Mycoplasma bovis as an agent of mastitis, pneumonia, arthritis and genital disorders in cattle

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Summary: Bovine diseases due to Mycoplasma bovis can cause considerable economic losses in cattle production. While the pathogen is principally responsible for therapy-resistant mastitis on large dairy farms, on smaller farms the typical mycoplasma diseases are calf pneumonia and arthritis. Moreover, the pathogen is able to cause genital disorders. M. bovis infection can be controlled effectively only if appropriate measures are implemented at the earliest possible stage. Since immunoprophylaxis and antibiotic treatment are known to be ineffective, control measures must include the introduction of strict hygiene standards, the restriction of animal movement out of infected herds and the culling of clinically diseased animals and shedders of the mycoplasma (the latter only in the case of mastitis and genital disorders).

In this review, symptoms of the various diseases caused by M. bovis are described and characteristics of the course of infection are outlined. To clarify the origin and spread of the infection, the authors describe the main properties and reservoirs of the pathogen and summarise experimental evidence on modes of transmission to susceptible organs.

As effective diagnosis is a prerequisite for the introduction of early control measures, the advantages and disadvantages of currently used diagnostic methods are discussed in detail. It is a serious shortcoming if testing for mycoplasmas is not included in routine bacterial examination of clinical samples. As a consequence, some M. bovis infections will remain undetected and outbreaks cannot be controlled properly. Finally, practical recommendations are given for prevention and control, including the formation of mycoplasma-free herds.


INTRODUCTION

Among the more than twenty species belonging to the class Mollicutes which have been isolated from cattle so far, Mycoplasma bovis is the most important aetiological agent of bovine mycoplasmosis in Europe and North America. In areas with intensive milk production and a high concentration of dairy livestock, M. bovis-associated
diseases, particularly mastitis, occur predominantly as herd enzootics, causing considerable economic losses because of drops in milk production (21, 39). On the other hand, in regions characterised by smaller cattle units, *M. bovis* is diagnosed mainly as the agent of pneumonia and arthritis in calves and young cattle, whereas mycoplasmal mastitis is rare. The main economic consequence of these infections is a reduction in daily weight gain.

The fact that, in many countries, testing for the presence of mycoplasmas is not included in routine bacterial examination of clinical samples indicates that certainly not all cases caused by *M. bovis* are actually recognised as such. In these circumstances, appropriate control measures are not usually taken and negative economic effects may be aggravated.

In this review, the symptoms and courses of the various diseases and disorders caused by *M. bovis* are described. Diagnostic approaches are compared and the importance of rapid detection methods for this often underestimated agent is discussed. Furthermore, epidemiological evidence and practical experience concerning the effects of various control measures are summarised and recommendations on controlling outbreaks are given.

**CHARACTERISTICS AND SYMPTOMS OF DISEASES DUE TO MYCOPLASMA BOVIS**

*M. bovis* is capable of producing subacute to acute inflammation of various organs, including the udder, joints (in calves and young cattle) and respiratory or genital tracts (in young and adult cattle). There is no evidence that environmental factors have any major influence on the course of disease, especially in the cases of mastitis and arthritis (13, 32, 33).

**Mastitis**

In an infected herd, mycoplasmal mastitis usually affects more than 20% of the cows independently of the stage of lactation; even dry cows can develop the disease. Depending on the dose of infection, the incubation period usually ranges from two to six days. Once the disease persists in a herd, the infection may extend over several weeks and a number of clinically healthy cows may shed the agent through milk. The main symptoms of mastitis due to *M. bovis* include a great alteration in milk consistency, a rapid decrease of milk yields down to a few millilitres within three to five days, quick spread of the infection from one udder quarter to the others, and lack of response to any antibiotic treatment, so that most cases finally lead to agalactia. Clinical symptoms tend to be most severe after new outbreaks and in cows in early lactation immediately after parturition. The consistency of secreted milk can vary from watery to purulent. A clear liquid over a precipitate of fibrin flakes is also typical. The overall number of cells in the milk increases dramatically. Conventional bacteriological investigation of milk samples, which does not include tests for mycoplasmas, is often negative. Moreover, mycoplasmal mastitis is characterised by the failure of cows to recover from the disease during the continuing lactation period. After two weeks, the affected quarters become atrophic, and even in a further lactation the animal would not be able to produce nearly as much milk as previously. As a consequence, infected cows have to be sent for slaughter. Although this represents a
major economic loss for the milk producer, there is no other effective control measure available at present. Morbidity largely depends on herd size and can vary from 10%, if effective control measures are taken, to 20% to 50%, if inadequate anti-epizootic measures or no measures at all are applied (51).

