



Isolation and molecular identification of *Moraxella ovis* and *Moraxella* spp. from IKC in sheep in India

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**Veterinary Type Cultures Collection,
NRCE, Hisar**

Introduction

- Infectious Keratoconjunctivitis (IKC) is a contagious disease that is common in domestic small ruminants and wild goats (*Caprinae*).
- Domestic sheep can act as reservoir hosts and are the probable source of infections in wildlife, for which the impact of IKC is comparatively higher.
- Tremendous economic losses stem from inappetance and poor weight gain in affected animals suffering from ocular pain and visual impairment.
- IKC has also been associated with decreased twinning rate in sheep, increased case of pregnancy toximia, starvation, weight loss, blind ewes trampling the lambs, and thus decreased economic return.

Contd.

- *Moraxella ovis* have been suspected to be involved in pathogenesis & etiology of Bovine Keratoconjunctivitis also (Cerny et al, 2006).
- Taxonomy of *Moraxella* spp. is in flux however little is known about its biodiversity in India.
- Search of Literature on (*Branhamella*) *Moraxella ovis* returns almost negative reports.
- Two reports from Himachal Pradesh on Large ruminants.
- However, no reports with molecular confirmation and no Indian Type strains of *Moraxella ovis* are available for comparison/study.

Isolation of *Moraxella ovis* and *Moraxella* spp. from keratoconjunctivitis in J & K sheep

- From Gulmarg near LoC at Ayesha post, J & K, samples were collected from a grazing sheep flock of about 200 sheep.
- Significant isolations of *Moraxella ovis* and *Moraxella* spp. from Keratoconjunctivitis in sheep.
- We confirm for the first time, isolation and molecular confirmation of *M. ovis* and *Moraxella* spp. isolates from IKC cases in sheep in India.
- Out of a free-ranging nomadic herd, cases of IKC in sheep were randomly sampled.

Isolation and Biochemical Tests

- Conjunctival swabs for bacterial culture were plated on 5% Columbia blood agar and MacConkey agar.
- Out of twenty isolates 10 oxidase-positive isolates which showed microscopic morphology of Gram-negative cocci in pairs were biochemically processed.
- Three non-motile, nitrate positive, indole negative and non-saccharolytic isolates were tentatively identified as *Moraxella spp.*

Severe conjunctivitis, blepharitis, lacrimation, small foci of corneal opacity developing is seen



