Bovine Pinkeye—Etiology and Pathogenesis

Richardo F. Rosenbusch, *D.V.M., Ph.D.*
Veterinary Medical Research Institute
Iowa State University
Ames, Iowa 50011

Summary

Significant advances have been made in the clarification of the etiology and pathogenesis of bovine pinkeye. The well established role of *Moraxella bovis* as a causative agent of the disease is now further clarified by an understanding of predisposing causes commonly involved in this disease. Of importance are ocular mycoplasmal infections, particularly by *Mycoplasma bovoculi* which clearly enhance the colonization of cattle eyes by *Moraxella bovis.* Dual infections are always seen in natural cases of the disease, and further, dual experimental infections will reproduce pinkeye, even if low doses of these organisms are deposited on the unaltered corneas of experimental calves. Additional requirements to produce pinkeye experimentally are the use of strains of *M. bovis* expressing adhesive pili and a surface-bound hemolysin. *In vitro* models and mouse pathogenicity models developed to study these phenomena add to our knowledge of the pathogenesis of this disease.

General considerations

Bovine pinkeye has long been known as an infectious disease of summer months, caused by *Moraxella bovis* infections (2). Difficulties in consistently reproducing the condition by inoculation of cattle with cultures of this organism (2), and lack of success in immunization attempts (3, 4) have led to widespread speculation as to the involvement of other agents either as primary or associated causes of the disease (5). Association of conjunctivitis with uncultivable organisms that resembled rickettsias is noted in early reports (6), but it isn't until 1969 that mycoplasmas are shown to present the large numbers in cattle eyes (7). Since this first report, investigators have documented the presence of several species of mycoplasmas in bovine eyes (8, 9, 10, 11, 12). A newly described species, *M. bovoculi* is found to be the predominant mycoplasma in cattle eyes and is found to have a wide geographical distribution. Studies performed in Iowa, show that this mycoplasma is responsible for the production of a seasonal conjunctivitis (13) distinct from the keratoconjunctivis characteristic of bovine pinkeye.

Conjunctivitis as a clinical entity

Epizootic conjunctivitis was studied in a survey of 19 Iowa farms during the springs of 1979 and 1980. As shown in Table 1, conjunctivitis could be associated with the presence of *M. bovoculi* in those farms. Another species of mycoplasma, *Ureaplasma diversum,* was found with lower frequency, but always assciated with *M. bovoculi* infections.

TABLE 1. Survey of 19 Iowa Farms for Organisms Involved in Pinkeye.

		Organisms recovered		
Herd clinical	Nr farms evaluated	M. bovoculi	U. diversum	Pathogenic M. bovis
Normal	3	0	0	0
Conjunctivitis	11	11	4	1
Pinkeye	5	5	2	5

In contrast, pinkeye was associated with the presence of pathogenic *Moraxella bovis.* This infection was superimposed to mycoplasmal infections, and as before, *M. bovoculi* was the predominant mycoplasma. Herds with normal eyes (no cattle with conjunctivitis or keratitis) were a minority in this survey, and generally were small dairy operations kept as closed herds. In this survey, a complete study was also made of the bacterial and viral flora found in cattle eyes. Most cattle eyes contained a limited bacterial flora and could frequently yield swab samples free of aerobic bacteria. In healthy cattle, viruses could not be detected in eye swab samples. Cattle with clinical signs of respiratory disease commonly yielded eye swab samples with IBR, BVD, PI-3 and RSV singly or in combination. Bovine adenoviruses or chlamidia were not detected in these herds and could not be responsible for the conjunctivitis outbreaks. These results are similar to those reported in Danish studies (14), but are in contrast to others reported from Australia (15), where bovine adenoviruses were described as playing a major role. Besides the 2 species of mycoplasma mentioned above, other mycoplasmas considered apathogenic were isolated sporadically in our surveys. Mycoplasmas associated with bovine respiratory disease were commonly isolated from the eyes of cattle with respiratory disease (see Table 2).