**Arthritis, pneumonia and genital disorders**

*M. bovis* is often associated with arthritis of calves and young cattle when enzootic mycoplasmal mastitis already persists in the population of mother cows and general exposure to the pathogen is high. However, *M. bovis* can also be present in the respiratory tract and can cause pneumonia or arthritis in the absence of mastitis in the herd. The main symptoms of arthritis due to *M. bovis* comprise acute severe lameness resulting from polyarthritis (especially in the carpal and tarsal joints), mostly connected with severe pneumonia ('pneumonia-arthritis syndrome'), an increase of the rectal temperature to 41°C and failure of antibiotic treatment (54, 55). Therapy resistance and retarded growth mean that affected animals have to be sent for slaughter. As with mastitis, the duration of the incubation period ranges from two to six days and depends on the degree of mycoplasma exposure prevailing in the herd. Morbidity to mycoplasmal arthritis is strongly influenced by the incidence of mastitis and pneumonia due to *M. bovis*, i.e. the general infection pressure. In the absence of effective anti-epizootic measures against mycoplasmal mastitis, morbidity to arthritis can also reach 20% to 50%.

The clinical picture of respiratory and genital diseases caused by *M. bovis* is indistinguishable from both enzootic pneumonia and common fertility disorders. Generally, the severity of pneumonia increases when secondary infections with pasteurellae, staphylococci or streptococci prevail in the population (7, 24, 64). In these conditions, pneumonia becomes therapy resistant. The incubation period is the same as for mastitis. It is still unclear why certain animals may carry the agent in the respiratory tract without actually developing any symptoms of disease. While the incidence of pneumonia can be as high as 100%, genital disease due to *M. bovis* is normally found only in a few individuals. In any case, reduced daily weight gains of pneumonic animals or fertility disorders can cause a marked deterioration in the economic performance of a production unit.

**DIAGNOSIS**

**General approach**

For farmers and veterinarians, the appearance of clinical symptoms as described in the previous section is normally the first indication of mycoplasma infection in a cattle population. As symptoms of mycoplasmal mastitis are largely non-specific, the clinical picture and also post-mortem findings can result in a merely tentative diagnosis. Only rapid and specific laboratory diagnosis can serve as a basis for effective control measures. Nevertheless, each *M. bovis* infection requires the individual consideration of appropriate control measures as well as the diagnostic approach. Apart from hygienic measures, *M. bovis* mastitis is controlled by culling all shedders. Thus, the identification of cows which carry the agent in their milk (i.e. single-animal diagnosis) at the earliest possible stage of the disease is a crucial prerequisite for any improvement. In the case of arthritis and pneumonia, control consists of trade
restrictions, which are imposed or lifted on the basis of screening and monitoring data from the respective population (i.e. herd diagnosis). The occurrence of genital disorders also demands early identification of animals shedding *M. bovis* through the genital tract (i.e. single-animal diagnosis) to support the usual control measures, i.e. decontamination of semen and, if vesiculitis or orchitis appear, culling of shedders.

**Collection of samples**

To ensure optimal recovery of the agent, fresh samples of milk, synovia, nasal or genital discharge or pathologically altered tissue must be obtained. Clean and hygienic conditions are essential during the entire sampling operation. If the sample becomes contaminated, e.g. by bacteria or other micro-organisms, cultivation of mycoplasmas may become impossible, even if the concentration of inhibitors (thallium acetate, penicillin) in the broth is raised. Furthermore, immediate and direct transport to the laboratory is another important precondition for successful mycoplasma isolation. In the summer months, samples should be refrigerated during transport. Whenever possible, samples should be processed in the laboratory immediately after arrival, since any delay may cause the death of mycoplasma cells and/or overgrowth with bacteria. Material from organs with pathological lesions should be investigated for mycoplasmas only in a fresh state. Optimal recovery can be achieved when, immediately after the death of the animal, specimens are collected and transferred into liquid broth without delay. Nasal or genital swabs should be protected from drying up. For this purpose, it is recommended to inoculate the liquid broth with the swabs immediately after sample collection (15, 45).