Keratoconjunctivitis in Sheep- J & K



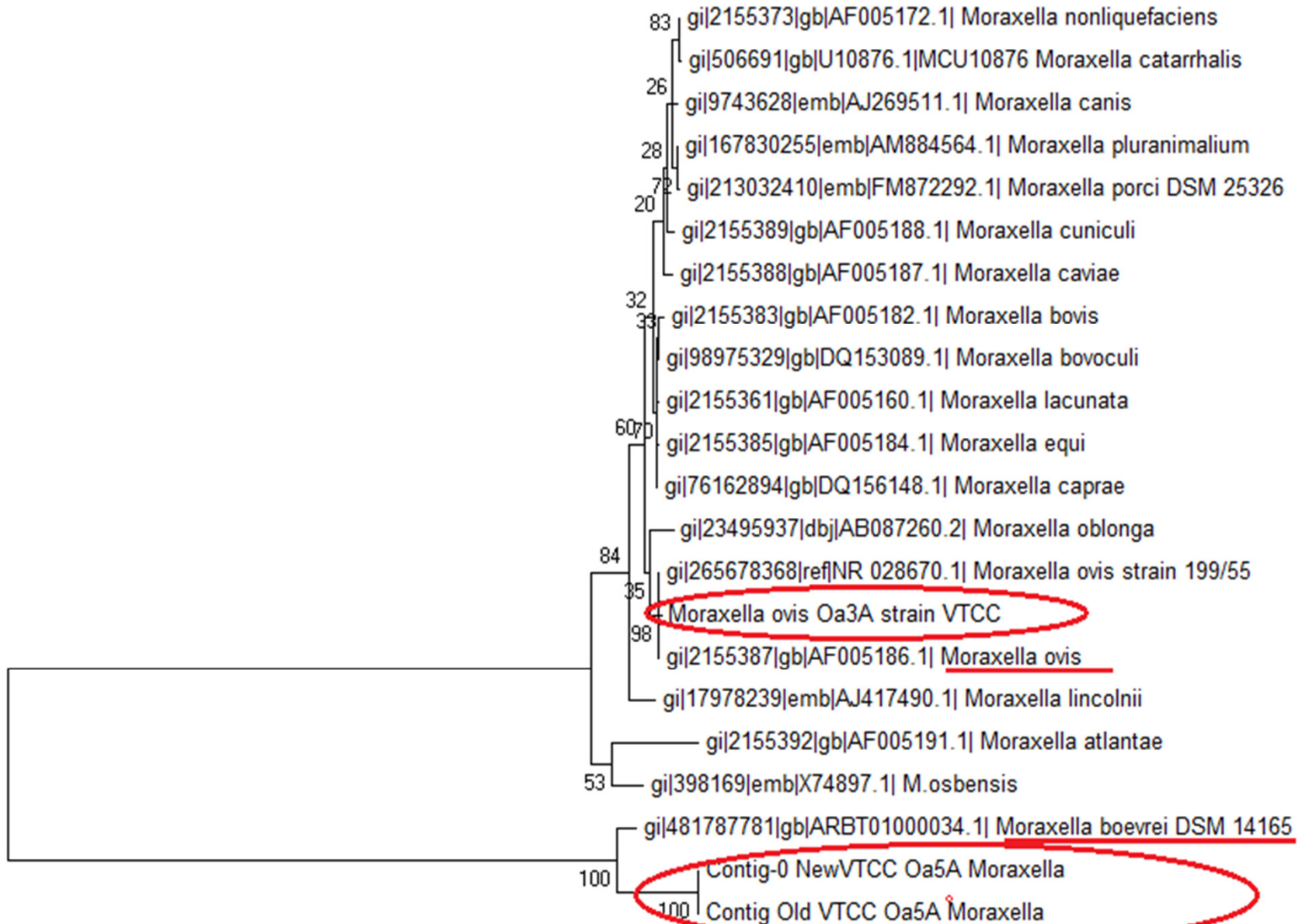
Molecular Identification

- Two isolates subjected to 16S rRNA PCR sequencing.
- Clones of 16S rRNA gene were sequenced to complete >1.5 bp, which was subjected to phylogenetic analysis
- Morphological, biochemical, molecular and phylogenetic evidence identified isolates as *M. ovis* and *Moraxella* spp.
- This isolate with first molecular confirmation of *M. ovis* isolation from sheep in India, and has been accessioned in VTCC repository.
- *Moraxella ovis* VTCCBAA497
- *Moraxella* spp. VTCCBAA498

Phylogenetic Analysis Evidence

- By RDB database analysis sequence showed a sequence similarity of 99.24% with *Moraxella ovis* ATCC33078, and formed a cluster with *M. ovis* strains.
- However the other isolate *Moraxella* spp. clustered close to *Moraxella boevis* DSM14165.
- Sequence alignment of all other *Moraxella* spp. taxa and our 16S rRNA sequences were analysed and P. tree was made.

