B-5349
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RESEARCH ARTICLE Indian Journal of Animal Research

Molecular Confirmation of Moraxella Bovis Associated Infectious Keratoconjunctivitis in Ruminants

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ABSTRACT

Background: Infectious keratoconjunctivitis is a significant problem causing a greater level of economic losses in production animals. The affected animals show progressive cloudiness of eye, severe congestion of ocular mucous membrane with inflammation of cornea, ocular discharges vary from serous to purulent and causes temporary or permanent blindness in affected animals

Methods: A total number of 238 conjunctival swabs were collected from the suspected cases (93 sheep, 98 goats and 47 cattle) of different age groups in both symptomatic and asymptomatic animals during the period of 2021-2023 (both winter and summer season in livestock farms of Tamil Nadu, SBRS -Ooty, PGRIS-Katuakkam, Tirvananamalai, Sheep Research Station-Potaneri, Tiruvallur district). The samples were subjected to bacterial isolation and identification. The bacterial organism isolated from the swabs were characterized by cultural, biochemical and molecular tests.

Result: The *Moraxella species* was successfully isolated from 9 animals (N=5 in cattle and N=4 in sheep). In addition to this *Staphylococcus* and *Klebsiella*, (N=34), *Streptococcus* (N=10), *Pseudomonas* (N=8) and concurrent infection of *Tricophyton verrocosum*, *Aspergillus*. The genomic DNA was extracted from positive culture of *morexalla* and PCR amplification was performed with specific primers. The amplified PCR product were subjected to partial gene sequencing which showed 99% homology with other, *Morexalla bovis* isolates. Antimicrobial sensitivity test of the isolates revealed that *Moraxella* was sensitive towards Chloramphenicol (100%), Ciprofloxacin (100%), Gentamicin (100%), Tylosin (100%) and sensitive to Oxytetracycline (80%), Endrofloxacin (80%), Gatifloxacin (80%) and Metronidazole (70%). All tested isolates were resistant to Norfloxacin, Vancomycin, Ampicillin /Sulbactam, Methicillin and Sulphadiazine.

Key words: Antibiotic sensitivity test, Fungus, *PCR,* Pink eye.

INTRODUCTION

Infectious keratoconjunctivitis is a significant problem causing a greater level of economic losses in production animals. The affected animals shows progressive cloudiness of eye, severe congestion of ocular mucous membrane with inflammation of cornea, ocular discharges vary from serous to purulent and causes temporary or permanent blindness in affected animals (Baker *et al.,* 2001 and Abdullah *et al.,* 2014). There are more than 10 different types of pathogens including fungus causing Infectious keratoconjunctivitis in ruminants. The *Moraxella* along with other pathogens was a major pathogen causes Infectious keratoconjunctivitis in small and large ruminants (Abdullah *et al.,* 2015; Gelormini *et al.,* 2017 and Zheng *et al.,* 2019). The frequent isolation of different bacteria like *Staphylococcus*, *Streptococcus, E. coli, Klebsiella* and *Pseudomonas* from ruminants with signs of Infectious keratoconjunctivitis may be attributed to the synergistic action between environmental risk factors and opportunistic pathogens habitat eye (Sağlam *et al.,* 2018). In certain cases, *Moraxella spp* alone isolated from outbreaks of Infectious keratoconjunctivitis in sheep and goat flocks (Karthik *et al.,* 2017 and Athira *et al.,* 2018).