**Culture methods**

In routine diagnosis, *M. bovis* isolation and differentiation are still performed mostly using conventional culture methods (23), especially in single-animal diagnosis. When the liquid medium is inoculated with a sample, it is important to limit the inoculum size to either a volume of 0.2 ml, i.e. the amount of milk adhering to a glass stick, or to pinhead size if organ material is used. Oversized inoculum may result in acidification of the broth caused by organic matter or bacterial contaminants. Subsequent incubation of the cultures takes two to five days, followed by plating onto mycoplasma agar and incubation of these plates for three to five days. Modified Hayflick’s medium (20) is recommended for cultivation. It would be very helpful to laboratories if the complete medium became commercially available. To confirm a case, the isolates have to be identified as *M. bovis*, which should be done by serological methods, preferably epi-immunofluorescence or by the growth inhibition test. However, this sequence of operations is laborious and, because of the slow growth of the mycoplasmas, rather time-consuming, so that final results become available only after several days. This may be too late to single out individual infected animals or introduce other early control measures. Another drawback is that false negative findings may emerge as a result of bacterial contamination of samples. Nevertheless, culture methods will remain important for routine diagnosis as their high specificity and sensitivity (10^1-10^2 colony forming units [CFU]/ml) ensure reliable detection of *M. bovis* from a wide variety of clinical specimens. *M. bovis* isolation by culture can be a useful tool for single-animal diagnosis and will serve as the basis for control of *M. bovis* mastitis until more rapid routine assays are available. Furthermore, culture methods are suitable for the simultaneous detection of several pathogenic mycoplasma species which may be present in the same sample (35, 41, 51).
Detection of antibodies

Antibodies to *M. bovis* in blood and milk sera can be detected by indirect haemagglutination, film inhibition and enzyme-linked immunosorbent assay (ELISA) (46). However, neither the antigen for indirect haemagglutination nor ELISA test kits are commercially available, so that results cannot be compared between different laboratories and standardisation is impossible. This is certainly one of the reasons why antibody detection is carried out in only a few research laboratories. The ELISA developed in the laboratory of the authors uses whole-cell antigen to coat the solid phase (14) and has proved highly specific. The sensitivity of this ELISA is greater by two orders of magnitude than that of film inhibition and indirect haemagglutination. The procedure is normally less labour-intensive and time-consuming than culture methods (results become available within two days). The potentially high sample processing rate of the ELISA technique makes this test a useful tool for herd diagnosis of arthritis and pneumonia and for regular screening of *M. bovis*-free herds in connection with the animal trade, as well as for livestock monitoring in general. There are two main factors limiting the use of antibody detection ELISAs. First, antibody titres to *M. bovis* emerge only 10 to 14 days after the onset of disease, so that infection by the pathogen cannot be detected during the incubation period. Secondly, the sensitivity of this method is insufficient to identify shedders.

Protein-based methods

In recent years, antigen detection by ELISA has been improved in terms of specificity through the use of monoclonal antibodies (MAbs) (12, 27, 40). In particular, cross-reactions with *M. californicum* and *M. bovigenitalium*, which frequently occur in assays using polyclonal capture antisera, could be excluded by using MAbs recognising characteristic proteins of *M. bovis*. Heller *et al.* developed a highly specific antigen capture ELISA (27), capable of detecting $10^3$ CFU/ml from milk samples after 48 h pre-incubation. The MAb is directed against a surface protein later characterised as an adhesion factor (58, 60). Ball *et al.* described a similar ELISA based on a different MAb (2). Although assays of this kind proved suitable for identifying diseased cows as well as high-level shedders, it is certainly a disadvantage that no such ELISA has become commercially available, but has to be worked out by laboratory investigators themselves.

