General Sessions

Moderators: Del Miles, Sheila McGuirk, Daryl Nydam

Transmission of *Mycoplasma bovis* and the Syndromes that Result in Beef and Dairy Cattle

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Abstract

Infections with *Mycoplasma bovis* can result in several syndromes of importance for dairy and beef cattle of all ages. These diseases continue to require the attention of practitioners due to poor response to treatment and the marginal effectiveness of prophylaxis. We explore here parameters of transmission of the infections, and how practitioners can use this knowledge to develop management strategies. General concepts about transmission of the mycoplasma are followed with specific description of several different mycoplasma mastitis clinical presentations. Issues with transmission and mastitis are outlined. Pneumonia and polyarthritis, and middle ear infections in dairy calves are described next and transmission of infection differences are highlighted. Examples of presentations of pneumonia and polyarthritis in beef calves are presented together with discussion of some of the transmission questions that are commonly raised when considering this disease.

Introduction

Infections with *Mycoplasma bovis* will present themselves with several well-defined clinical syndromes. All of these can potentially be seen in dairy or beef cattle, yet several of the syndromes will preferentially be seen in specific age groups of beef or dairy cattle. Due to the infectious nature of these syndromes, modes of transmission as well as susceptibility of the cattle population at risk dictate which syndrome will be frequently encountered. Here we explore these modes of transmission, and correlate them with specific susceptibility attributes of dairy and beef cattle of various age groups.

Transmission of *M. bovis* is affected by several variables. Cattle are usually susceptible to infection by multiple routes, although the infectious dose of agent needed to establish infection may vary widely among portals of entry. It is accepted that 100 cfu (colony forming units) of *M. bovis* can initiate a nasal or mammary infection,[1] while 10^6 cfu or more are needed to set up a lower respiratory tract infection, and much higher doses for systemic infection by the oral route.[10] Contagiousness of the infection is determined by the capacity of the pathogen to replicate at the portal, time-delay in becoming a contagious carrier, and duration of the contagious stage. Nasal infection will need five days to reach peak infectivity at 10^8 cfu/ml, and shedding will continue for several months. Mammary infection will need 2-3 days to reach peak levels of 10^9 cfu and shedding in secretions will continue for the duration of lactation, although it is intermittent after the first month.[7] Dry quarters may shed mycoplasma (milking claw applied in error), and flare-ups at freshening are seen in many cows. Thus, natural or disease-enhanced dissemination of fomites from the colonized portal play a major role in successful contagion. In addition, environmental contamination is presumed to occur as deduced from seeding trials,[12] although there is lack of well-documented evidence of its occurrence in the field. Transmission of the infection must also be contrasted with clinical presentation. It is known that the proportion of cattle infected with *M. bovis* well exceeds that of clinically affected cattle, and in some cases, infection is documented without clinically relevant presentations (the over-diagnosis problem).[5]

Depending on the portal of entry, dissemination of mycoplasma from the portal may or may not be necessary before clinical signs ensue. This dissemination can occur by normal host defense mechanisms (migration of mycoplasma-loaded dendritic cells and macrophages) and/or by specific virulence attributes of the pathogen.

As occurs with other pathogenic mycoplasmas, virulence factors of *M. bovis*, while inferred to exist, are still poorly defined. In Table 1, several strains of *M. bovis* with diverse pathogenic potential are compared in a model of intratracheal infection in naïve weaned dairy calves. When a dose of 10^{10} cfu (colony forming units) is presented at the tracheal bifurcation, some strains will rapidly establish mycoplasmemia and febrile response . Strains may also destroy the tracheal escalator and not allow recovery from the nose in this model. All evidence to date suggests that even though these variations in virulence exist among strains of *M. bovis*, specific types of strains are not associated with specific syndromes.[13] Rather, any strain can cause each syndrome, with more or less severity, depending on the presence and strength of expression of these yet unknown virulence factors. Following are descriptions of the more common syndromes seen with *M. bovis* infections in cattle.

