DAIRY VETERINARY NEWSLETTER

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Pinkeye – Is it Getting Worse? Is There Yet Another Causative Organism (a Mycoplasma) Involved?

Pinkeye. Historically a summer problem, the conjunctivitis and keratoconjunctivitis of calves - or sometimes cows or bulls - was a disease that I remember well from when I first was in veterinary practice in Indiana. Humid, hot summer, tall grass or weeds, dust and flies are among the contributing causes of pinkeye. However, sometimes none of those factors are obvious and there can still be outbreaks of pinkeye. I certainly treated a lot of calves with subconjunctival injection of penicillin or penicillin and dexamethasone, and then we glued eye patches on them. I have always found it interesting that many animals with pinkeye have one eye that looks worse than the other, which is fortunate if one wants to use eye patches. After you applied the patches, tried to let the glue dry, and then let the calf go, sometimes the patches were rubbed off pretty quickly, but usually they would wear off or be removed by the producer only after the passing of a few weeks, and often the eye was healed up pretty well. A colleague of mine used to say that besides protecting the eyes from light, flies, dust, etc., the eyes were out of sight of people so nobody worried about them until the patches came off. This was often a successful plan of treatment. The same eye patches are in use today. Even more than 30 years ago, pinkeye vaccine was widely used to attempt to prevent the disease complex (see more below).

Pinkeye can cause rupture of eyeballs, permanent cone-like projection of the cornea, or permanent bluish-white corneal opacity resulting in blindness or poor eyesight. Many cases just cause considerable discomfort to, and poor performance by, the affected animals for several weeks or more.

During summer 2013 I have seen more cases of, and heard more about pinkeye in dairy calves than I have in some time. A veterinarian I know told me recently that a large dairy heifer raising facility he runs, as well as many dairy farm clients he works for, have each dealt with hundreds to thousands of cases of pinkeye this summer. And the perception is that it is getting worse.

The classical cause of pinkeye is *Moraxella bovis*, a Gram-negative bacterium from the family *Moraxellaceae*, described for more than 60 years. It is an opportunistic pathogen found in tears and nasal secretions of clinically normal cattle, including those with no history of pinkeye. Transmitted by mechanical vectors including face flies, it has no known other reservoir species besides cattle. The length of time per day that bovine eyes are exposed to UV radiation is positively associated with susceptibility of the cornea to infection. Together with more long grasses, dust, and flies, this helps explain why pinkeye is more prevalent during warm summer weather. (White faced cattle, especially with Hereford breeding, are considered more susceptible not only because they have no eyelid pigmentation, but Hereford genetics results in less antibacterial property of tears compared with other cattle breeds.) An excellent review of *Moraxella bovis* is

found in Postma et al., Comp Immunol Microbiol Infect Dis, 2008. Many calves vaccinated with pinkeye bacterins containing subunits of *Moraxella bovis*, usually pilin and/or cytotoxins of the bacteria, which have been available and widely used for decades, still contract pinkeye. One factor contributing to this observation is antigenic variation in pilin among different strains of *Moraxella bovis*, but all identified strains of *Moraxella bovis* to date have antigenically conserved cytotoxin.

***Moraxella bovoculi*, a more recently reported cause of pinkeye**

First reported by Angelos et al., Int J of Systematic and Evol Microbiol, 2007, a newly described species of bacteria was isolated from dairy and beef calves with pinkeye in 2002, *Moraxella bovoculi*, a Gram-negative coccus. *Moraxella bovoculi* was isolated from pinkeye cases either alone or in combination with *Moraxella bovis*. The same investigative group studied 101 Angus, Hereford, or Angus-Hereford crossbred beef calves from 5 to 7 months old, all with no signs of pinkeye at onset of the study, from a herd with a historically high incidence/prevalence of pinkeye keratoconjunctivitis. Calves were randomized into 3 blocks and treated with either adjuvant alone, *Moraxella bovis* cytotoxin bacterin, or *Moraxella bovis* cytotoxin and pilus bacterin; each group had 33 or 34 animals but the exact totals were not reported. Eyes were examined weekly for 18 weeks, naturally occurring corneal ulcers were stained with fluorescein dye and scored using a standard scoring system, and swabs from ulcerated eyes were cultured for etiologic bacteria.

Study results were reported in Angelos et al., Vet Microbiol 2007. After 9 wk, 24% to 33% of each group had corneal ulcers; after 18 wk, 35% to 53% of each group had corneal ulcers, all not significantly different (P = 0.37 to 0.54). Proportion of calves requiring at least one pinkeye treatment (adjuvant alone was not the highest) ranged from 29% to 44%, also not significantly different (P = 0.39 to 0.70). Times until onset of first ulcer or until corneal healing were also not different among the treatment groups. However, despite statistical non-significance, it was interesting to me that *Moraxella bovoculi* was the only isolate from 4/11 (36%) of positive eye cultures from the adjuvant alone group, but it was the only isolate from 19/25 (76%) of positive eye cultures from the two *Moraxella bovis* vaccinated groups. Of the calves contracting pinkeye keratoconjunctivitis despite *Moraxella bovis* bacterin immunization, most had *Moraxella bovoculi* infections.

The same research group studied a new *Moraxella bovoculi* bacterin made from its cytotoxin carboxy terminus. In a similar study to that above, 127 Angus, Hereford, or Angus-Hereford crossbred beef calves from 5 to 7 months old, again only calves with no signs of pinkeye at onset of the study, were randomized into 2 groups and administered either adjuvant alone or the bacterin. Eyes were examined weekly for 15 weeks and corneas were scored and eye swabs were cultured as in the previous study.

