Review article

Assessing and tackling IBK problems in dairy herds

John A. Angelos, DVM, PhD, DACVIM (Large Animal)

Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis

Correspondence: jaangelos@ucdavis.edu

Abstract

The purpose of this review is to provide research updates related to the pathogenesis and prevention of infectious bovine keratoconjunctivitis (IBK; pinkeye), and to review commonly recognized IBK risk factors. While there has been some progress made in what we know about the pathogenesis of *Moraxella* species commonly associated with IBK, much is still poorly understood about why *Moraxella* spp. which can be found in eyes of normal cattle, may or may not cause clinical IBK. Unlike beef cow-calf operations, dairy management practices present both challenges and opportunities when considering IBK prevention. Having an understanding of IBK risk factors and applying general principles of disease control can help practitioners sort out IBK problems in dairy cattle and develop more effective IBK control plans.

Keywords

Moraxella bovis; *Moraxella bovoculi*; Infectious bovine keratoconjunctivitis; IBK; pinkeye

Introduction

The most commonly encountered eye disease amongst dairy and beef cattle is infectious bovine keratoconjunctivitis (IBK; pinkeye). Corneal ulceration in a classic IBK case is typically associated with corneal edema, blepharospasm/photophobia, and lacrimation. The clinical course varies depending on severity and may lead to relatively minor corneal scarring with minimal effects on vision to complete blindness in cases of severe corneal damage with or without corneal rupture. A series of review articles published in 2021 cover many aspects of IBK including: case definition and diagnosis,[1] causation,[2], disease prevalence,[3] treatment,[4] prevention,[5] potential risk factors,[6-9] ocular immune responses,[10] and future research directions.[11]

Disease and Risk Factors

Worldwide average prevalence rate estimates for IBK are from 0.88% for *Bos indicus* cattle to 5.1% for *Bos taurus* cattle.[3] In a US study of case records of nearly 42,000 calves over a 20 year period, Herefords were considered to be most susceptible to IBK.[12] Peak incidence is typically associated with warmer months[12] which coincides with commonly recognized IBK risk factors such as flies, ultraviolet irradiation, and mechanical trauma, commonly from plant awns. In addition, trace mineral deficiencies are generally thought to be risk factors as well, particularly copper and/or selenium deficiency. In one study from the UK, a permethrin-based pour-on was compared to permethrin-based insecticide impregnated ear tags for their effectiveness against IBK; in that study no difference was reported between groups suggesting that both treatments were similarly effective at reducing IBK.[13]

Trace minerals such as copper and selenium are considered to have an important role in supporting cattle immune health, and although unproven, supplementation with copper and selenium are generally considered to be important for IBK prevention in animals raised in copper and selenium deficient areas. One in vitro study found that neutrophils from copper deficient cattle had reduced superoxide dismutase and hydrogen peroxide production when compared to neutrophils from copper replete animals, but the phagocytic and bactericidal activities of neutrophils from copper replete and deficient animals were not significantly different.[14] During the course of IBK endogenous anti-inflammatory lipids and hydroperoxyl glycerophospholipids in tear film were reported[15] suggesting that ocular infections associated with IBK may result in lipid peroxidation; if true, trace minerals important in bodily antioxidant functions may be important in a host response to IBK.

The most common bacteria that have been associated with IBK include *Moraxella bovis* (*M. bovis*) and *Moraxella bovoculi (M. bovoculi)*. Roles for non-*Moraxella* organisms in IBK such as *Mycoplasma* spp. and infectious bovine rhinotracheitis (IBR) virus are also often discussed and debated, and recent reviews of these other organisms have been published.[6] The *Mycoplasma* species that has often been associated with IBK is *Mycoplasma bovoculi* and should not to be confused with *Moraxella bovoculi* which is also listed as '*M. bovoculi*' in scientific literature. High-throughput nucleic acid sequencing has added new information on microbial population dynamics in bovine eyes, however, major differences in microbial populations between calves with and without IBK were not observed.[16] Bovine ocular flora has also been assessed longitudinally and these investigations demonstrate that the left eye and right eye ocular microbiome of individual animals are similar and are slow to reestablish after perturbation.[17]