Mastitis

Mastitis caused by *M. bovis* will be seen in both dairy and beef cattle. In dairy cows where the problem is most commonly reported, several forms can be described. Acute mastitis associated with herd expansion is seen infrequently although it is a costly presentation. A midwest dairy herd of 130 cows was commingled with 180 purchased cows, and introduced into a new milking parlor. Within a week, a few cows were reported with single non-secreting quarters. Rapid progression to full agalactia followed, and affected cows were febrile. By 15 days, there were 12 cows with severe mastitis. These cows were from the home herd as well as the purchased herd, and in both early and late lactation. Affected cows appeared to respond to antibiotic treatment with slight recovery of milk secretion, then relapsed with severe mastitis, and most of these also presented polyarthritis involving knees, hocks and shoulder joints, as well as signs of pneumonia. Culture from affected quarters milk yielded *M. bovis*, and segregated milking of all abnormal milk cows was instituted. At one month of commingling there were 20 cows dead or salvaged, and bulk tank samples from all "clean strings" tested negative for mycoplasma. At three months, composite samples from all cows in the hospital pen yielded 25% culture-positive cows, and the entire hospital group was culled. At 3.5 months, bulk tank samples from the "healthy group" tested positive for *M. bovis* and it was decided to culture all remaining cows individually. The five culture-positive cows identified were also culled. Overall losses (from all causes) by four months were 115/310 cows, or 37% of initially lactating cows. Bulk tank samples taken at 5, 6 and 7 months after commingling were negative. Serology done on all remaining cows at 6 months after commingling revealed that 70% were seropositive, evenly dispersed among the home and purchased herds. No samples were taken to determine transmission patterns in this outbreak, although the ELISA serology data clearly indicated that some of the cows in the home and purchased herd were initially naïve to *M. bovis* infection, and remained so throughout the episode. In our hands, ELISA serology will detect exposure within 2-3 weeks, such as nasal colonization, even in absence of disease. Nose-to-nose transmission here presumably evolved independent of mammary infection. Transmission between quarters and among udders would have occurred primarily through milking parlor activity. Nose-to-udder transmission could have accounted for the initial cases, and for cases at 3.5 months in the "healthy group".

Recurrent mastitis can be seen in herds that have recovered from acute mycoplasmal mastitis. These herds will undergo three or more episodes a year,[2] each one involving some cows that have mild alterations in milk secretion (abnormal quantity of flecks). These are often freshening cows, and the infection may not be detected if sampling is only done from the bulk tank. The herds often have increasing SSC and many cows with environmental mastitis. It is a good idea to review treatment protocols in farms with recurrent mycoplasmal mastitis. Eliminate the practice of compounding mastitis treatment tubes!

Heifer mastitis occurs in herds that bring in bred replacement heifers from commercial sources. These heifers present with mastitis that is resistant to treatment at freshening, and they can be a source of infection for the herd. In these frequent situations, all fresh cows and all clinically affected cows need to be cultured for mycoplasma and kept separate from the main herd until results are at hand. Positive cows need to be culled.

Table 1. Intratracheal pathogenicity of *Mycoplasma bovis* strains for naïve 8 to 12-week-old Holstein calves.

Strain	No. of calves	Clinical signs		Culture positive from			Lung lesion score
		Fever	Arthritis	Nose	Blood	Joints	
M23	6	6/6	0/6	0/6	6/6	1/6	2.4±1.2
M45	4	1/4	0/4	3/4	0/4	0/4	0.5±0.2
428E	5	2/5	1/5	5/5	1/5	1/5	14.4±4.7

Separating hospital and fresh cows into multiple strings can reduce mycoplasma transmission among them.

Mycoplasma mastitis diagnosis will often only be done at the *Mycoplasma* genus level (whether culture or PCR is used). These incomplete diagnostic results may add more questions than they answer for the practitioner. In Table 2, mycoplasma species known to cause bovine mastitis are listed, together with their common presentations and transmissibility. Two species of mycoplasma are involved in acute outbreaks and recurrent mastitis presentations of significant severity. These are *M. bovis* and *M. californicum*, with the former being the most frequent. Both species are highly transmissible. Other species are not as transmissible to udders, and some are highly transmissible but not very pathogenic. These characteristics need to be taken into account when analyzing species-specific diagnostic results, clinical presentations and sampling practices.

