure 1**). Correspondingly growth was also noticed on nutrient agar while MacConkey does not yield any growth. Other three samples do not yield any growth on all the three media used for isolation. Microscopic examination of the colonies revealed gram negative bacilli organisms. Biochemical tests like catalase and oxidase yielded positive results while other tests yielded negative result. Antibiotic sensitivity test revealed that the isolate was sensitive to all the antibiotics used in the drug sensitivity test. PCR reaction yielded an amplicon size of ~650 bp which may correspond to *M. bovis*, *M. boevrei*, and *M. caprae* (**Figure 2**). Sequences result was matching 99% with the *M. bovis*.

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 sheep bacterial origin namely *Listeria monocytogenes*, *Moraxella ovis*, and *Mycoplasma conjunctivae* are predominant [5]. Several cases of keratoconjunctivitis in sheep goes unnoticed hence incidence of this condition is not clear. This investigation report the isolation of *Moraxella* sp. from two affected sheep and the isolate has 99% similarity with *M. bovis*. Though *M. bovis* is the predominant organism of IBK in cattle its frequency of isolation from sheep is less. *M. ovis* has been documented by several

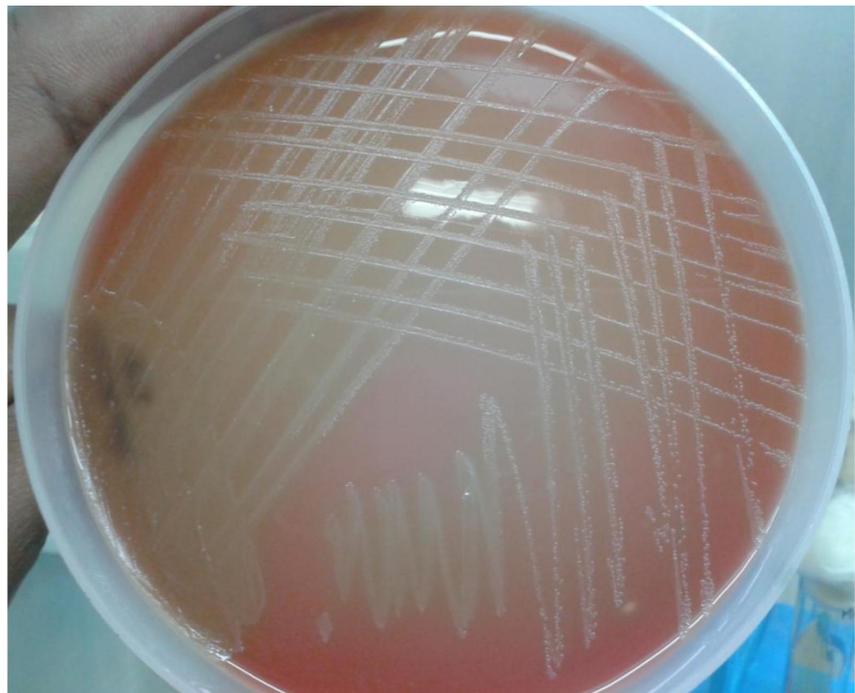


Figure 1. Blood agar showing haemolytic colonies.

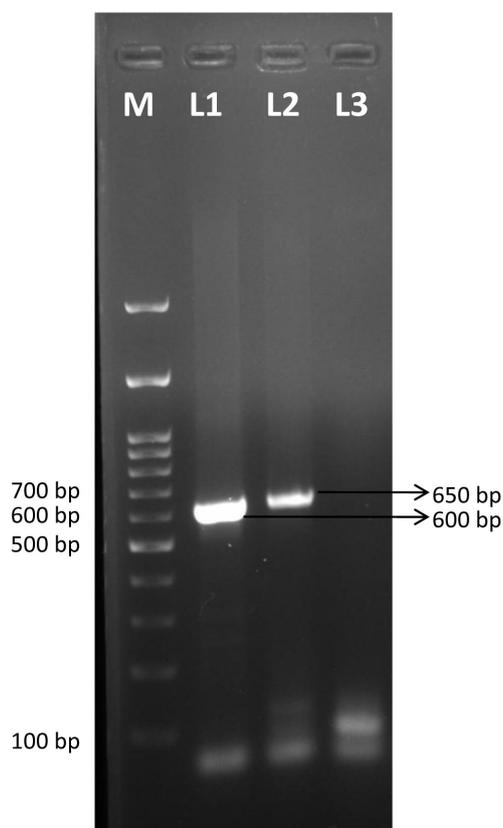


Figure 2. Agarose gel image of 16S-23S intergenic spacer region PCR for detection of *Moraxella* sp. M-100 bp ladder (New England Biolabs; Cat No. N3236S). L1-*Moraxella bovoculi* (GenBank sequence id. KX121047) showing specific band at ~600 bp. L2-Sheep eye swab sample showing band at ~650 bp (specific for *Moraxella* sp.).

authors from sheep [7] [9] [17]. Several authors though report the isolation of *Moraxella* sp. from sheep there is no clear picture regarding its role in keratoconjunctivitis in sheep [18] [19] [20]. There are reports of isolation of *S. aureus*, *Corynebacterium* spp. and *E. coli* from cases of keratoconjunctivitis in sheep [5]. Antibiotic sensitivity testing results showed that the isolate was sensitive to all the antibiotics used which was in concordance with earlier reports [9]. There are only few reports on *Moraxella* sp. from India [10] [11] [12] [13]. Hence further studies has to be carried out in this part of the world to know the exact status of *Moraxella* infection and also to carve out a better prevention measure to minimize the economic loss to the farmers. Vectors namely house fly and face fly plays major role in transmission of the pathogen hence vector control should also be carried out to effectively control the infection [6].

4. Conclusion

Current investigation reports the presence of *Moraxella* sp. in sheep causing keratoconjunctivitis. Hygienic farm practices can minimize vector control thereby can prevent vector transmission of *Moraxella* sp. Further studies need to be carried out to know the virulence factors of *Moraxella* sp. causing keratoconjuncti-

vitis in sheep.

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Conflict of Interest

The authors declare that they have no competing interest.

Disclosure Statement

The authors declare that they have no competing interest.

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