Moraxella bovis

Moraxella bovis has generally been considered to be an important cause of IBK as the disease can be experimentally reproduced with *M. bovis* ocular infections as well as with intracorneal injections of extracts derived from hemolytic *M. bovis*. The hemolytic activity of pathogenic strains of *M. bovis* is linked to expression of an RTX (repeats in the structural toxin) toxin encoded in an RTX operon[18,19]representing a pathogenicity island.[20] Non-hemolytic isolates of *M. bovis* are considered to be nonpathogenic and nonhemolytic isolates that were examined in one study did not have an RTX operon.[20] The host cell receptor that *M. bovis* cytotoxin binds to has not been characterized, however, if it follows the pattern exhibited by other RTX toxins, that receptor is likely a β2 integrin.[21-23] Unlike *M. bovis* pili which are known to be highly variable (see below), *M. bovis* cytotoxin is highly conserved,[24] even amongst the 2 genotypes of *M. bovis* that were recently described.[25]

The pathogenicity of *M. bovis* is also associated with expression of proteins (pili) that allow *M. bovis* to stick to corneal surfaces. Until the recent discovery of multiple genotypes of *M. bovis*[25] there were considered to be only 7 different pilus serogroups in *M. bovis*,[26] however, the ability of *M. bovis* to invert its pilin gene is believed to create antigenic variability and could allow it to evade host immune responses. In vivo switching between pilus forms has been observed and raises the possibility that certain pili are important for colonizing bovine corneal surfaces while other pili types are more involved in keeping bacteria established in and around the eye.[27]

That nonpiliated *M. bovis* are able to stick to different cell types raises the possibility that additional proteins besides pili could be used in adherence to ocular surfaces. Examples include filamentous hemagglutinin[28] which is important for maintenance of other mucosal surface-associated bacteria.[29,30]

Moraxella bovis also expresses numerous other degradative proteins that may be involved in host cell injury,[31-35] as well as iron acquisition proteins that are generally understood to be necessary for bacterial survival. These include transferrin binding proteins[36] and lactoferrin binding protein.[37] Iron binding factors that are likely to be siderophores and other outer membrane proteins (OMPs) are expressed when *M. bovis* grows in a low iron environment.[38]

More recent research has found that changes (shortening) of *M bovis* lipooligosaccharide results in slower in vitro growth, increased susceptibility to certain antibiotics, , and decreased adherence to some cell lines.[39]

Moraxella bovoculi

Moraxella bovoculi was identified in ocular secretions of IBK-affected beef and dairy cattle in the early 2000's.[40] *Moraxella bovoculi* also expresses a hemolytic RTX toxin and has an RTX operon.[41] Based on published studies and further supported by anecdotal experience, *M. bovoculi* is the most frequently isolated *Moraxella* spp. from eyes of IBK-affected cattle[42,43] and its presence has been associated with clinical signs of IBK.[43] Rigorous experimental challenge studies published to date have not demonstrated that *M. bovoculi* can cause corneal ulceration. [44] An *M. bovoculi* pilin gene has recently been characterized, however, unlike *M. bovis* pili, it demonstrates high sequence similarity across geographically diverse isolates.[45] It is currently not known whether or not an *M. bovoculi* pilin is important in adherence on the corneal/ocular surface. An *M. bovoculi* lipooligosaccharide has also been described.[46]

Moraxella bovis and M. bovoculi can form biofilms[47,48] and disruption of *M. bovis* pili with magnesium chloride prevents biofilm formation.[47] Lysozyme also appears to negatively affect formation of biofilm.[48] For *M. bovis*, biofilm formation imparted greater tolerance to antibiotic exposure.[47]

Two genotypes are known to exist amongst *M. bovoculi*[49,50] where genotype 1 is associated with IBK-affected cattle and genotype 2 is associated with the nasopharynx of cattle without IBK. Genotype 2 strains lacked RTX-toxin and antibiotic resistance genes. These findings suggest that interspecies recombination occurs in *M. bovoculi* and results in high genetic diversity amongst *M. bovoculi*.[49] Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) used by diagnostic laboratories for bacterial species identification can differentiate between *M. bovoculi* genotypes as well as *M. bovoculi* possessing RTX toxin operons.[51,52] The increasing availability of whole genome sequence data for *Moraxella* species associated with eyes of cattle[50,53,54] should provide many new avenues for investigations into the pathogenesis of these organisms in cattle.