Pneumonia and Polyarthritis in Dairy Calves

With the increasing size of modern dairies has come the practice of feeding discard milk to calves. The practice has shown economic advantages, as well as improved weight gain and health status among calves.[6] Discard milk from mycoplasma shedders can cause explosive presentations in calves.[4] Calves fed contaminated milk will present with pneumonia within two weeks of birth, and may also exhibit polyarthritis starting at three weeks. Large dairies that feed discard milk will routinely pasteurize the product, so that these outbreaks are now associated with pasteurizer malfunction, or post-pasteurization recontamination. The latter can occur in calf ranches that purchase pasteurized discard milk from multiple sources. Transmission in outbreaks is by oral route, and it has been shown that in very young calves *M. bovis* can establish intestinal infection after oral infection. This leads to septicemia and infection of multiple organs, in addition to pneumonia and polyarthritis.[8] The infectious dose needed for these oral transmissions is not known precisely, but it is presumed that it is much higher that what is needed for nasal or mammary infection.[10] Of note, colostral anti-mycoplasmal antibody, although usually plentiful, is not protective against infection.

Transmission concerns can have important economic repercussions. A Professional Heifer Association farmer has been raising dairy heifers and bulls from commingle sources. A bull calf presented with pneumonia and polyarthritis at six weeks of age. A joint tap sample was used to provide a positive diagnosis of *M. bovis*. Shortly after, several heifer calves presented with nasal discharge, bronchitis and a few had head tilt. The farmer had serology done on all calves at entry to the farm, and calves were all seropositive for *M. bovis*. Confronted with buyers reluctant to purchase heifers from him, he claimed that *M. bovis* was only present in the bull calf. The example serves to illustrate that commingle operations raising dairy heifers are a significant risk for dairy farms. The risk can presumably be reduced or controlled by raising these heifers in small isolated groups, and testing nasal swab samples for *M. bovis*, rather than relying on serology. Dairy farms that receive heifers from an integrated operation that does not commingle are at much lower risk of introducing a strain of *M. bovis* of higher virulence than the resident strain found in the noses of their cows.

Middle Ear Infections

Middle ear infections with *M. bovis* can be seen in calves of all ages, and also in cows, including dairy and beef cattle. However, reports of high incidence outbreaks are primarily associated with large dairy heifer farms, dairy beef calf farms, or veal farms. The condition is observed in either bucket or nipple-fed newborn calves. Calves present with conjunctivitis within one week of arrival, and then with droopy ears starting at two weeks. Calves evolve to bilateral otitis media and head tilt, become febrile and pneumonic. The conditions are re-
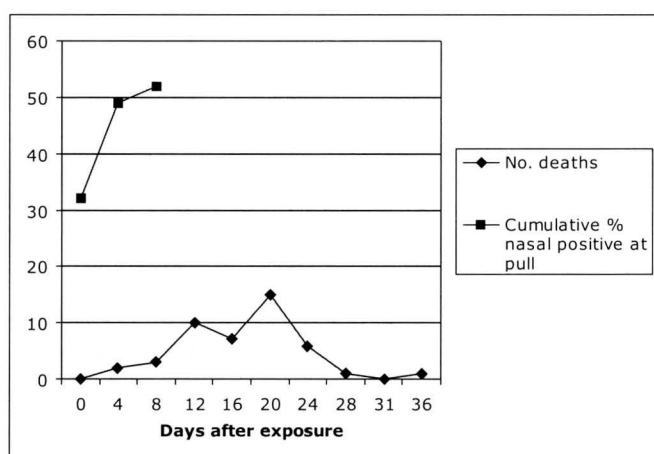
Table 2. Mycoplasmas causing bovine mastitis: clinical signs and transmission.