Moraxella are Gram negative diplococci, non-motile, catalase and oxidase positive and the infection were easily transmitted between the animals by direct contact with nasal and ocular discharges and by flies as mechanical transmitter (Ojo *et al.,* 2009 and Athira *et al.,* 2018). *Moraxella ovis* produces heat-labile exotoxins causing both hemolysis for bovine RBCs and cytotoxic activities against corneal tissues leads to permanent blindness (Cerny *et al.,* 2006). *Moraxella spp* form biofilm *in vivo* but the biofilm easily broken by the action of lysozyme naturally present in tears (Ely *et al.,* 2018). The prevalence rates of *Moraxella* associated infectious keratoconjunctivitis have been observed by several authors (Van Halderen *et al.,* 1994; Naglić *et al.,* 2000 an d Karthik *et al.,* 2017). The variations in results of antimicrobials susceptibility were noticed

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How to cite this article: Kalaiselv, G., Balakrishnan, G., Raman, A., Jayanthi, N., Ramya, R. and Soundararajan, C. (2024). Molecular Confirmation of Moraxella Bovis Associated Infectious Keratoconjunctivitis in Ruminants. Indian Journal of Animal Research. 1-6. doi: 10.18805/IJAR.B-5349.

Submitted: 12-03-2024 **Accepted:** 02-09-2024 **Online:** 22-11-2024

between *Moraxella spp* isolates of different regions. This may refer to differences in genotypic characters of isolates (Loy and Brodersen, 2014). The Cure rate of IKC was 100% by using various types of antibiotics locally and systemically by injection along with topical antibiotic eye preparations (Pandey, 2018).

MATERIALS AND METHODS

The study was carried out in different district of Tamil Nadu, India, both winter and summer season during 2021-2023. A total number of 238 conjunctival swabs were collected from the suspected cases (93 sheep, 98 goats and 47 cattle) of different age groups in both symptomatic and asymptomatic animals during disease investigation in the period of 2021-2023 (both winter and summer season, Table 1). The samples were subjected to bacterial isolation and identification. Aseptically, conjunctival swab was taken from each suspected case and the swabs were immediately inoculated in sterile tubes containing sterile nutrient broth and transported in ice pack to Central University Laboratory, Madhavaram Milk Colony, Chennai, Tamil Nadu.

The suspected samples were inoculated in nutrient broth and incubated at 37°C for 24 hr in nutrient broth. A loopful of culture from each tube was streaked onto 5% blood agar, BHI, Mac Conkey and SDA agar (Himedia - India) plate and incubated at 37°C for 48 h with daily examination for any bacterial and fungal growth. The Staphylococcus, Strptococcus, Pseudonmoas, Klebsiella Trichophyton and Moraxella were isolated from the samples (Table 2). The suspected Moraxella colonies were picked-up, subcultured and subjected to Gram's staining and lactophenol cotton blue staining followed by catalase test, indole production test, oxidase test, motility test, sugar-fermentation test, Simmon's citrate test. The suspected colonies genomic DNA were extracted by Phenol Chloroform and Isoamyl alcohol and 0.2 M NA^{OH} method. The purity and concentration of DNA were assessed by using nano drop. The PCR amplification was performed by using *Moraxell a*specific primers F-5′GTGAAGTCGTAAC AAGGTAGCCGT-3′ and reverse primer R-3′ACCGACGC TTATCGCAGGCTATCA-5′ with cyclic condition of 95°C for 5 min, 95°C for 30 sec, 55°C for 45 sec, 72°C for 30 sec and 72°C for 7 min for 30 cycles. The gel electrophoresis was performed with 1% agarose gel. The positive samples showed amplification size of 650 bp. The amplified PCR product were purified with Quiagen gel purification kit and subjected to partial gene sequencing. The antibiotic sensitivity test was performed with Mueller-Hinton agar (Himedia-India), all the *Moraxella* isolates were tested for their antimicrobials sensitivity based on the method prescribed by Bauer *et al.* (1966) using commercial antimicrobial discs (Himedia - India). Ampicillin plus Sulbactam 20 µg, Vancomycin 30 µg, Ciprofloxacin 30 µg, Gentamicin 10 µg, Oxytetracycline 30 µg, Sulfamethoxazole 30 µg, Endrofloxacin 30 µg, metronidazole 30 µg Gatifloxacin 30 µg and Tylosin 30 µg were used. The affected eye washed with 2% Boric acid lotion, topical eye ointment (Terramycin®), eye drops contains Gentamicin, Chloramphenicol and Microflox-DX were applied 3 times daily and systemic antibiotic and antiinflammatory and vit ADE3 supplement were suggested for the positive animals for 5 successive days.