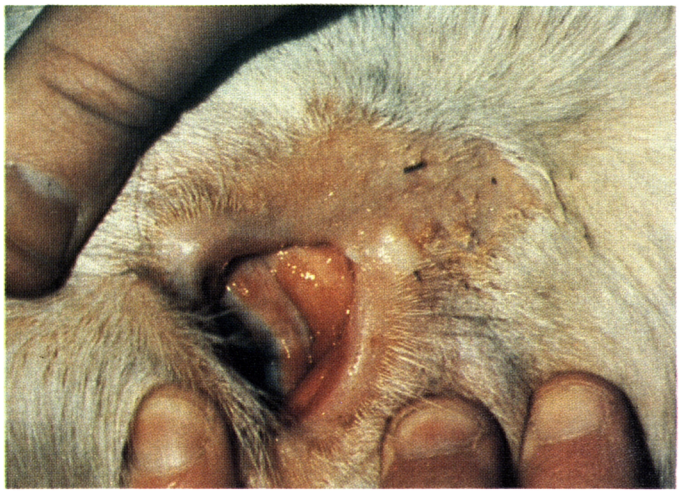
TABLE 2. Mycoplasmas Isolated from Bovine Eyes.

Mycoplasma species	Clinical condition
Mycoplasma bovoculi	Conjunctivitis
Ureaplasma diversum	Conjunctivitis
Mycoplasma verecundum	Conjunctivitis
Mycoplasma bovis	Respiratory disease
Mycoplasma bovirrhinis	Apathogenic
Acholeplasma laidlawii	Apathogenic
Acholeplasma oculi	Apathogenic

Clinically, conjunctivitis appeared usually in early spring during calving. Newly born calves had healthy eyes, but developed a watery conjunctivitis 5 to 7 days after birth. These calves remained with conjunctivitis through the summer and fall, the secretions becoming serous and forming small yellow crusts on the medial canthus of the eyes (see Figure 1). Many calves showed normal eyes during late winter, especially if they remained in the same farm. Adult cattle also showed signs of conjunctivitis, with a peak in early spring and clearing of the symptoms by winter. Older cows were resistant or showed only minimal signs.

When pure cultures of *M. bovoculi* or *Ureaplasma diversum* were instilled into the conjunctival sacs of healthy calves, conjunctivitis followed within 3 to 7 days after infection (13). All cattle infected with *Mycoplasma bovoculi* had abundant mycoplasmas tightly adhering to conjunctival epithelial cells. These could be shown by fluorescent antibody staining of conjunctival scrape-smears (see Figure 2). Mycoplasmas also adhered to the corneal surface, but did so in lower numbers. Recovery of *Mycoplasma bovoculi* was also possible from the nasal cavity, pharnyx and tonsils of infected cattle, but never from regional lymphnodes, trachea, bronchi or the genital tract.

FIGURE 1. Calf with mycoplasmal conjunctivitis. Congestion and serous to purulent secretions.



Keratitis models

It became apparent to us that it was crucial to determine what interactions occurred between this newly described mycoplasmal conjunctivitis and classical pinkeye. A calf model was developed where calves were inoculated by instillation of pure *M. bovoculi* cultures or cultures of other select mycoplasmas isolated from bovine eyes. After 6 days, when mycoplasmal colonization was maximal, calves were inoculated by the instillation of *Moraxella bovis* cultures (16). Results of these experiments are shown in Table 3. All calves were raised in individual isolators from birth and were

FIGURE 2. Conjunctival epithelial cells obtained by scraping. Fluorescent antibody staining shows numerous adherent **Mycoplasma bovoculi.**

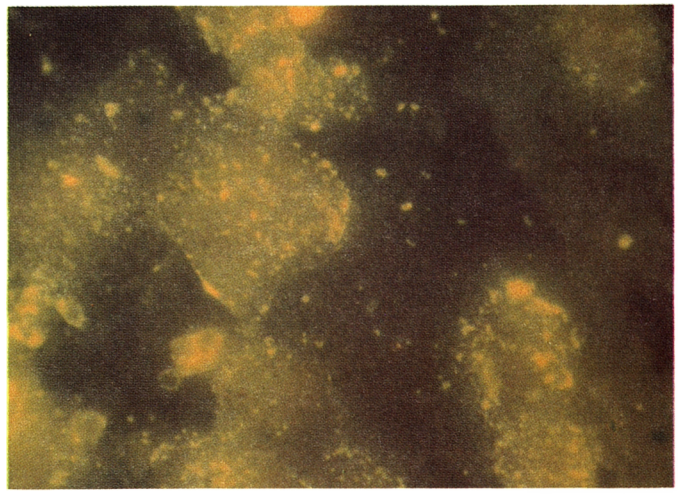


TABLE 3. Colonization of **Moraxella bovis** and Development of Keratitis in Calves.