ML reconstruction of 18 taxa and up to 1453 bp of the 16S rRNA gene

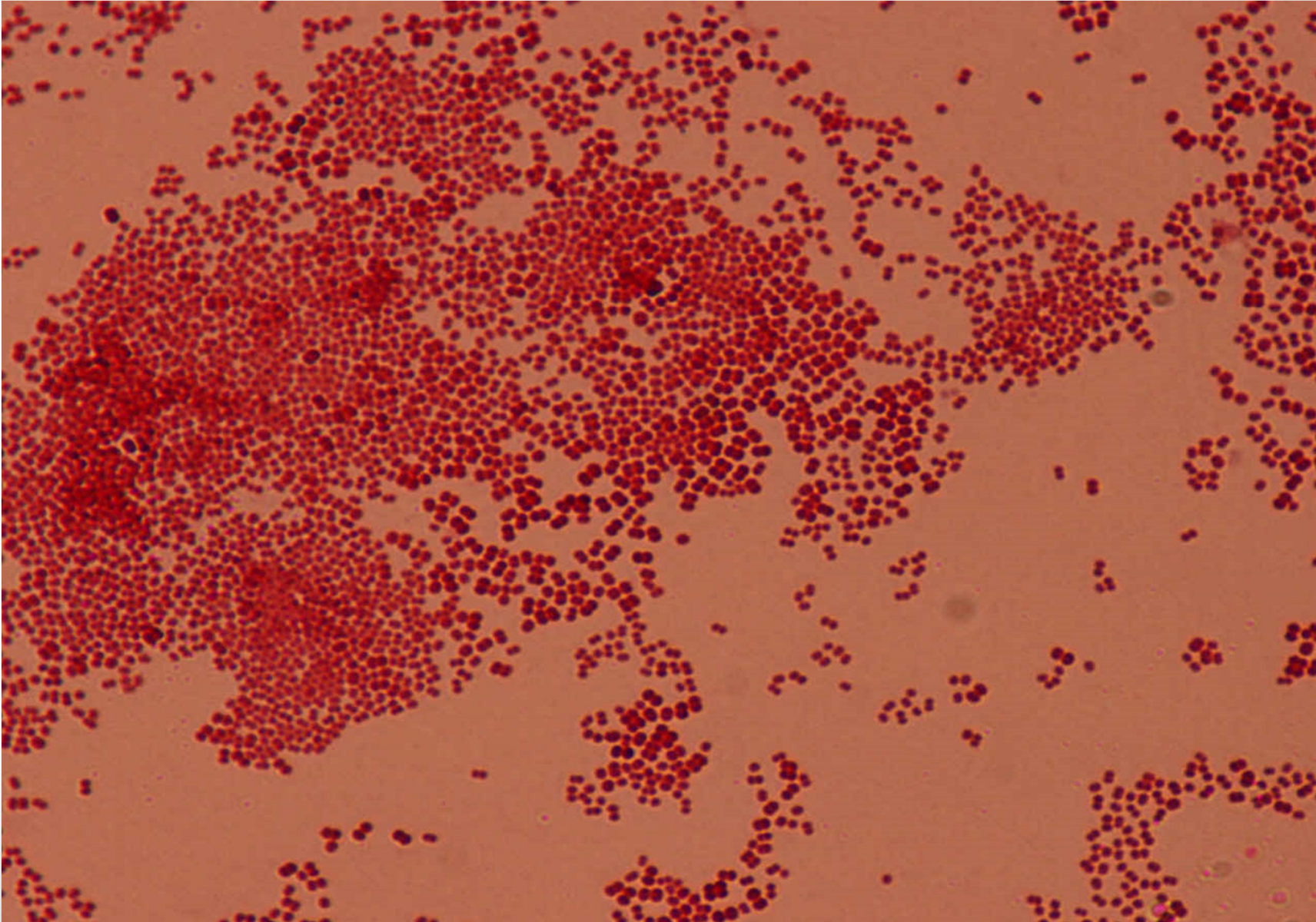


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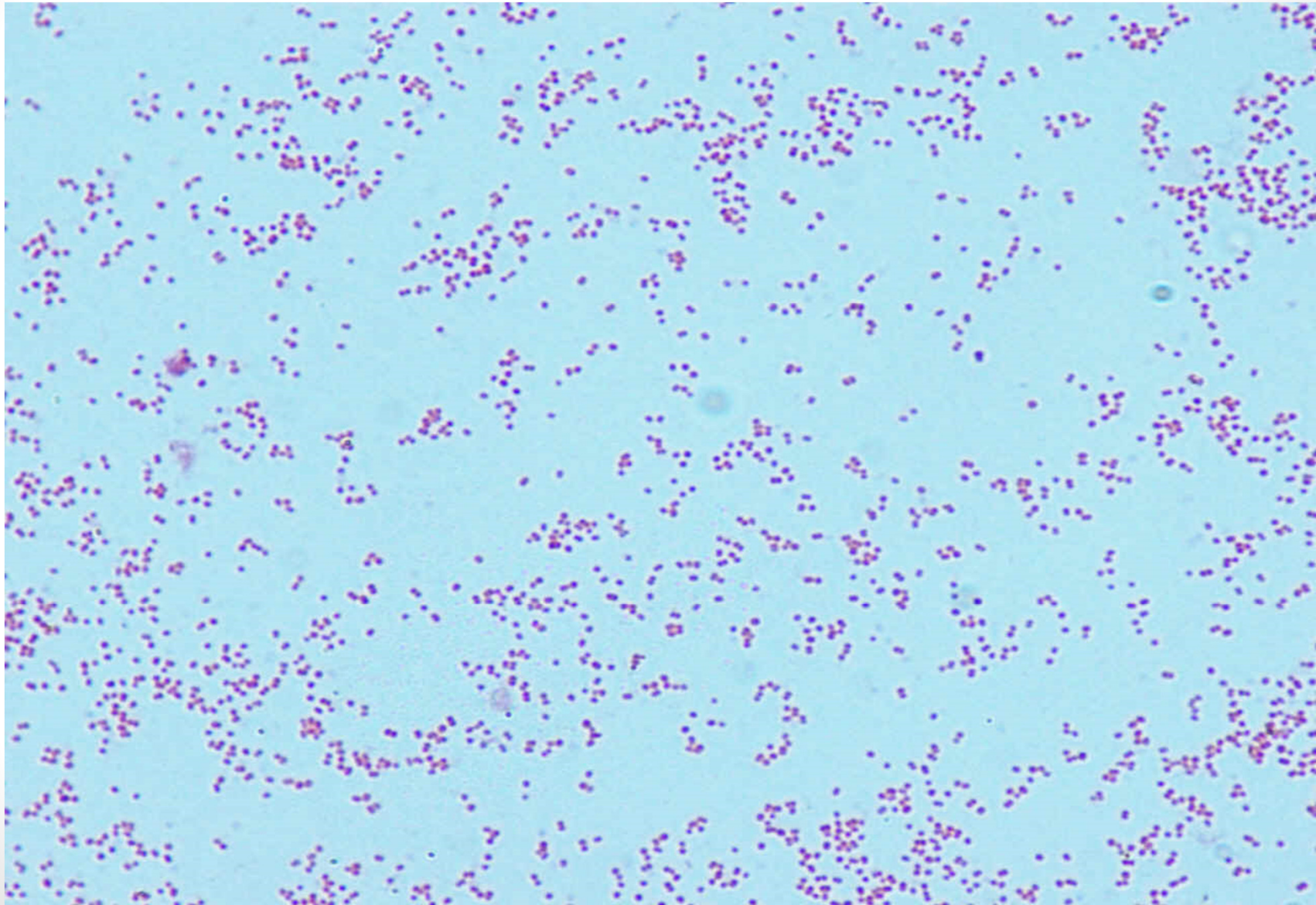
Antimicrobial Sensitivity

- The *M. ovis* isolate was resistant to penicillin, ampicillin, and cloxacillin, 3rd generation cephalosporins (Cefotaxime/ceftazidime) and sulpha drugs (Nitrofurantoin & trimethopirim).
- It was susceptible to sulphafurazole, gentamicin, ciprofloxacin, tetracycline, nalidixic acid, kanamycin, chloramphanicol, amikacin, and tetracycline.
- The *Moraxella* spp. isolate was interestingly not resistant to any of the antimicrobials tested above.