RESULTS AND DISCUSSION

The examination of eye of suspected animals showed severe of conjunctivitis, keratitis with different degrees of corneal opacities either unilateral bilateral and the ocular discharge was mucopurulent and blepharospasm in clinical cases of infectious kerato conjunctivitis (Fig 1 and Fig 2). Some positive cases showed fever, tachycardia and tachypnea, nasal discharge, corneal ulcers, restlessness in affected flock. The blood agar plate showed small (1 mm), friable, grayish-white colonies surrounded with narrow zone of complete hemolysis (Fig 4) and Yellow colour mucoid colonies were seen in BHI agar (Fig 3). The big radiating blackish colonies in SDA agar and lactophenol cotton blue staining of fungal culture shows septate hyphae with chains of conidial spore (Fig 5 and 6). In gram staining showed gram's negative diplococci and biochemically colonies showed positive for oxidase test and catalase tests and negative for indole test, motility test, Simmon's citrate test

Table 1: Samples details.

Particulars	Sheep and goat	Cattle
Number of samples collected from animals	191	47

Table 2: Microorganism isolated from different samples.

Name of organism	Number organism isolated from sheep and goat	Number organim isolated from cattle
Moraxella	4	5
Staphylococcus and *Klebsiella*	12	22
Streptococcus	7	3
Pseudomonas	5	3
Tricophyton verrocosum, *Aspergillus* and *Moraxella*	5	4

and glucose fermentation tests. The bacteriological cultural examinations, biochemical identification and molecular confirmation revealed that *Moraxella* species were successfully isolated from 9 animals (5 cattle and 4 sheep).

The molecular confirmation of positive isolates showed 650 bp amplification in 1% agarose gel (Fig 7). The partial gene sequencing of purified PCR product showed 99% homology with other *morexalla* isolates (Fig 8). The partial gene sequence were aligned in gene alignment tool and blasted in NCBI which showed 99.47% homology with *Moraxella boviculi* isolates and 94% homology *Moraxella nasovis* isolates.The partial gene sequence were

Fig 1: The examination of eye of suspected sheep showed severe of conjunctivitis, keratitis with different degrees of corneal opacities bilateral mucopurulent ocular discharge.

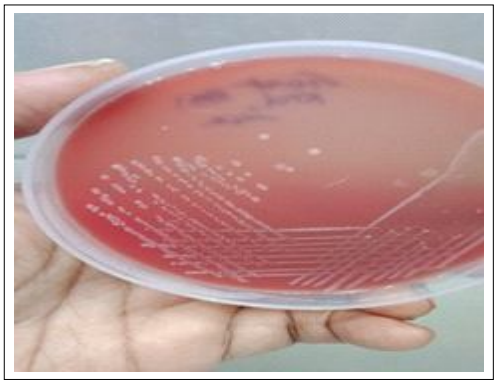


Fig 4: The blood agar plate showed small (1 mm), friable, grayish-white colonies surrounded with narrow zone of Complete hemolysis.

Fig 2: The examination of eye of suspected cattle showed severe of conjunctivitis, keratitis with different degrees of corneal opacities with unilateral mucopurulent ocular discharge.

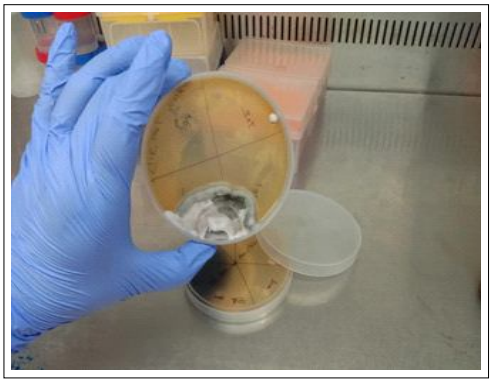


Fig 5 : Eye swab of suspected animals inoculated in SDS agar showed big radiating blackish colonies.