Challenge	Nr of calves	Keratitis Nr of calves	Keratitis Nr of eyes	Nr of calves with M colonization 7d	Nr of calves with M colonization 21d
M	4	0	0	3	1
U+M	6	0	0	4	2
B+M	6	6	10	0	6
b+M	2	0	0	1	1

M **Moraxella bovis,** strain 118F, 10^6 CFU/eye (colony forming units/eye)
U **Ureaplasma diversum,** strain U233, 10^7 CCU/eye (color changing units/eye)
B **Mycoplasma bovoculi,** strain FS8-7, 10^8 CCU/eye
b **Mycoplasma bovis,** strain 79-27784, 10^8 CCU/eye
+M Calves were inoculated with M six days after mycoplosma inoculation

repeatedly tested to ensure their mycoplasma-free status. Control calves left free of mycoplasmas could not colonize *Moraxella bovis* and did not develop pinkeye. Calves infected with *Mycoplasma bovis* or *Ureaplasma diversum* responded as did the mycoplasma-free controls. Calves preinfected with *M. bovoculi* permitted extended colonization by *Moraxella bovis* and all (6 out of 6) developed pinkeye. These early experimental results were corroborated in further testing, and careful field observations in several herds showed the same picture, an early spread of mycoplasmal conjunctivitis followed by introduction of *Moraxella bovis* and concomitant pinkeye.

The *Moraxella bovis* strain used for experimental infection was capable both of expressing pili and hemolysin in culture. All field isolants of *Moraxella bovis* obtained from pinkeye outbreaks also had these characteristics (17, 18, 19). With an *in vitro* adherence model using intact bovine corneas, we could demonstrate that pilus expression was

required for adherence of *Moraxella bovis* to the cornea; nonpiliated mutants could not adhere (20). Hemolysin activity could be associated to a membrane protein of *Moraxella bovis* (21), and infection of cattle eyes resulted in a rapid and easily detectable serological response against this hemolysin. It could be shown that even in outbreaks where only a low percentage of cattle were affected by pinkeye, *Moraxella bovis* infection rapidly spread to all animals in the group after introduction by carrier cattle, as judged by serological response to the hemolysin (22).

To further understand the role of pili and hemolysin, a mouse model was developed and used on various strains of *Moraxella bovis*. When inbred C57/B16 mice had their corneas scarified and infected with piliated and hemolytic *Moraxella bovis*, keratitis was reproduced in all mice. Bacteria defective in either pilus or hemolysin expression could not produce the disease, confirming that the mouse model was measuring the *Moraxella bovis* virulence factors involved in bovine pinkeye.

Conclusions

Practitioners need to be alert to the signs of mycoplasmal conjunctivitis, even though treatment to eliminate the mycoplasmal infection has not been successful and is not recommended at this time. Conjunctivitis by itself does not always lead to pinkeye, introduction (or spread from carrier cattle) of *Moraxella bovis* is needed to produce pinkeye in *M. bovoculi* infected herds. Presence of *Moraxella bovis* should be suspected in all animals of a herd with pinkeye, whether or not certain animals exhibit clinical signs or even yield ocular samples culturally negative for the organism. In addition to face fly transmission of the disease, attention should be placed on the role of carrier cattle in introduction of *Moraxella bovis* to herds.