Sodium dodecyl sulfate Polyacrylamide gel electrophoresis (SDS-PAGE) of *M. bovis* strains will usually reveal only minor variations in whole-cell protein patterns (52, 57), in contrast to the findings for many other bacteria. On the other hand, pronounced inter-strain antigen variations could be demonstrated on Western-blotted protein patterns incubated with polyclonal antisera (52). This method can be used for epidemiological surveys, in which the source of an outbreak must be identified. Whereas, in diagnostic practice today, the application of these protein-related methods remains confined to special problems, a resurgence of immunoblot procedures can be anticipated as soon as major virulence factors of the agent are identified and the corresponding MAbs become available.

Deoxyribonucleic acid-based methods

In recent years, the most significant progress in diagnostic techniques has been made with deoxyribonucleic acid (DNA)-based methods. As with *M. bovis*, early attempts
focused on the use of DNA probes. Several investigators used plasmid probes containing random genomic fragments to identify *M. bovis* field strains by dot blot hybridisation (28, 37). The specificity of detection was satisfactory with cross-reactions confined to *M. agalactiae* or *M. arginini*, respectively. In a different approach, synthetic oligonucleotide probes derived from a region of the 16S ribosomal ribonucleic acid (rRNA) gene were proposed (38). However, both variants lacked the necessary sensitivity and dot blot hybridisation procedures proved too cumbersome for routine diagnosis.

The polymerase chain reaction (PCR) will certainly emerge as a widely accepted detection method for *M. bovis* in the near future. In addition to high sensitivity and specificity, the decisive argument in favour of PCR is rapidity. Even when direct use of biological samples for PCR is impossible and liquid cultures have to be prepared, the final result will still be available within one day of delivery of the culture, with sensitivity comparable to and specificity higher than that of culture methods (28, 59). Nasal fluid can be subjected directly to PCR, thus enabling the diagnostician to provide results on the same day (29). The investigation of milk samples for mycoplasmas is more difficult because of the presence of large amounts of protein and other PCR inhibitors. To overcome these problems, Hotzel *et al.* (29) developed a pre-enrichment procedure which includes trypsin digestion, detergent extraction and selective DNA binding on a filter membrane. After releasing mycoplasmal DNA from the membrane, this DNA is amplified by PCR. The detection limit was $5 \times 10^2$ CFU/ml milk from standard agarose gels and $5 \times 10^1$ CFU/ml after Southern hybridisation. The maximum time required for this multi-step procedure is 24 h. PCR assays have the potential to replace most conventional methods in the diagnostic laboratory as soon as they can be conducted at comparable cost.

**THE CAUSATIVE AGENT**

After its first description as an agent of mastitis (26), *M. bovis* was designated *M. agalactiae* subsp. *bovis* because of a wide-ranging analogy to the clinical picture of contagious agalactia in goats and sheep, which is caused by *M. agalactiae*. In 1976, the organism was elevated to species rank and given its present name (1). Although there are many indications of a close taxonomic relationship between *M. bovis* and *M. agalactiae*, in particular the frequent cross-reactions in serological assays, they remain strictly confined to their respective host animal species. A detailed study recently conducted by Gummelt has provided interesting new evidence about this complex relationship (25). Phenotypic properties are largely identical and both species share an unusually high number of common antigens. Nevertheless, it is possible to differentiate between them on the basis of whole-cell protein profiles. In genotypic terms, *M. bovis* was clearly distinguished from *M. agalactiae* by DNA-DNA hybridisation and PCR fingerprinting.

The virulence factors of *M. bovis* and mechanisms of pathogenesis are still largely unknown. In this respect, the recently discovered ability of the agent to vary the expression of a family of membrane surface proteins with high frequency (3, 56) will possibly deliver the clue to future understanding of the action of this species in the host.
M. bovis is not ubiquitous, but is rather common in cattle populations of enzootically infected regions. The occurrence of this agent in a herd is always connected with cases of mastitis, arthritis and/or pneumonia.