Mycoplasma species	Occurrence	Clinical signs	Transmission
M. bovis	Very frequent	Mastitis, arthritis, pneumonia	High
M. californicum	Frequent	Mastitis, arthritis, pneumonia	High
M. bovigenitalium	Frequent	Mastitis	Low
M. alkalescens	Infrequent	Mastitis	Low
M. canadense	Sporadic	Mastitis	Very low
M. dispar	Sporadic	Mastitis	Low
M. sp. serogroup 7	Frequent	Mild mastitis	High
Acholeplasma sp.	Sporadic	Mild mastitis	High
M. arginini	Sporadic	Mild mastitis	High

sistant to antimicrobial treatment unless started very early. Also, response to vaccination is generally poor. Transmission is by oral route when a calf is fed contaminated colostrums, or during prolonged and commingled transportation to rearing farms. It has been observed that in very young calves, infection by any route results in elevated colonization by mycoplasma in tonsils and pharyngeal lymphoid tissues. This may be due to preferential homing of activated macrophages to these sites, and these cells will carry live mycoplasma on their surfaces. Local immunosuppression will result from direct killing[15] or inhibition of lymphocytes in these tissues. These calves develop very high concentrations of *M. bovis* in the pharynx and ascending infection of middle ears is presumed. All outbreaks of middle ear infections in young dairy calves have been associated with *M. bovis* to date.

Pneumonia and Polyarthritis in Stocker Calves

Pneumonia and polyarthritis caused by *M. bovis* can be seen infrequently in pre-weaned beef calves, and then under conditions of high stress and exposure to infection (forced commingling as occurs in dry lot calving).[3] Most occurrences of pneumonia and polyarthritis are seen in stocker calves purchased from order buyers and then shipped long distances. Recent studies document that calves from Southeastern states arrive at small stocker operations in these same states naïve to *M. bovis*. Commingling of susceptible calves with a few infected calves then leads to rapid dissemination of the infection. In Figure 1, rapid nasal infection is demonstrated among calves at arrival to feedyard after they have been commingled during transport with a known infection source. This can set up an acute presentation, where clinical manifestations are seen as early as two weeks after arrival, and the disease course is completed by 4-6 weeks. The following is an example of one such outbreak.

A midwestern lot received 200 light beef calves averaging 450 lb that were shipped directly from a western ranch. Shipment occurred in early November in a snowstorm. After processing and vaccination with multivalent inactivated vaccines, the calves were treated uneventfully for respiratory disease during the first 10 days. Shortly after, some calves became lame, and by three weeks 25% of them were lame, febrile, had clear nasal secretions and presented moist, occasional coughing. The calves were pneumonic and did not respond to antimicrobial treatments. Lame calves had swollen hocks and knees. Several of them had subcutaneous swellings on their backs that were edematous on palpation, and a few of them were ulcerated, draining serous exudates. Several of the affected calves lost condition rapidly, even while continuing to eat, and 15 of them eventually died. On necropsy, severe bronchopneumonia was seen, with numerous small coagulative necrosis lesions disseminated throughout the lungs. *M. bovis* was recovered from noses of affected and healthy calves and from lung lesions. Immunohistochemistry proved that the small coagulative necrosis lesions had *M. bovis* antigen accumulations. All deaths were reported from the third to the sixth week after arrival. At seven weeks, a group of healthy home-raised calves were placed in nose-to-nose contact across the fence with the affected calves group. No disease was noted in this group of calves, and nasal swab samples were obtained from them at 12 weeks (from receipt of the calf shipment). Isolates of *M. bovis* obtained from the lungs and noses of affected calves were of a single genomic fingerprint, proving that a single introduction of infection was made.[3] The healthy home-raised calves yielded two different genotypes of *M. bovis* from their noses, the one associated with the outbreak as well as a different one. Even though polyarthritis was a hallmark of this case, with 25% of calves becoming lame, the number of calves with pneumonia was estimated to be around 85%. Overall, only 20% of *M. bovis* outbreaks present with lameness[14] while therapy-resistant pneumonia with only moderate loss of appetite are the common signs. Similar presentations in heavier feedlot calves are less frequent than in stockers, and mortality is low or nil.

