Bovine mastitis due to mycoplasma

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Summary: Since the original report in 1960, bovine mastitis due to mycoplasma has become an important problem in much of the world. Mycoplasma bovis is the principal cause, but several other mycoplasma also may be involved, including M. californicum, M. canadense, M. bovigenitalium, M. alkalescens and others.

Most transfer of infection occurs at milking time and carrier cows may introduce infections into previously uninfected herds. The respiratory system is often involved as is the reproductive tract. Semen used in international trade has been suspected of transmitting the disease. Inclusion of appropriate antibiotics in semen extenders to kill mycoplasma and ureaplasma is strongly advised, especially in international commerce.

Diagnosis is generally made by culture of milk, semen or other material. Treatment is unsatisfactory. Control depends upon isolation of infected animals. At times, slaughter may be advised.

KEYWORDS: Cattle diseases - Diagnostic techniques - Disease control - Epidemiology - Mastitis - Mycoplasma.

Bovine mastitis due to mycoplasma was first reported from England in 1960 (9). This single outbreak was due to Mycoplasma bovigenitalium, a species not reported from bovine mastitis again in England for many years. Shortly thereafter, an outbreak of serious mastitis due to a mycoplasma was observed in Connecticut, USA (12). Because of the clinical similarity of the Connecticut outbreak to contagious agalactia of goats and sheep caused by M. agalactiae, this organism was tentatively called M. agalactiae var. bovis (12), later modified to M. agalactiae subsp. bovis (22). In recognition of serological differences, Jain, Jasper and Dellinger (13) suggested M. bovimastitidis as a more appropriate designation. This name appeared later in a number of publications (14). Finally, following extensive studies, full species status was recognized and the organism was officially designated M. bovis (1).

In 1964, a series of herd outbreaks of mastitis was reported from the state of New York, USA, due to mycoplasma serologically identical to the Connecticut strain (8). Outbreaks in New York state have continued sporadically to the present time. An isolated herd outbreak due to M. bovis was also reported from Israel in 1964 (2) and a large herd outbreak occurred in California, USA, in 1964 (18), also due to M. bovis. Mycoplasma mastitis became an important problem in California and several other states (14).

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Since these early reports, mastitis due to *M. bovis* has been reported from most European countries, especially from Eastern Europe, Canada, and rarely from Latin America (14). *Mycoplasma bovis* has been reported from mastitis in cattle in China and Japan and from buffalo in India, although no information is available on extent of the disease in these countries.

The most common mycoplasma causing mastitis is *M. bovis* and other species are apparently uncommon causes of mastitis in most parts of the world (14). In California, about 50% of mycoplasma isolated from bovine mastitis are *M. bovis*. About 40% are due to *M. californicum* or *M. canadense*. The remainder are usually due to *M. bovigenitalium*, *M. alkalescens*, or unclassified species. *Acholeplasma laidlawii* may appear sporadically in quarter milk or bulk-tank milk samples, but these are believed usually to be contaminants from the environment (14).

**EPIDEMIOLOGY**

Most transfer of mycoplasma infection within herds occurs at milking time by means of fomites such as milking machines, teat cups and hands (15). It is known that many new herd infections occur from introduction of cows with infected udders. However, the disease may suddenly appear in previously clean herds without introduction of cows. Heifers in clean, or infected, herds may be infected at calving.

Since *M. bovis* is widely prevalent as a resident of the bovine respiratory tract of apparently normal cows, transfer from the lungs by hematogenous or other routes has been postulated (15). Once an udder infection is established, rapid spread within a herd can occur.

Treatment of mastitis either in lactating or dry cows provides a good opportunity for spread to other cows. If rigid sanitary precautions are not followed, spread by contaminated equipment, medication or hands in infected herds readily occurs. Only a few organisms, 77 or less, will cause infection (14). Since infected secretion may contain $10^7$ mycoplasma per ml, minor contamination may result in transfer of infections.

Although only rare isolations were made from the ampullae or seminal vesicles, the prepuce and distal urethra were found to be sites of colonization by various mycoplasma and ureaplasma in the genital tract of apparently normal bulls (10, 16). This results in infected semen and may be one means of domestic and international dissemination of these organisms. *Mycoplasma bovis* in semen can survive some antibiotic treatments, semen diluents and deep freeze storage of AI straws. Use of infected semen has been shown to result in lowered conception rates, especially from the first service, increased services per conception, prolonged diestrum, uterine pathology, prolonged calving interval and all attendant economic losses (20). Infection spread from experimental cows to control cows in the same housing, many of them developing mastitis.