The pathogenicity of M. bovis was demonstrated in various experiments. To investigate virulence in the bovine udder, 30 different M. bovis strains were applied intracisternally to 91 cows. This resulted in severe clinical mastitis in each animal (47, 50). There were no apparent differences in virulence, although whole-cell protein patterns as generated by SDS-PAGE revealed some interstrain variation. Even M. bovis strains isolated from the milk of cows without any symptom of mastitis showed high virulence for the udder in experimental infection. This means that latently infected cows probably play an important role in the spread of the causative agent. Surprisingly, M. bovis isolates from nasal swabs taken from both clinically healthy and diseased calves and young cattle also turned out to be highly virulent in the udder. The same applies to isolates from the lungs and inflamed joints. These observations suggest that calves and young cattle can also contribute to the spread of the agent, and could even be the origins of an infection chain. The high virulence of M. bovis towards joints and the respiratory tract of calves could be demonstrated by experimental intra-articular, intra-tracheal and endobronchial inoculation (22, 24, 48). Furthermore, intrateruterine application produced abortions in cows and intravesicular application led to vesiculitis, epididymitis and orchitis in bulls (8, 36).

Another characteristic feature of M. bovis is host specificity. Since the microorganism is highly adapted to cattle, detection of M. bovis in small ruminants is a very rare event. Experimental inoculations into mice and specific pathogen-free piglets failed to produce any evidence of disease or colonisation by the agent. Only in sheep was it possible to generate pneumonia and mastitis by inoculation of the respiratory tract of specific pathogen-free lambs and the udder of ewes, respectively (10, 11).

It is often suggested that M. bovis cannot persist in the environment outside the host animal for a longer period because of high susceptibility to dryness. However, the investigations of the authors regarding tenacity, which were conducted with artificially contaminated sterile materials, demonstrated that, at 4°C, M. bovis survived on sponges for 57 days, in milk for 54, on straw for 20 and on wood and in water for 17 days. At 20°C, the survival period on these materials dropped to one to two weeks, and at 37°C to one week. In deep-frozen bovine semen processed for artificial insemination, the agent remained infective for years (42).

Disinfectants based on formalin or peracetic acid solutions are very effective in killing M. bovis. The authors conducted dilution tests in broth, milk and water which showed that, even at low concentrations, these disinfectants killed the mycoplasmas within a short time. Iodophores are also known to be effective and can therefore be recommended for teat dipping. However, disinfectants based on hypochlorides, which are used very often during milking and for the disinfection of milking machines, proved unsuitable because relatively high concentrations and long periods of exposure are required. Generally speaking, the efficacy of any disinfectant will be reduced in the presence of biological material, such as milk and nasal or genital discharge (34, 49).

In regard to antibiotics, M. bovis is known to be susceptible to a number in vitro, e.g. tylosin, turimycin, spectinomycin, lincomycin, oxytetracycline, tiamulin and enrofloxacin (62). Unfortunately, the efficacy of antibiotic drugs in vivo is insufficient so they cannot be used for therapeutic purposes (32, 51).
THE RESERVOIR

Clinically healthy calves and young cattle harbouring *M. bovis* in the respiratory tract were observed to shed the agent through nasal discharge for months; some even for years. Another important reservoir is found in the male and female genital tracts. Infected bovine semen used in artificial insemination can also be a source of new infections. In populations already affected by mastitis, clinically healthy cows shedding the pathogen in their milk represent a permanent reservoir (43).

Sheep can acquire *M. bovis* from infected cattle and should also be taken into account as living vectors (9). Finally, considering the tenacity of this agent outside the animal, contamination of the environment, such as the air in the cow-shed, tools and litter, should not be underestimated in this respect.

MODE OF TRANSMISSION

Infected cattle sporadically shed *M. bovis* through milk or through mucus of the respiratory and genital tracts. At the onset of clinical disease, the level of shedding is substantially elevated. When transmission occurs, the released mycoplasmas will again colonise the udder, respiratory or genital tract in another host animal.

Udder

The bovine udder is infected upwards only through the teat canal, as could be demonstrated in a number of experimental infections (44). Haematogenic or retrograde lymphogenic infection was also suggested, but has not been proved so far. Intravenous, subcutaneous, intramuscular, oral and intrauterine inoculation of *M. bovis* cultures did not facilitate colonisation of the host tissue by the agent and failed to produce mastitis. Before the udder becomes infected, the agent must propagate massively around this organ and the barrier function of the teats must be impaired. Factors contributing to disturbances of the barrier function of the teat seal include high population density and inadequate feeding and rearing conditions, as well as unsatisfactory hygiene, e.g. manure on tails. As will be discussed below, the milking process also creates conditions enabling mycoplasmas to enter the host organism. All these factors are normally more relevant in larger farms. Indeed, *M. bovis* mastitis is more common in larger units, with 1,000 or more cows per shed, but nevertheless also occurs in small herds. Generally speaking, the risk of infection, the incidence of the disease and the necessity to cull infected and/or diseased cows are much higher in large units (44, 63).

