

Comparison of the microbiota of healthy calf eyes compared to eyes with active pinkeye infection

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Introduction

Pinkeye, also known as infectious bovine keratoconjunctivitis (IBK), is a highly contagious disease, causing inflammation of the cornea and conjunctiva of the eye that can lead to ulcerations and occasionally blindness. This is a multifactorial disease caused by *Moraxella bovis*, *Moraxella bovoculi*, bovine herpesvirus 1 (BHV1), and multiple *Mycoplasma* species. Factors instrumental in causing eye irritation and allowing for infection include excessive ultraviolet light, face flies, wind, plant material, dust, and pollen. Agents involved in pinkeye infection colonize the eyes and nasal cavities of affected animals. Control and prevention of the disease includes vaccination, fly control, environmental management, and antibiotic treatment. The economic impact resulting from pinkeye infections includes decreased weight gain, decreased weaning weight, decreased milk production, and increased treatment costs, which were estimated to be \$150 million in the United States alone in 1993. There are 8 commercially licensed pinkeye vaccines manufactured by 3 companies. These vaccines only include *Moraxella bovis*. There is little to no publically available data supporting the efficacy of these vaccines.

Materials and Methods

Twenty-nine 4 to 5 month old Holstein calves were eye swabbed and their eye microbiota analyzed. Ten of the calves were deemed clinically healthy by a veterinarian and displayed no clinical signs of pinkeye infection. Nineteen of the calves displayed clinical signs of pinkeye including photophobia/light sensitivity, blepharospasms, ocular discharge, corneal opacity, ulceration and corneal neovascularization. Eye swabs were taken using BD Universal Viral Transport Swabs for viruses, *Chlamydia* spp, *Mycoplasma* spp, and *Ureaplasma* spp for culturing and qPCR testing. An additional swab was taken and stored in MTM to preserve the nucleic acids for metagenomics analysis. The swabs were aerobically cultured, cultured for *Mycoplasma* spp, tested by qPCR for bovine coronavirus (BCV), BHV1, bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV),

Influenza type D virus (IDV-B), parainfluenza virus type 3 (PI3), *Mycoplasma bovis*, *Mycoplasma bovoculi*, and the metagenome was sequenced to determine the differences in the microbiota of healthy calf eyes compared to eyes with active pinkeye infection.

Results

The healthy eye swabs cultured were 40% positive for *Moraxella bovoculi* and 20% positive for *Moraxella bovis*; no other significant bacteria was isolated. Swabs were all negative for BCV, BHV1, BRSV, BVDV, and PI3 by qPCR analysis. One swab was positive for IDV-B. All were negative for *Mycoplasma bovis* by qPCR and culturing, 70% were positive by qPCR and culturing for *Mycoplasma bovoculi*; no other *Mycoplasma* spp were isolated.

The active pinkeye infected eye swabs were 63% culture positive for *Moraxella bovoculi* and 58% culture positive for *Moraxella bovis*. Additionally, 1 swab that did not culture either *Moraxella bovis* or *Moraxella bovoculi* was positive for *Pasteurella multocida*. All of the swabs were negative by qPCR for BCV, BHV1, BRSV, BVDV, IDV-B, and PI3. Among the *Mycoplasma* spp, all swabs were positive by qPCR for *Mycoplasma bovoculi* and 89% of the swabs cultured positive; however, they were negative for *Mycoplasma bovis*. One of the swabs cultured positive for *Mycoplasma arginini*.

Metagenomics analysis showed *Moraxella bovoculi* in all samples in addition to *Moraxella bovis*, *Mycoplasma bovoculi*, *Escherichia coli*, *Campylobacter* spp, *P. multocida*, and BVDV in few samples.

Significance

The metagenomic analysis and culture isolation results concur with pinkeye pathogenesis. It is recommended to develop a vaccine for *Moraxella bovoculi*, *Moraxella bovis*, and *Mycoplasma bovoculi*.