Gram-negative plump cocci of *Moraxella ovis* in pairs



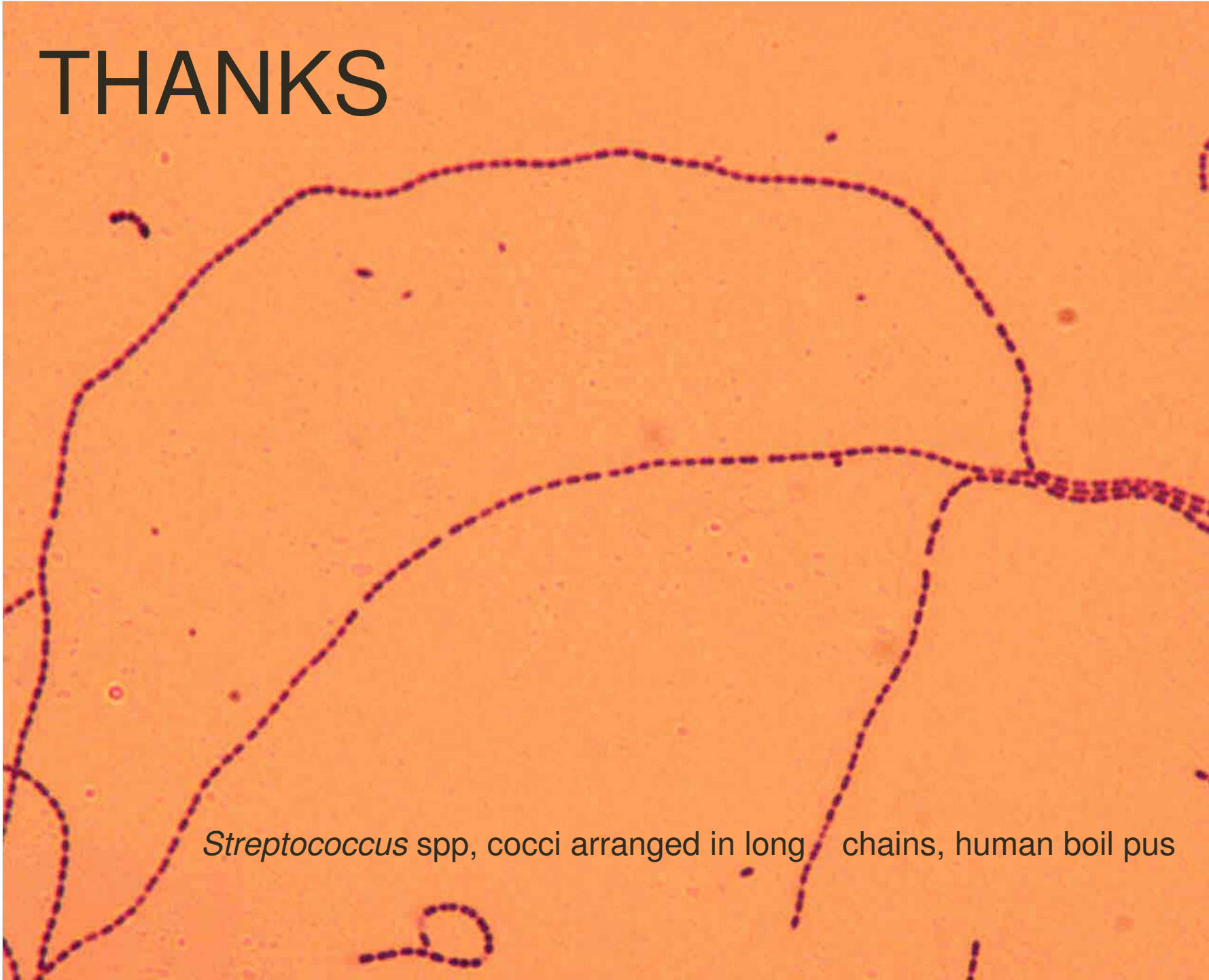
Gram Negative minute cocci of *Moraxella* spp.



Conclusions

- We have demonstrated isolation and identification of *Moraxella ovis* and *Moraxella* spp. from cases of Infectious Keratoconjunctivitis in sheep in India.
- Our results showed presence of *Moraxella ovis* and other *Moraxella* spp. in this part of the country.
- The strains are of clinical significance and are showing antimicrobial resistance.
- We did molecular confirmation of *M. ovis* from IKC cases in sheep.
- Cultures are Accessioned in VTCC and are available for research.

THANKS



Streptococcus spp, cocci arranged in long chains, human boil pus