Once the first cow in a herd becomes diseased with mastitis, other animals quickly become infected during milking. Even an infective dose as low as 100 mycoplasma cells, when applied intracisternally, proved sufficient to enable colonisation of the udder tissue and, consequently, to trigger a galactogen-ascending infection (5, 47). In the same series of experiments, contact animals which were not artificially infected became as severely diseased as those that were artificially infected (47).

The most favourable conditions for infection of the udder exist during the milking process and for some time afterwards, when the teat canals are still open. It is important to know that a cow diseased with *M. bovis* mastitis sheds between $10^5$ and $10^8$ CFU per ml of milk, but considerable shedding also occurs before the onset of the
clinical stage \((10^3 \; \text{to} \; 10^6 \; \text{CFU/ml})\). Therefore, milking machines, particularly contaminated milking cups, cloths to wash the udder, the hands of the milker, and also reflux of milk from infected neighbouring cows within the pipeline are of great importance for the transmission of the infective agent. Contaminated resting places in the boxes can also be responsible for the transmission of \textit{M. bovis}. Furthermore, \textit{M. bovis} can be transmitted iatrogenically from udder to udder when rules of aseptic operation during the intracisternal application of antibiotics are neglected. In summary, it should be emphasised that the most important infection source is the clinically healthy shedder when milked on the milking stand.

**Respiratory tract and joints**

\textit{M. bovis} has a high affinity to the epithelial cells of the broncho-alveolar region. Infection of the respiratory tract occurs predominantly through droplets set free during the coughing of infected animals. \textit{M. bovis} can also reach the airways on contaminated dust particles. As soon as respiratory disease has fully developed, general infection pressure increases considerably and infection spreads at an accelerated rate. The authors conducted experiments in which the agent appeared in the nasal fluid of calves 24 hours after their first contact with infected animals. Field investigations demonstrated that, seven days after the first detection of \textit{M. bovis} in a herd, the infective agent could be isolated from the nasal swabs of most other calves \((48, 50)\).

Joints of calves and young cattle can become infected through haematogenic dissemination (mycoplasmaemia), but usually only when mastitis and/or pneumonia already prevail in calves or their mothers, so that mycoplasma exposure in the herd is very high \((54, 55)\).

**Genital tract**

Infection of the male genital tract probably progresses upwards through the prepuce with mycoplasmas transmitted from a contaminated environment or by the licking of shedder animals. In experimental studies, \textit{M. bovis} inoculated into the prepuce or urethra was shown to ascend to the testes producing orchitis, vesiculitis, decrease of semen quality, and shedding of the agent in semen \((36)\). The possibility of male genital tract infection by haematogenic dissemination or mycoplasmaemia has not been finally proved so far.

The female bovine genital tract is also infected by the pathogen in the ascendant way. Infection may originate from the environment and much more so from artificial insemination with contaminated semen \((19, 53)\). Whether the bovine female genital tract can be colonised by mycoplasmaemia resulting from \textit{M. bovis} mastitis is still unclear. On the one hand, \textit{M. bovis} was isolated from internal organs, the uterus of slaughtered cows and from aborted foetuses during and after mastitis. On the other hand, intravenous inoculation of \textit{M. bovis} cultures did not produce any infection of the uterus \((8, 50)\).

**Transmission cycle**

Experimental and field studies have revealed that there is a closed cycle of \textit{M. bovis} infection from the infected cow to the foetus or post-partum over the new-born calf to young cattle and thus the next generation \((50)\). During mastitis, \textit{M. bovis} is transmitted to foetuses through the uterus. Furthermore, newborn calves can become
infected through milk or through the environment. The agent is capable of persisting in the respiratory tract, where it remains infective as the young cows mature and can even be transmitted to the next generation. Moreover, there are possibilities for horizontal transmission at all stages of the development of cattle.

