Persistence of *Mycoplasma bovis* in the Mammary Gland of Naturally Infected Dairy Cows

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Abstract

Observations on persistence of *Mycoplasma bovis* intramammary infections, the effect of the infections on milk production in the current lactation and in subsequent lactations, and the risk of *M. bovis* infected cows housed and milk together with infected cows, were made on 4 New York State dairy herds with outbreaks of mycoplasma mastitis. The dairy management at each farm decided to maintain several infected cows in the herd, milking them either last or with a separate milking unit, because a majority of the cows had either high milk production or were registered with a high genetic value. The results of their decisions plus implications for dairy practitioners will be discussed in this presentation.

Introduction

Bovine mycoplasmal mastitis has been persistently diagnosed in the State of New York since it was first detected in 1963.[1] Mycoplasma mastitis usually appears in dairy farms as an acute syndrome, although chronic and subclinical forms have been frequently seen. It is unlike most other acute forms of mastitis because it lacks systemic signs. Control of bovine mycoplasmal mastitis has traditionally relied on detection of infection by bacteriological culture of milk samples, and subsequent culling of infected animals.[2,3] Dairy farmers often ask veterinarians questions like "If a mycoplasma cow is always a mycoplasma cow, will milk production be regained after the clinical episode or in the next lactation?" and "How dangerous is it to keep mycoplasma cows in the same environment with uninfected animals?" Thus, the objectives of this study were to follow up a group of cows diagnosed as having *Mycoplasma bovis* mastitis in the current lactation through the dry period and subsequent lactations to determine presence of *M. bovis* in their milk; the effect of *M. bovis* udder infection on milk production in the current lactation and in subsequent lactations; and, the risk of *M. bovis* infected cows for uninfected cows housed and milked together with infected cows.

Material and methods

Four commercial cooperator dairies (A, B, C, D) that had a mean of 90, 320, 95 and 180 milking cows, respectively, were included in the study. On Dairy A, 4 of 5 first lactation cows with intramammary infections (IMI) from which *M. bovis* was isolated, were studied for 2 consecutive lactations and 2 nonlactation periods. Five consecutive composite milk samples were obtained from the affected cows on a monthly basis during the second lactation while in the third lactation, three cows were sampled every day for 4 to 6 months. Four samples of mammary secretions from each cow were obtained during each of the 2 nonlactation periods. On Dairies B and C, 8 of 40 and 3 of 7 cows, respectively, with *M. bovis* IMI were studied for a whole lactation. Seven consecutive composite milk samples were obtained from the affected cows on a monthly basis after detection of IMI. On Dairy D, 13 of 18 cows with *M. bovis* IMI were studied for 2 consecutive lactations. Between 7 and 15 consecutive milk samples were obtained from the cows on a monthly basis.

For isolation of mycoplasma, 0.1 ml of each milk sample was spread onto a whole plate of a modified Hayflick agar medium and 3 ml of milk samples were inoculated into 3 ml of modified Hayflick broth, and recultured on Hayflick agar at 2, 4, and 6 days of incubation. Mycoplasma plates were incubated at 37°C in 10% CO_2 in a moist chamber and examined at 3, 5, 7 and 10 days for mycoplasmal growth. If no colonies were observed after 10 days, the culture was diagnosed as negative. Mycoplasmas were speciated by an indirect immunoperoxidase test[4] using blank susceptibility test disks soaked with antiserum for each species of mycoplasma known to cause mastitis in cows[5], and antiserum for *Acholeplasma laidlawii*.

Results

After detection of *M. bovis* IMI, affected cows in Dairies A, B, and C were always milked last while management on Dairy D elected to milk mycoplasma cows with a separate milking unit.[6,7]

Dairy A (90 cows): One cow affected with a pneumonia-mastitis-arthritis syndrome died.[6] Of the 4 animals that were studied during their second lactation,

1 cow was culled due to low milk production (although it always cultured negative for *M. bovis*), 2 cows apparently eliminated the *M. bovis* IMI during the first dry period, and the remaining cow always shed *M. bovis* intermittently. The organism was consistently isolated from this cow during the two dry periods and in the first 5-15 days of the two studied lactations. In contrast, during the remainder of both lactations, *M. bovis* was not isolated from the infected cow for intervals in the 2-20 day range. For those 3 cows, mean milk production per cow/lactation was 9,500 kg. Two annual whole herd surveys and monthly bulk tank milk cultures showed that the remaining cows in the herd were mycoplasma-free.

Dairy B (320 cows): Thirty-two cows were culled due to either low milk production or high somatic cell counts (SCC). Of the 8 animals that were followed up, 4 cows apparently eliminated the *M. bovis* IMI during the dry period, and the remaining 4 cows always shed *M. bovis* intermittently. However, these infected cows were seldom positive on consecutive monthly samplings. For those 8 cows, mean milk production per cow/lactation was 8,200 kg. A whole herd survey and monthly bulk tank milk cultures showed that the remaining cows in the herd were mycoplasma-free.

Dairy C (95 cows): Four cows with *M. bovis* IMI ceased milk production and were culled. *Mycoplasma bovis* was isolated once from the 3 remaining cows during the dry period and they shed the organism a few times in the milk during the monthly samplings. Mean milk production per cow/lactation was 8,400 kg. Monthly bulk tank milk cultures were always negative for mycoplasma organisms.