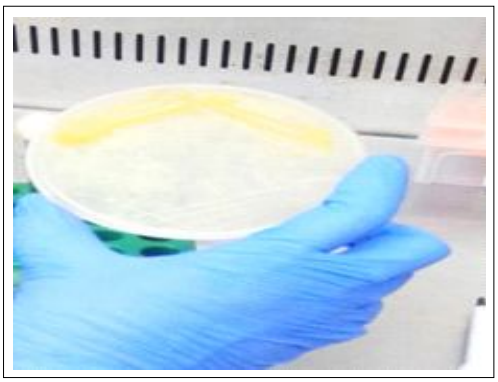


Fig 3: Yellow colour mucoid colonies were seen in BHI.

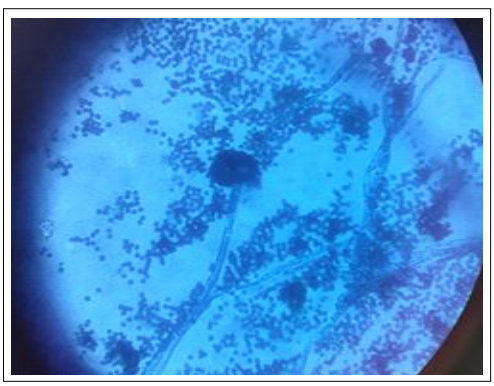
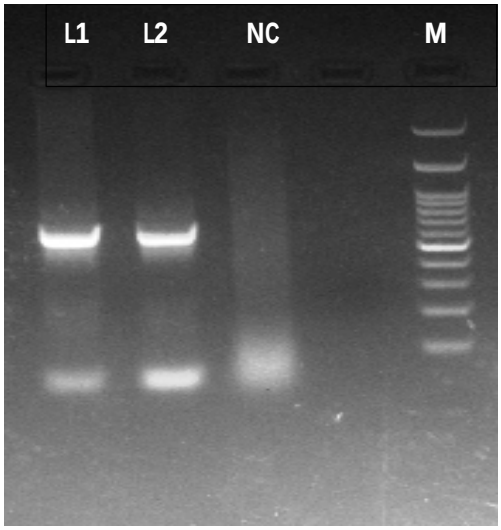


Fig 6: The lactophenol cotton staining of SDA culture samples.

submitted to gene bank (Gen Bank Accession number OQ862535). Antimicrobial sensitivity test of positive isolates revealed that *Moraxella* was 100% sensitive to Chloramphenicol, Ciprofloxacin, Gentamicin and Tylosin and 82% sensitive to Oxytetracycline, Endrofloxacin, Gatifloxacin and Metronidazole. All tested isolates were resistant to Norfloxacin, Vancomycin, Ampicillin - Sulbactam, Methicillin and Sulphadiazine. The affected animals were treated with specific antibiotic and other supportive drugs.



L1and L2- M.bovis positive sub conjunctival swab culture samples, L3- Negative control, M- 100 bp ladder.

Fig 7: 1% agarose gel electrophoresis showed 650 bp amplification size in positive samples.

India is the largest animal population and many farmers depends on animal husbandry practice for their livelihood. The cattle, sheep and goats occupied source of Meat and milk production and important source of incomes to the country. The infectious keratoconjunctivitis representing a considerable health problem for cattle, sheep and goat flocks leads to greater economic loss (Hidson and Winter, 2008). The observed clinical findings of suspected cases strongly refer to infectious keratoconjunctivitis. Similar clinical findings were reported previously (Ojo *et al.,* 2009 and Karthik *et al.,* 2017). Various type of microorganisms were the causative agents of infectious keratoconjunctivitis in small ruminant, *Moraxella* and *Mycoplasma* appear to be the most important one causing infectious keratoconjunctivitis in small ruminants (Åkerstedt and Hofshagen, 2004). In the present study *Moraxella* was successively isolated from 9 cases of 138 examined animals. The low isolation rate of *Moraxella* may give an indication that infectious keratoconjunctivitis is a multi-complexes and *Moraxella* is not the only pathogen responsible for infectious keratoconjunctivitis in ruminants and caused by different microorganisms and the presence of risk factors is crucial (Van Halderen *et al.,* 1994; Jansen *et al.,* 2006; Åkerstedt and Hofshagen, 2004 and Pandey, 2018).