In a field survey, *M. bovis* was isolated from the nasal mucus of newborn calves descending from mastitic cows. Some weeks later *M. bovis* was also isolated from contact animals, up to two years old. Detection of antibodies to the agent confirmed that this was real infection, rather than mere colonisation of the upper respiratory tract. Later, the infective agent could not be found in any sample from pregnant heifers. However, after parturition *M. bovis* was isolated sporadically from blood, amnion, milk, nasal and cervical fluid, as well as from newborn calves. The presence of the agent in blood, amnion and cervical mucus immediately after parturition, and also in the endometrium of slaughtered mastitic cows and in different organs of viable foetuses, particularly demonstrate haematogenic dissemination and vertical transmission of *M. bovis* infection through genital organs and amnion to the foetus and, finally, to the newborn calf. It seems certain that mycoplasmas retain their virulent properties throughout this cycle. When cattle were artificially infected intracisternally, with twelve different *M. bovis* strains isolated from the progeny of dams with a history of mastitis, all strains without exception produced severe mastitis (50). These data also indicate that young cattle and heifers carrying the agent may be the source of mastitis in adult cows.

Finally, it must be emphasised that *M. bovis* is principally disseminated by uncontrolled trade of infected calves and young cattle or by animal movement along the production chain. As a consequence, the cattle populations of a particular region become gradually infected (41).

**THE HOST POPULATION**

Although mycoplasmal mastitis can occur at any age or stage of lactation, cows are most susceptible after parturition and at maximum lactation. These are the periods when the disease is most frequent and severe. Susceptibility to pneumonia and arthritis has no peaks during particular periods, but can be increased considerably through direct or indirect contact with mastitic populations. Outbreaks of mastitis, pneumonia and arthritis trigger the production of humoral antibodies, which can be detected in blood or milk sera. In addition, a cell-mediated immune response develops as well. But, in spite of this, no protective immunity emerges. There are, however, reports of natural resistance lasting approximately 60 days in the aftermath of recovery from mastitis (4, 5). With increasing duration of enzootic disease, the number of clinically healthy shedders in the herd will usually rise. As discussed above, these shedders are of particular importance for the epizootic process.

**PREVENTION AND CONTROL**

There is no effective immunoprophylaxis for the prevention and control of *M. bovis* mastitis. In the case of pneumonia and arthritis, vaccination of calves reportedly reduced losses under experimental conditions (4, 5, 16, 17, 18, 30, 31, 61). The
respective experiments were carried out 10 to 15 years ago but, until now, no
evidence about vaccination under field conditions has emerged nor have any
M. bovis vaccines become commercially available. Since diseases caused by M. bovis
are refractory to therapy, animal health control measures must be given priority for
prevention and control. Such measures should aim at preventing further spread of the
agent and avoiding the epizootic occurrence of mycoplasmal mastitis (6, 15, 32, 51).
They will not, however, lead to eradication of M. bovis infections. According to
present knowledge, this can only be achieved by complete replacement of all cattle in
infected populations with animals from M. bovis-free territories.

Recommended measures for the protection of Mycoplasma bovis-free territories

Any measures to control M. bovis infection should be directed particularly towards
the protection of hitherto M. bovis-free territories and herds. The introduction of
international animal health control certificates for the international animal trade and
the prevention of cattle movements from infected or suspected herds into free
populations would be required to achieve this aim. Once therapy-resistant pneumonia
and arthritis occur in herds of calves or young cattle, random samples must be taken
immediately. Likewise, milk samples from cows with therapy-resistant mastitis must
be tested for M. bovis in the laboratory. The following measures are recommended to
reduce the danger of infection:

- remove all cows with therapy-resistant mastitis from the herd
- ensure proper hygiene during milking and correct functioning of milking
  machines, as well as conducting intermediate disinfections of milking devices with
  efficient disinfectants
- avoid overcrowding
- observe a high level of hygiene in all sheds.