References

1. Henson, J.B., Grumbles, L.C. 1960. Infectious bovine keratoconjunctivitis. I. Etiology. Am. J. Vet. Res. *21*:761-766. 2. Pugh, G.W., Hughes, D.E. 1971. Infectious bovine keratoconjunctivitis induced by different experimental methods. Cornell Vet. *61*:23-45. 3. Hughes, D.E., Kohlmeier, R.H., Pugh, G.W., Booth, G.D. 1979. Comparison of vaccination and treatment in controlling naturally occurring infectious bovine keratoconjunctivitis. Am. J. Vet *Res.*40:241-244. 4. Webber, J.J., Selby, L.A. 1981. Effects of *Moraxella bovis* vaccination schedules on experimentally induced infectious bovine keratoconjunctivitis. Am. J. Vet. Res. *42*:1181-1183. 5. Wilcox, G.E. 1968. Infectious bovine keratoconjunctivitis. A review. Vet. Bull. *38*:349-360. 6. Coles, J.D.W.A. 1936. A rickettsia-like organism of the conjuctivial epithelium of cattle. J.S. Afr. Vet. Med. Assoc. *7*:221-225. 7. Langford, E.V., Dorward, A.J. 1969. A mycoplasma isolated from cattle with infectious bovine keratoconjunctivitis. Can. J. Comp. Med. *33*:275-279. 8. Gourlay, R.N., Thomas, L.H. 1969. The isolation of large colony and T-strain mycoplasmas from cases of bovine keratoconjunctivitis. Vet. Rec. *84*:416-417. 9. Gourlay, R.N., Leach, R.H., Howard, C.J. 1974. *Mycoplasma verecundum*, a new species isolated from bovine eyes. J. Gen. Micro. *81*:475-484. 10. Jack, E.J., Moring, J., Boughton, E. 1977. Isolation of *Mycoplasma bovis* from an outbreak of infectious bovine kerato-conjunctivits. Vet. Rec. *101*:187. 11. Goto, K., Hashimoto, K., Kishima, M. 1979. Isolation of mycoplasmas from infectious bovine kerato-conjunctivitis. Bull. Azabu Vet. Coll. *4*:211-217. 12. Kelly, J.I., Jones, G.E., Hunter, A.G. 1983. Isolation of *Mycoplasma bovoculi* and Acholeplasma oculi from outbreaks of infectious bovine keratoconjunctivitis. Vet. Rec. *112*:482. 13. Rosenbusch, R.F., Knudtson, W.U. 1980. Bovine mycoplasmal conjunctivitis: experimental reproduction and characterization of the disease. Cornell Vet. *70*:307-320. 14. Friis, N.F., Pedersen, K.B. 1979. Isolation of *Mycoplasma bovoculi* from cases of infectious bovine keratoconjunctivits. Acta Vet. Scand. 20:51-59. 15. Wilcox, G.E. 1970. The aethiology of infectious bovine keratoconjunctivitis in Queenland. 1970. 2. Adenovirus. Austr. Vet. J. *46*:415-420. 16. Rosenbusch, R.F. 1983. Influence of mycoplasma preinfection on the expression of *Moraxella bovis* pathogenicity. Am. J. Vet. Res. *44*:1621-1624 17. Pedersen, K.B., Froholm, L.O., Bovre, K. 1972. Fimbriation and colony type of *Moraxella bovis* in relation to conjunctival colonization and development of keratoconjunctivitis in cattle. Acta Pathol. Microbiol. Scand. (B) *80*:911-918. 18. Henson, J.B., Grumbles, L.C. 1968. Infectious bovine keratoconjunctivitis. III. Demonstration of toxins of *Moraxella (Hemophilus) bovis* cultures. Cornell Vet. *51*:835-839. 19. Pugh, G.W. Jr., Hughes, D.E. 1968. Experimental bovine infectious keratoconjunctivitis caused by sunlamp irradiation and *Moraxella bovis* infection: correlation of hemolytic ability and pathogenicity. Am. J. Vet. Res. *29*:835-839. 20. Jackman, S.H., Rosenbusch, R.F. 1984. *In vitro* adherence of *Moraxella bovis* to intact corneal epithelium. Curr. Eye Res. *3*:1107-1112. 21. Ostle, A.G., Rosenbusch, R.F. 1984. *Moraxella bovis* hemolysin. Am. J. Vet. Res. *45*:1848-1851. 22. Ostle, A.G., Rosenbusch, R.R., 1985. Immunogenicity of *Moraxella bovis* hemolysin. Am. J. Vet. Res. *46*:1011-1014. 23. Berkebile, D.R., Hall, R.D., Webber, J.J. 1981. Field association of female face flies with *Moraxella bovis*, an etiological agent of bovine pinkeye. J. Econ. Entomol. *74*:475-477.