The concurrent infection of *trichophyton verocussum*, *Aspergillus* and *Moraxella* in affected flocks will be confirmed by culture isolation and identification. Mycotic keratitis is caused by filamentous fungi and occurs in

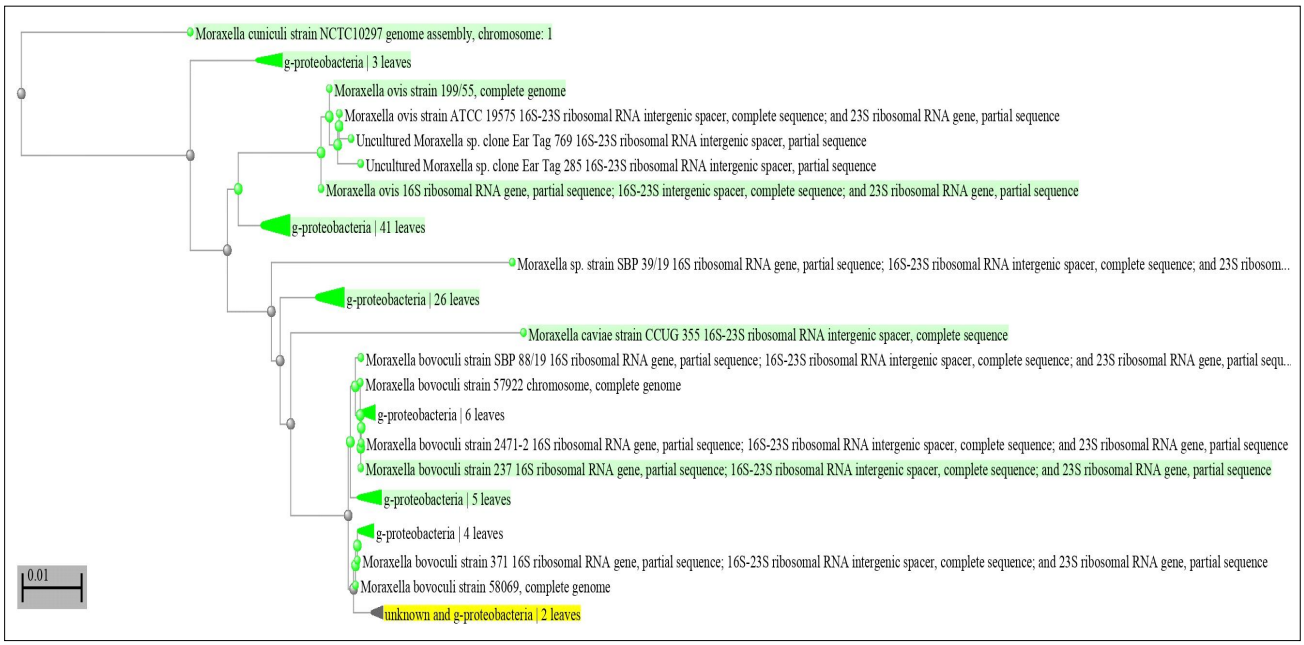


Fig 8: Partial gene sequencing *of Moraxella bovis* shows 99% homology with other existing isolates.

conjunction with trauma to the cornea. Eye trauma is the cause of fungal keratitis in temperate areas by *Fusarium*, *Alternaria* and *Aspergillus* (Aristimuno *et al.,* 1993). Keratitis caused by yeasts such as the *Candida* spp. almost always occur in previously abnormal eyes, like dry eye, chronic corneal ulceration or corneal scarring.