Dairy D (180 cows): Five cows were culled due to either low milk production or high SCC (>6,000,000 cells/ml).[7] Of the 13 animals studied in this herd, 2 cows were culled due to low milk production (although they always cultured negative for *M. bovis*), 9 cows apparently eliminated the *M. bovis* IMI during the first dry period, and the remaining 2 cows, shed the organism only twice during the samplings. For those 11 cows, mean milk production per cow/lactation was 8,650 kg. A whole herd survey showed that the remaining cows in the herd were mycoplasma-free.

Discussion

Although severe mycoplasmal mastitis herd outbreaks have been described in New York and elsewhere,[1,2] often related to blitz therapy against *Streptococcus agalactiae*,[1] low morbidity has been the case during the last 10 years in the State of New York. In the herds studied, outbreaks were limited to 5.5% (Dairy A), 12.5% (Dairy B), 7.4% (Dairy C), and 10% (Dairy D) of the cows.

A majority of the cows studied had either a high milk production in their first lactation or were registered with a high genetic value. We could not fully answer the aforementioned questions of the dairy farmers, therefore the dairy management at each farm decided to maintain several infected cows in the herd. Changes aimed at reducing SCC and controlling the spread of *M. bovis* mastitis included culture for mycoplasma of cows with clinical mastitis, all heifers at calving, cows calving that were dry at the time of our herd survey, biweekly culture of BTM samples, and new replacement cows prior to commingling with the herd. As a result, 18 of the 28 cows apparently eliminated the infection spontaneously and 15 of the 18 cows fully recovered milk production. The remaining 10 *M. bovis*-positive cows also recovered their expected milk production. Differences with respect to milk production between *Mycoplasma*-negative and *M. bovis*-positive cows that were kept on the 4 dairies were not observed, but the low number of *M. bovis*-infected cows precluded statistical analysis. Based on information provided in a recent review paper,[8] it seems that this is the first report of spontaneous and complete recovery of a *M. bovis* mastitis.

The diagnosis of an intramammary infection (IMI) is subject to error. The most widely accepted criteria for IMI diagnosis is that an IMI exists when the same pathogen is isolated from 2 samples or 2 of 3 consecutive samples taken at least one day apart.[9,10] The culture results of all samples obtained from the 10 cows chronically infected and that shed *M. bovis* intermittently, showed that even the use of the aforementioned criteria for diagnosis of *M. bovis*-IMI instead of the common single milk sample use by veterinary practitioners can easily lead to a false negative diagnosis. Following *M. bovis* naturally infected cows for 1 and 2 complete lactations has shown that a negative culture can occur frequently despite of the sampling method used. This may be an explanation for the moderate correlations obtained between percentage of cattle infected in the herds and isolation of mycoplasma from BTM.[11] Despite limitations on the efficiency of using bacteriologic culture of BTM for detection of mycoplasma infected herds,[11] we believe that the procedure is useful for initial herd screening and routine surveillance for *Mycoplasma*.[1]

Based on our experience with these 4 herds and several others that we have worked with, identification and segregation of infected cows rather than culling may be an alternative in herds experiencing mycoplasmal bovine mastitis. In a large Florida dairy herd, selective culling of low production and mastitic cows and keeping the remaining subclinical *Mycoplasma*-infected cows in a separate subherd, proved to be effective to control the disease and limit economic losses.[12] Although it has been recommended that uninfected animals should not

be housed and milked together with cows suffering from *Mycoplasma* mastitis,[5] it is of special epidemiological interest that cows that were milked last although sharing the same barn (Dairies A, B, C) or cows milked with separate milking units (Dairy D) seemed to have posed no risk for the remaining cows in the herd. This observation confirmed previous published reports that infection is mainly spread from infected to uninfected cows by milker's hands and milking machines.[2,3,5,13] However, mycoplasmal mastitis outbreaks in New York State have been frequently associated with respiratory problems[1,6] and airborne transmission suspected among animals housed in poorly ventilated barns.[1,6,14]

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Oxytetracycline Residues in Milk After Intrauterine Infusion of Dairy Cows With Retained Fetal Membranes

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Abstract

Our objectives were to establish the duration, by high performance liquid chromatography, of milk residues of oxytetracycline (OTC) after intrauterine infusion of 5 g OT of cows with retained fetal membranes. We also determined the predictive values positive (PVPT) and negative (PVNT) of the Delvo-P (Gist Brocades), Cite Probe (Idexx), Charm Farm, and Charm II (Charm Sciences) tests for OT residues above 30 ppb, and above the stated minimum detection level of each test. Cows with mastitis were not included. Milk samples were collected from 50 cows at 24 hour intervals during treatment, and at 12 hour intervals after the cessation of treatment. The results of this work plus important implications for dairy practitioners will be discussed in this presentation.

Oxytetracycline (OT) is widely used as an intrauterine infusion for the treatment of retained fetal membranes (RFM) in dairy cattle. It has a broad spectrum of antimicrobial activity and remains active in the presence of organic debris (Olson et al 1984). However, it is not approved by the Food and Drug Administration (FDA) for any use in lactating dairy cattle. OT is used as an intrauterine infusion in lactating cattle under the Extra Label Use Provisions set forth by the FDA (FDA Compliance Policy Guide 7125.06). One of the stipulations of these provisions is that the prescribing veterinarian inform the producer how long after the cessation of treatment the milk must be withheld from sale to ensure that treatment residues are not present in the milk. Because OT is not approved for lactating cattle, there are no milk withholding times listed on the label. Veterinary practitioners have commonly recommended that milk be withheld only during treatment.

Recent public concern about residues in milk and meat in general, and OT in particular, has prompted the