Three new *Moraxella* spp. were recently characterized: *Moraxella oculi* (isolated from a conjunctival swab of a cow with IBK),[55] *Moraxella nasibovis* (from the nasal cavity of a cow with respiratory disease),[56] and *Moraxella nasovis* (from sheep with respiratory disease).[56] None of these *Moraxella* spp. exhibited hemolysis.

IBK treatment

The efficacy of *Bdellovibrio* spp., a predatory bacterial species, against *M. bovis* has been evaluated in calves that were experimentally infected with *M. bovis*; this therapy was not effective.[57] Based on results of a review and meta-analysis of antibiotic efficacy studies published between 1996 and 2016, it is clear that antibiotic treatments can be effective in treating IBK.[58] One study of a non-antibiotic therapy for IBK evaluated hypochlorous acid (Vetericyn Plus™ Pinkeye Spray); results showed that it decreased pain and healing time in calves experimentally infected with *M. bovis*.[59] The role of iodine on growth of *M. bovis* in tear fluid has been evaluated in kelp-fed dairy cows; in that study bacterial growth in vitro in tears was not inhibited as a result of feeding kelp.[60] An aminoglycoside resistance gene has been identified in an *M. bovoculi* isolated from an IBK-affected steer in Nebraska,[61] however, the practical implication of this is not clear considering expected low levels of aminoglycoside treatment in cattle in the USA. Nanocapsules containing cloxacillin are reported to have efficacy in treating IBK.[62] Eyepatches used in conjunction with oxytetracycline and flunixin meglumine improved healing rates of steers with naturally occurring IBK.[63]

Vaccine research

Most randomized controlled field trials that have been published evaluating autogenous *M. bovoculi*,[64] autogenous *M. bovis*,[65] commercially available *M. bovis*,[66] and conditionally licensed *M. bovoculi* vaccines[67]have not been reported to be effective at preventing IBK in the herds where these vaccines were tested. One recent study that evaluated 3 vaccine treatments (an autogenous vaccine comprised of antigens from *M. bov*is, *M. bovoculi*, and *Mycoplasma bovoculi*; a commercial *M. bovis* vaccine; and an adjuvant-only control) over a 5-year period in Nebraska in ~1200 calves reported a numerically lower (but not significantly lower) cumulative incidence of IBK in the autogenous combination vaccinated calves.[68] A recently published study in beef calves in northern California evaluated an experimental intranasal vaccine based on recombinant *M. bovis* cytotoxin adjuvanted with Carbigen® (MVP adjuvants, Phibro Animal Health, Omaha NE USA) versus an adjuvant control.[69] In that study cytotoxin vaccinates had lower metrics of disease severity compared to animals in the adjuvant control group.

Identifying bacteria associated with IBK

When diagnosing possible infectious organisms involved in cattle with IBK a variety of factors should be considered: the numbers of animals and which animals to sample; sampling site on the eye (cornea versus subconjunctival cul-de-sac); whether special specimen handling media are required and what a particular diagnostic lab needs for different testing methods (e.g. MALDI-TOF, standard aerobic culture; mycoplasma culture; molecular diagnostics); and whether isolates should be saved for possible use in autogenous vaccine formulations. With molecular diagnostic methods it is possible to identify *M. bovis*, *M. bovoculi*, *Mycoplasma bovis*, *Mycoplasma bovoculi*, and bovine herpesvirus type 1 (BHV-1) from eye samples.[70]

Tackling IBK problems in dairy herds

In making IBK prevention recommendations it is important to consider multiple factors such as: reducing dust and potential foreign bodies that might cause mechanical eye injury leading to IBK; fly control; possible associations with bedding types; and trace mineral supplementation. If making a vaccine recommendation it is also important to get a good history regarding previous use of IBK vaccines (product type (commercial vs autogenous); bacterial organisms covered in previously used vaccines; and timing of vaccination (it is generally recommended that a vaccine series be initiated at least 4 weeks before the expected IBK onset in a particular group or herd). Evaluation of these aspects of history as well as knowledge of risk factors can help practitioners in designing/changing IBK control plans.

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