In this study the prevalence of *Moraxella sp.* among the cattle, sheep and goats was confirmed by PCR. The *Moraxella ovis* was isolated from 85.7% of affected sheep with IKC (Saglam. *et al.,* 2018). *Moraxella* was isolated from 100% of diseased goats (N=8) (Ojo *et al.,* 2009). Variation in prevalence rates depends upon differences in the number of examined animals and distinction in managemental and environmental conditions in different localities where the studies were conducted. The different environmental predisposing factors are greatly influence for *Moraxella* and the presence of corneal hurt by mechanical factors or ultraviolet irradiation is important for occurrence of the disease (Dubay *et al.,* 2000). However, *Moraxella* was isolated as a single pathogen from goats with characteristic signs of IKC (Athira *et al.,* 2018). The corneal dryness and corneal abrasion caused by aero-sandy particles carried by wind, as well as exposure to high percentage of ultraviolet rays which may predispose for *Moraxella* colonization during dry and summer season.

The therapeutic management of affected population with right antibiotic will provide significant results usage of Oxytetracycline systemically and topically come in accordance with the results of antimicrobial sensitivity test as the sensitivity of *Moraxella* to Oxytetracycline was intermediate. The susceptibility rate of *Moraxella* of small and large ruminants to Oxytetracycline ranged from 80% to 91% and the haphazard use of Oxytetracycline in veterinary practice contributed to the emergence of resistant strains (Maboni *et al.,* 2015). Using of Oxytetracycline topically as eye ointment and systemically by injection gives better results with a rare relapse rate. Currently, *Moraxella* isolates were 100% sensitive to Tylosin and Gentamicin and *Moraxella* were resistant to Penicillin, Ampicillin, Cloxacillin and Chloramphenicol while being 100% susceptible to Gentamicin and Ofloxacin by disk diffusion technique (Ojo *et al.,* 2009).

In our study, *Moraxella* isolates were 100% sensitive towards Chloramphenicol, Ciprofloxacin, Gentamicin, Tylosin and 82% sensitive to Oxytetracycline, Endrofloxacin, Gatifloxacin,Metronidazole. All tested isolates were resistant to Norfloxacin, Vancomycin, Ampicillin Sulbactam, Methicillin and Sulphadiazine. The antibiotic sensitivity test will vary according to the geographical location and strain of pathogens.

Bovine alphaherpes virus-^{1} (BoHV-^{1}) is a highly contagious virus is responsible for causes conjunctivitis of one or both the eye, upper respiratory tract infection known as infectious bovine rhinotracheitis (IBR), reproductive tract lesions in cows, infectious pustular vulvovaginitis (IPV) and in bulls infectious balanoposthitis (IBP) and new-born systemic infection. The Glycoprotein D is significantly immunogenic and subunit vaccine candidate give protection against BoVH-^{1} and also BoVH-^{5}. The g^{E}-live or killed marker vaccine has proven to be promising in inducing protective immune response and in such animals gE based ELISA are used to differentiate infected animals from vaccinated animals . Analysis of genes of g^{B}/ g^{C}/ g^{D} and g^{E} by PCR plays an important role in diagnosis and cost effective epidemiological studies of the virus (Rashmi *et al.,* 2023).

The antioxidant and micronutrients are plays important roles for body functions especially during transitional period in ruminants and improve feed intake, cell functions, carbohydrates, protein and metabolism. The animal production, reproduction were improved by increasing the immunity. The antioxidant micronutrients change feed intake, nutrient digestibility, rumen fermentation and energy production in ruminants leads to prevent bacterial, viral and fungal infection (Mohammed *et al.,* 2024).