Pfützner and colleagues have made extensive studies on transmission of *M. bovis*, especially from generation to generation. In a recent research (21), *M. bovis* was isolated from viable fetuses and progeny of cows with *M. bovis* mastitis. *Mycoplasma bovis* was also isolated from the semen of young bulls. Newborn calves were shown to develop respiratory infections postpartum which persisted to sexual maturity and even first calving (21). Bennett and Jasper (4) had previously shown a high percentage
of young calves fed *M. bovis*-infected milk to have *M. bovis* respiratory infection (19.8-47.4%), many of which persisted for at least 9 months. Calves from herds without mycoplasma mastitis had low rates of *M. bovis* infection (3.9-8.7%) for up to 5 months (3). *Mycoplasma arginini, A. laidlawii* and other mycoplasma were also present. Lateral transmission of respiratory infection occurs (3, 21), and it may be airborne (17). The organism may also be present in the vagina (17, 21).

The observations on semen contamination with mycoplasma, its subsequent deleterious effect on reproduction and possible role in spreading *M. bovis* and other types of mycoplasma mastitis led to recommendations that semen be treated with mycoplasmacidal antibiotics. Lincopectin-specinomycin, when added to semen extender, is effective against *M. bovis* and other mycoplasma (24); however, ureaplasma are resistant and strains of *M. bovigenitalium* are sometimes resistant to several times the normal use concentrations (19). The combination of lincomycin-specinomycin and tylosin was found to be effective against mycoplasma and ureaplasma in semen (24).

In California, the risk of large herds (> 350 cows) having a mycoplasma-positive bulk-tank sample was 15 times greater than that of small herds (<350 cows) (23). The reason for this is thought to be a combination of several factors of management commonly found, but poorly understood in larger California herds.

**CLINICAL FINDINGS**

Mycoplasma mastitis should be suspected (13, 14) whenever there is:

1. An increase in severe purulent mastitis cases, with cows showing little other evidence of illness.
2. Mastitis cases that typically involve more than one quarter (often all quarters).
4. Abnormal secretions with tannish or brownish discoloration and sandy or flakey sediments in watery or serous fluid. After a few days the secretion may be seropurulent.

Although severe mastitis as described above is regarded as typical, especially of *M. bovis* mastitis, the degree of severity is variable, even for *M. bovis* infections, and becomes progressively less for infections due to *M. californicum* (ST-6), *M. canadense, M. bovigenitalium, M. alkalescens* and other mycoplasma (15). Swelling and firmness may be marked, hemorrhage is uncommon, and gangrene does not occur.

Duration of inflammation is variable and is often prolonged (weeks to months) with *M. bovis* infection. Milk production may cease for that lactation (14) and purulent secretions sometimes may still be present at the onset of the next lactation.

Cows that appear to recover after several days or weeks can shed mycoplasma in the milk, perhaps intermittently, up to at least 13 months (14, 18). The variable duration of clinical signs and shedder status contributes to the difficulty in predicting the outcome of infected quarters or determination of complete bacteriologic recovery. For this reason, cows diagnosed positive for *M. bovis* should probably be considered to be positive for life even though this may not be the case.
Arthritis in *M. bovis*-infected dairy cows is uncommon but does sometimes occur (14). Arthritis due to *M. bovis* rarely occurs in dairy calves but is somewhat more common in young feedlot calves. Arthritis due to *M. alkalescens* in newborn calves in the absence of known infected dams has also been observed (15). An especially interesting observation was the finding of arthritis due to *M. alkalescens* in 84 of 450 embryo transfer calves in two herds (25).

**MICROBIOLOGICAL DIAGNOSIS**

Diagnosis of mycoplasma mastitis is usually made by isolation of the mycoplasma on a petri plate containing modified Hayflick medium, of which there are several variations (11). One liter of the medium we use is prepared as follows:

- Distilled water 750 ml
- Difco PPLO medium without crystal violet 21 g
- Difco Noble agar (omit agar for liquid medium) 15 g

Autoclave 15 minutes at 15 PPI.

Add sterile components aseptically:

- Yeast extract, preferably fresh 50 ml
- 1 M K$_2$HPO$_4$ 20 ml
- Ampicillin 500 mgm
- DNA (0.2% solution) (salmon origin) 10 ml
- Horse serum (heat inactivated) 150 ml

Adjust pH to 7.8.

One liter of medium will make 66 plates.

For sampling areas such as the respiratory tract or the vagina which may have a varied bacterial flora, including fungi, 5 ml of 10% thallium acetate may be added to the above formula, or 25 mg/liter of fungizone may be used.

Best results are usually obtained by streaking milk samples in 0.1 ml to 0.3 ml amounts directly onto agar plates. Quarter samples may be streaked on one-quarter areas of a 7-cm petri plate, composite samples over a one-half plate and bulk-milk samples over an entire plate (14). Growth may be seen after three days of incubation at 37.5°C in a moist candle jar or a moist 10% CO$_2$ incubator, but 5-7 days of incubation is desirable for the full development of colonies. Incubation should proceed 7-10 days before plates are diagnosed as negative. Plates are examined for colonies under 20× to 40× magnification.