Results were reported in Angelos et al., Vet Res Commun 2010. After 8 wk, 40% of controls and 37% of vaccinates had corneal ulcers; after 15 wk, 52% and 45%, respectively had corneal ulcers, all not significantly different (P = 0.69 and 0.43, respectively). Times until onset of first ulcer or until corneal healing were also not different among the treatment groups. As might be expected, from the 61 initial ulcers, *Moraxella bovis* was isolated from 24/29 (83%) of *Moraxella bovoculi* vaccinates' ulcers, but from only 20/32 (63%) of controls' ulcers. These proportions of *Moraxella bovis* isolation approached statistical significance (P = 0.09).

The same veterinarian I know with the heifer raising facility recently told me that their practice has used *Moraxella bovis* bacterins, and made and used bacterins containing *Moraxella bovoculi* in addition. In comparisons of large groups of calves of equal size, they observe a trend toward reduced pinkeye, but there is still a high prevalence of pinkeye regardless of bacterin used. The above study results and anecdotal reports suggest that if any etiologic agents of pinkeye are included in bacterins administered to calves, the

proportion of pinkeye cases caused by those bacteria may be reduced, but other agents "take up the slack" and the overall prevalence of pinkeye is not reduced when conditions are right for keratoconjunctivitis. How is it that bacterins combining antigens, which are immunogenic, against both *Moraxella bovis* and *Moraxella bovoculi*, are still not sufficient to make significant reductions in the incidence/prevalence of pinkeye in calves? Is there yet another important etiologic agent of pinkeye? The answer may at least partly lie in the form of a pathogen that many veterinarians including myself consider something "new" that we have not heard of before, but that has actually been described for many years. However, as a cause of pinkeye it has not attracted much attention until recently (see below).

Is there yet another "new" bovine pinkeye causative bacteria?

It now appears that another cause of keratoconjunctivitis in calves is *Mycoplasma bovoculi*, a mycoplasma bacterium first reported in 1973 by Langford and Leach. A major literature search I did looking for "mycoplasma" in many species just a couple of years ago when helping with a review paper did not turn up this organism, and I was not familiar with it. It has only been studied sporadically for the last 40 years, and has not been considered a major cause of keratoconjunctivitis in calves; indeed some studies have suggested that it did not cause the disease even when inoculated into the eyes of calves. It was also reported in some other studies to produce milder keratitis than the other agents discussed above. In 1992, ter Laak et al. reported in Zentralbl Veterinarmed B that a study of 270 cows and 35 calves from 27 herds found *Mycoplasma bovoculi* as one of the most common mycoplasmas among many *Mycoplasma* spp. that were tested for in the nares of asymptomatic animals, found in nasal swabs from 23% of calves and 20% of cows with no clinical signs of disease.

However, more recently there are reports of outbreaks of pinkeye keratoconjunctivitis where the major isolate from calves' eyes was *Mycoplasma bovoculi*, including some where no other causative agents of pinkeye were detected. Therefore, while this is not a newly classified bacteria, it has only recently attracted much attention as possibly being an important component of the pinkeye keratoconjunctivitis disease complex.

Speculation is growing that inclusion of pilin and cytotoxin antigens from *Moraxella bovis*, cytotoxin antigens from *Moraxella bovoculi*, and antigens from *Mycoplasma bovoculi* into one three-agent bacterin may be needed to help reduce the incidence/prevalence of pinkeye during outbreak conditions. However, whether other opportunistic pathogens will simply take advantage of increased UV radiation, dust, long grass and face flies, as has been the case so far with those above, and therefore cause pinkeye remains to be seen.

Environmental and pest control conditions remain vitally important

One of the dairy farms I visited this summer that had major pinkeye problems in calves was a well run farm that had good overall sanitation and management. Nevertheless, their calves that were approximately 2 to 5 months old had all been moved because of overcrowding to a back corner pen. There was stacked up old manure, weeds growing out of some of the manure and bedding in the stack, and a large population of face flies under the only roof providing shade to the pen. Many calves also had substantial ringworm lesions. The calves largely had to spend their time in the sun during the day. The calves had all been vaccinated with *Moraxella bovis* bacterin. We discussed the importance of the environmental factors and that bacterin would not overcome them. The calves were growing well and did not appear in poor overall health except for the pinkeye and ringworm; this was not an "abuse" situation. The farm I referred to above was really quite clean and a great place to show anyone how dairy cows can and should be taken care of. Yet imagine if a school group or some other components of society were to observe that pen of calves and their eyes. It is important to note that pinkeye problems this summer are not confined to calves housed in situations like this.

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However, the extensive pinkeye associated with the above conditions reinforces the point that shade, good bedding and reduction of dust, mowing or other control of tall grass or weeds, and of course face fly control are important to reducing pinkeye. Given the ubiquitous nature of the pinkeye etiologic agents we already know about in bovine eyes and tears, this will always remain true.

I have always enjoyed summer. We used to say in practice that the longer daylight hours extended the typical practice day, but we still liked it. My summer schedule at Utah State always gets busier with farm visits, summer projects, etc. than during any other season, by design. I hate to see it end. This year as the weather cools off and what has been an exceptionally hot and dry summer comes to a close, one bonus will hopefully be the end of pinkeye season, sooner than later. Of course there is considerable evidence now that hot, dry summers and increasing UV light exposure per year are becoming more common. Perhaps the triple-bacterin for pinkeye that is being proposed will become an important tool in the future. Management of the environment and flies is sure to continue to be. I hope our readers are enjoying the summer and will also enjoy the coming of fall, ripe tomatoes, calving, back to school, football, etc.

Please let us know your comments and also suggestions for future topics. I can be reached at (435) 760-3731 (Cell), (435) 797-1899 M-Tues, (435) 797-7120 W-F or David.Wilson@usu.edu.

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