CONCLUSION

Infectious keratoconjunctivitis is a serious problem of ruminants located in hilly areas particulary dry summer season. *Moraxella* infection was confirmed both culture isolation and identification and PCR for the pathogen causing infectious keratoconjunctivitis. In this present study, the prevalence of *Morexalla* species among the cattle, sheep and goats was confirmed by PCR. However, further indepth detailed study needed for developing antibiogram and vaccine for infectious keratoconjunctivitis and further clinical studies needed for selecting systemic and topical antimicrobials agents for complete recovery of affected animals.

Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Abdullah, F.F.J.,Naidu, N.R.G.,Sadiq, M.A., Abba, Y., Tijjani, A., Mohammed, K., Chung, E.L.T., Norsidin, M.J.M., Lila, M.A.M., Haron, A. *et al.* (2015). Prevalence of moraxella ovis infection in goats under the ladang angkat programme, universiti putra malaysia: A cross-sectional study. IOSR Journal of Agriculture and Veterinary Science. 8 (11 Ver. I): 99-102. DOI: 10.9790/2380-0811199102.

Abdullah, F.F.J.,Radzuan, N.S.,Tijjani, A., Adamu, L., Abba, Y., Mohammed, K., Osman, A.Y., Roslim, N., Awang, D.N., Saharee, A.A. *et al.*, (2014). Stage II keratoconjunctivitis in a goat: A case report. IOSR Journal of Agriculture and Veterinary Science. 7: 16-18.

Åkerstedt, J. and Hofshagen, M. (2004). Bacteriological investigation of infectious keratoconjunctivitis in norwegian sheep. Acta veterinaria Scandinavica 45(1-2): 19-26.

Aristimuno, B., Nirankari, V. S., Hemady, R. K. and Rodrigues, M.M. (1993). spontaneous ulcerative keratitis in immunocompromised paients. Am. J. Ophthalmol. 115: 202-208.

Athira, G.R., Chandrasekaran, D., Arunaman, C.S., Senthil, N.R. and Vaiaramuthu, S. (2018). Successful management of keratoconjunctivitis in goats in chennai. Journal of Applied and Natural Science. 10(4): 1196-1198.

Baker, S.E., Bashiruddin, J.B., Ayling, R.D. and Nicholas, R.A. (2001). Molecular detection of mycoplasma conjunctivae in english sheep affected by infectious keratoconjunctivitis. Veterinary Record. 148: 240-241.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pthology. 45:493-496.

Cerny, H.E., Rogers, D.G., Gray, J.T., Smith, D.R. and Hinkley, S. (2006). Effects of moraxella (branhamella) ovis culture filtrates on bovine erythrocytes, peripheral mononuclear cells, and corneal epithelial cells. Journal of Clinical Microbiology. 44(3): 772-776. https://doi.org/10.1128/JCM.44.3.772-776.2006.

Dubay, S.A., Williams, E.S., Mills, K. and Boerger-Fields, A.M. (2000). Association of moraxella ovis with keratoconjunctivitis in mule deer and moose in wyoming. Journal of Wildlife Diseases. 36(2): 241-247.

Ely, V.L., Vargas, A.C., Costa, M.M., Oliveira, H.P., Pötter, L., Reghelin, M.A., Fernandes, A.W., Pereira, D.I.B., Sangioni, L.A. and Botton, S.A. (2018). Moraxella bovis, moraxella ovis and moraxella bovoculi: biofilm formation and iysozyme activity. Journal of Applied Microbiology.126: 369-376. doi:10.1111/jam.14086.

Gelormini, G., Gauthier, D., Vilei, E.M., Crampe, J.P., Frey, J. and Degiorgis, M.P.R. (2017). Infectious keratoconjunctivitis in wild Caprinae: merging field observations and molecular analyses sheds light on factors shaping outbreak dynamics. BMC Veterinary Research. 13: 67. DOI 10.1186/s12917-017-0972-0.

Hidson, J.C. and Winter, A.C. (2008). Manual of Sheep Diseases. The 2nd Ed., Pp 158-162. Blackwell Scientific.