Serological and biochemical tests are used to determine species, but some form of immunofluorescence is generally the most satisfactory (1, 3). Either direct or indirect immunofluorescence with incident or transmitted light may be used. The method currently in use (1) has been modified to the extent that 0.1 mg/liter of thimersol is added to the phosphate-buffered saline, incubation is increased to 75 minutes and the alternate method of washing agar blocks in place before removed to slides is generally used. By using plastic cylinders during staining several different antisera may be used at the same time on one plate. This method has the distinct advantage
that mixed cultures, not uncommon from milk, can readily be recognized. It is very important to determine species when mycoplasma are found in milk samples, as their significance varies greatly.

A number of serologically and immunologically based tests have been developed as measures of current or past infection or status of resistance, including plate agglutination, gel double-diffusion, indirect hemagglutination, growth and metabolic inhibition, complement fixation, latex agglutination, and radial growth precipitation (14). Enzyme-linked immunosorbent assay (6) is now the most satisfactory antibody assay method and can also be used to detect mycoplasma (4), although sensitivity is less than for culture. Tests for antibody indicate past or present infection, but they do not differentiate between them.

CONTROL OF MYCOPLASMA MASTITIS

Control of mastitis due to *M. bovis* generally means identification of infected cows by culture of composite milk samples of all milking and dry cows in the herd (14). All cases of clinical mastitis should be cultured and perhaps all cows at freshening, including heifers. We recommend that all *M. bovis*-infected cows be segregated and milked last for life (or at least through a succeeding lactation). Cows infected with other mycoplasma may recover and stop shedding during the same lactation. Cows with very severe mastitis may recover very slowly and may be candidates for slaughter. Slaughter of all infected cows may be indicated when only a few animals are infected. The exact mode of handling will vary from dairy to dairy based upon the owner’s attitudes and facilities, on the number of infections, etc. All actions should be based upon an understanding of the highly contagious nature of the infection, slow recovery, and the ineffectiveness of treatment. Extreme sanitation at milking time and during treatment is very important.

The good news is that mycoplasma infection in a herd can be eradicated. Culture of all clinical mastitis cases and of monthly bulk-tank milk samples should provide an early alert of new or missed infections in order to avoid serious new outbreaks in herds or areas where mycoplasma mastitis has been a problem.

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Résumé : Signalée pour la première fois en 1960, la mammite bovine causée par des mycoplasmes représente désormais un problème important dans de nombreux pays. Son principal agent est Mycoplasma bovis, mais elle peut aussi être provoquée par plusieurs autres mycoplasmes, notamment M. californicum, M. canadense, M. bovigenitalium et M. alkaescens.

La transmission de la maladie a lieu surtout au moment de la traite ; des vaches hébergeant des mycoplasmes peuvent introduire l’infection dans des troupeaux jusque-là indemnes. Le système respiratoire et les organes génitaux sont souvent atteints. La possibilité d’une transmission de la maladie par la semence utilisée dans les échanges internationaux a été suspectée. Il est fortement conseillé d’ajouter aux diluants de la semence des antibiotiques capables de tuer les mycoplasmes et les uréaplasmes, surtout en vue du commerce international.
Pour le diagnostic, on utilise en général une mise en culture du lait, de la semence ou d’autre matériel pathologique. Il n’existe pas de traitement satisfaisant. La prophylaxie repose sur l’isolement des animaux infectés. Dans certains cas, l’abattage peut être conseillé.


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LA MASTITIS BOVINA CAUSADA POR MICOPLASMAS. – D.E. Jasper.

Resumen: Señalada por primera vez en 1960, la mastitis bovina causada por micoplasmas representa desde entonces un problema importante en muchos países. Su principal agente es Mycoplasma bovis, pero puede también ser provocada por muchos otros micoplasmas, en particular M. californicum, M. canadense, M. bovigenitalium y M. alkalescens.

La transmisión de la enfermedad tiene lugar sobre todo en el momento del ordeno; las vacas portadoras de micoplasmas pueden introducir la infección en rebanos hasta ese momento indemnes. El sistema respiratorio y los órganos genitales suelen ser atacados. Se ha conjeturado la posibilidad de una transmisión por medio del semen utilizado en los intercambios internacionales. Se aconseja vivamente agregar a los diluyentes del semen antibióticos capaces de matar los micoplasmas y los ureaplasmas, sobre todo en función del comercio internacional.

Para el diagnóstico se suele utilizar un cultivo de la leche, del semen o de otro material patológico. No existe tratamiento satisfactorio. La profilaxis se basa en el aislamiento de los animales infectados. En ciertos casos, cabe aconsejar la matanza.


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REFERENCES