There are reports of delayed-onset pneumonia and polyarthritis in stockers. In these, the first sick calves are seen 2-3 months after arrival, and the disease courses over a one month period. There are no known reasons for this delayed onset. It is speculated that this

Figure 1. Number of dead calves and cumulative percentage nasal positive to *M. bovis* for 3-day intervals starting on designated day after exposure. Sampling done at the feedyard. A load of 720 calves was shipped from order buyer premises with two positive shedder calves per truck.

could occur if the calves mounted a short-lived immune response against *M. bovis* due to exposure or vaccination at commingling. In avian mycoplasma infections, delayed onset outbreaks are associated with environmental exposure.[9] Potentially, this could also occur in beef operations. Since there is increasing use of vaccination with *M. bovis* bacterins in stocker calves, the influence of vaccination on nasal shedding should be explored. Experimental data would support the notion that vaccination will not prevent nasal colonization,[11] although field evaluation of the effect of vaccination on nasal colonization or shedding is not available.

References

1. Boothby JT, Jasper DE, Thomas CB: Experimental intramammary inoculation with *Mycoplasma bovis* in vaccinated and unvaccinated cows: effect on the mycoplasma infection and cellular inflammatory response. *Cornell Vet* 76: 188-197, 1986.

2. Bushnell RB: Mycoplasma mastitis. *Vet Clin North Am Large Anim Pract* 6: 301-312, 1984.

3. Butler JA, Pinnow CC, Thomson JU, Levisohn S, Rosenbusch RF: Use of arbitrarily primed polymerase chain reaction to investigate *Mycoplasma bovis* outbreaks. *Vet Micro* 78: 175-181, 2001.

4. Butler JA, Sickles SA, Johanns CJ, Rosenbusch RF: Pasteurization of discard mycoplasma mastitic milk used to feed calves: thermal effects on various mycoplasma. *J Dairy Sci* 83: 2285-2288, 2000.

5. Ghadersohi A, Fayazi Z, Hirst RG: Development of a monoclonal blocking ELISA for the detection of antibody to *Mycoplasma bovis* in dairy cattle and comparison to detection by PCR. *Vet Immunol Immunopathol* 104: 183-193, 2005.

6. Godden SM, Fetrow JP, Fiertag JM, Green LR, Wells SJ: Economic analysis of feeding pasteurized nonsaleable milk versus conventional milk replacer to dairy calves. *J Am Vet Med Assoc* 226: 1547-1554, 2005.

7. Gonzales RN, Wilson DJ: Mycoplasmal mastitis in dairy herds. *Vet Clin North Am Food Anim Pract* 19: 199-221, 2003.

8. Maeda T, Shibara Y, Kimura K, Wada Y, Sato K, Imada Y, Ishikawa Y, Kadota K: *Mycoplasma bovis*-associated suppurative otitis media and pneumonia in bull calves. *J Comp Pathol* 129: 100-110, 2003.

9. Marois C, Savoye C, Kobisch M, Kempf I: A reverse transcription-PCR assay to detect viable *Mycoplasma synoviae* in poultry environmental samples. *Vet Micro* 89: 17-28, 2002.

10. Maunsell F, Donovan A, Risco C, Hernandez J, Brown M: Field evaluation of a *Mycoplasma bovis* bacterin in young dairy calves. 15th Congress of the International Organization for Mycoplasmology, Athens, GA, 2004, p. Poster 184.

11. Nicholas R, Ayling R, Stipkovits L: An experimental vaccine for calf pneumonia caused by *Mycoplasma bovis*: clinical, cultural, serological and pathological findings. *Vaccine* 20: 3569-3575, 2002.

12. Pfutzner H: Tenacity of *Mycoplasma bovis*. *Zbl Bakt Hyg A* 258: 38-41, 1984.

13. Poumarat F, Solsona M, Boldini M: Genomic, protein and antigenic variability of *Mycoplasma bovis*. *Vet Micro* 40: 305-321, 1994.

14. Spire M, Sargent J, Blasi D, Rosenbusch RF: Kansas survey of Mycoplasma in stockers. *Bovine Veterinarian* May-June: 12-16, 2002.

15. Vanden Bush TJ, Rosenbusch RF: *Mycoplasma bovis* induces apoptosis of bovine lymphocytes. *FEMS Immunol Med Microbiol* 32: 97-103, 2002.