Jansen, B.D., Heffelfinger, J.R., Noon, T.H., Krausman, P.R. and Devos, J.C. (2006). Infectious keratoconjunctivitis in bighorn sheep, silver bell mountains, arizona, USA. Journal of Wildlife Diseases. 42(2): 407-411.

Karthik, K., Manimaran, K., Mahaprabhu, R. and Shoba, K. (2017). Isolation of *Moraxella* sp. from cases of keratoconjunctivitis in an organized sheep farm of India. Open Journal of Veterinary Medicine**.** 7: 138-143. https://doi.org/10.4236/ojvm.2017.710014.

Loy, J.D. and Brodersen, B.W. (2014). Moraxella spp isolated from field outbreaks of infectious bovine keratoconjunctivitis: a retrospective study of case submissions from (2010) to (2013). Journal of Veterinary Diagnostic Investigation. 26(6): 761-768. DOI: 10.1177/1040638714551403.

Maboni, G., Gressler, L.T., Espindola, J.P., Schwab, M., Tasca, C., Potter, L., Castagna de Vargas, A.(2015). Differences in the antimicrobial susceptibility of moraxella bovis, m bovoculi and m ovis. brazilian Journal of Microbiology. 46(2): 545-549. DOI: http://dx.doi.org/10.1590/S1517-838246220140058.

Mohammed, A.A., Al-Suwaiegh, S., AlGherair, I., Al-Khamis, S., Alessa, F., Al-Awaid, S., Alhujaili, W.F., Mohammed, A. and Mohammed, A. (2024). The potential impacts of antioxidant micronutrients on productive and reproductive performances of mammalian species during stressful conditions. Indian Journal of Animal Research. doi: 10.18805/IJAR.BF-1773.

Naglić, T., Hajsic, D., Frey, J., Šeol, B., Busch,K. and Lojkić, M. (2000). Epidemiological and microbiological study of an outbreak of infectious keratoconjunctivitis in sheep. Veterinary Record.147: 72-75.

Ojo, O.E., Oluwole, O.A. and Adetosoye, A.I. (2009). Isolation of moraxella bovis from infectious keratoconjunctivitis in a flock of goats. Nigerian veterinary journal. 30(1): 56-59. DOI: 10.4314/nvj. v30i1.65162.

Pandey, G.S. (2018). An outbreak of infectious caprine kerato-conjunctivitis in a flock of goats-case report. Indian Journal of Animal Health. 57(1): 103-108.

Rashmi, L., Sharada, R., Ratnamma, D., Isloor, S., Chandranaik, B.M., Mohan, H.V., Devi, Y.S.R., Ranganatha, S. and Patil, S.S. (2023). Bovine alphaherpesvirus-1 (BoHV-1) infection in cattle: an overview of epidemiology, role of envelope proteins in disease and control. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-5136.

Sağlam, A.G., Erkiliç, E.E., Büyük, F., Kirmizigül, A.H., Gökçe, G., Balyen, L., Akyüz, E., Aydin, U, Özba, B. and Otlu, S. (2018). Moraxella ovis and mycoplasma conjunctivae isolation from an ovine infectious keratoconjunctivitis outbreak and fortified treatment approaches. Kafkas Univ Vet Fak Derg. 24(4): 551-556. DOI: 10.9775/kvfd.2018.19572.

Van Halderen, A., Van Rensburg, W.J.J., Geyer, A. and Vorster, J.H. (1994). The identification of mycoplasma conjunctivae as an aetiological agent of infectious keratoconjunctivitis of sheep in South Africa. Onderstepoort Journal of Veterinary Research. 61: 231-237.

Zheng, W., Porter, E., Noll, L., Stoy, C., Lu, N., Wang, Y., Liu, X.,Purvis, T., Peddireddi, L., Lubbers, B., *et al.* (2019). Amultiplex real-time PCR assay for the detection and differentiation of five bovine pinkeye pathogens. Journal of Microbiological Methods. 160: 87-92.