Within the framework of veterinary surveillance programmes, bulls should be tested
for mycoplasmas; animals shedding M. bovis should be culled and all stored semen
doses from the last negative finding onwards should be destroyed. Imported cattle and
semen should be tested for M. bovis during quarantine and released only if negative.
In addition, there is the possibility of preparing M. bovis-free semen doses for artificial
insemination, namely: by supplementing the semen extender with a mixture of
antibiotics, e.g. lincomycin, spectinomycin and tylosin.

Recommended measures in the case of outbreaks of Mycoplasma bovis mastitis

These measures can only minimise losses caused by M. bovis mastitis rather than
completely eradicate the infection. However, in the absence of proper measures, the
disease will take an enzootic course and lead to considerable economic losses.

When M. bovis mastitis breaks out, any movement of cattle, apart from slaughtering
and fattening, should be prohibited temporarily in order to prevent the spread of the
agent. A comprehensive diagnostic programme should be conducted which will form
the basis for effective control of the disease. In addition to repeated clinical
investigations of the udders of all cows and heifers, milk samples from all animals
suffering from mastitis and from cows post-partum should be tested for M. bovis.
Furthermore, repeated monitoring of the entire herd at short intervals is necessary to
detect all shedders.
Independently of the results of clinical investigations of the udders, all infected cows must be removed from the herd on the spot and immediately slaughtered. Additionally, all cows with therapy-resistant mastitis must be culled without delay; this is necessary because infected cows often do not shed *M. bovis* continuously and, therefore, may not be discovered by the first laboratory screening of milk samples. In order to decrease exposure to the pathogen, calves born during the enzootic should be used for fattening only. Culling all calves identified as carriers of *M. bovis* on the basis of positive nasal swabs or the presence of specific antibodies will not contain the outbreak. The spread of *M. bovis* via droplet infection occurs fairly rapidly, so that the removal of these animals upon availability of laboratory data comes too late. Calves diseased with *M. bovis* pneumonia should be treated with antibiotics to minimise clinical symptoms caused by secondary infections. The removal of animals with pneumonia and/or arthritis can sometimes be helpful to decrease infection pressure.

Hygienic measures play the most important role in the control of *M. bovis* mastitis. In order to avoid the spread of *M. bovis* during milking, intermediate disinfections of milking devices and udder cloths are essential, as is the washing of udders with peracetic acid preparations. Furthermore, total cleaning and disinfection of the milking stand must be carried out twice per shift. Teat dipping with iodophore solution supports anti-epizootic measures efficiently. Further requirements are proper milking hygiene, well-functioning milking machines and a high general level of hygiene on the premises, which includes regular cleansing and disinfection of boxes and stables. Grazing contributes to a reduction of exposure.

To prevent the spread of *M. bovis* and to protect territories free of the agent, cattle from infected herds should only be introduced into breeding units which have the same state of infection, not into new trade partners. The introduction of animals into fattening units can be permitted.

When all animals shedding *M. bovis* in milk are removed, and three consecutive laboratory investigations of the entire herd (at intervals of at least two months), as well as intermediate tests of milk samples (from animals with diseased udders), all yield negative findings, the herd can again be considered free from *M. bovis* mastitis. In spite of this, the agent will still be able to be sporadically isolated from the respiratory and genital tracts, i.e. there is still the possibility of a new outbreak. In order to contain further disease spread, no cattle from these populations should be introduced into *M. bovis*-free territories.

Since control of *M. bovis* mastitis is so complicated, the stamping-out policy should be practised in the case of a first outbreak. This approach is recommended if the herd is not too large and the agent has not yet been transferred to other populations.

The formation of *M. bovis*-free herds is possible in practice, as has been demonstrated in a field study (14). The measures, however, are laborious and expensive. At first, *M. bovis* mastitis was controlled successfully. Subsequently, a trial was carried out with a group of newborn female calves, which were immediately removed from their dams after parturition. These animals remained strictly separated from any other cattle during the whole rearing period. Extensive laboratory investigations (i.e. nasal swabs and specific antibodies) confirmed that the animals were free from *M. bovis*. When some members of the group were put into contact with cattle carrying *M. bovis*, antibodies were detected in those group members. These observations imply that the pathogen-free status can only be maintained as long as the animals are protected against contact with infected livestock.
Recommended measures in enzootically infected territories

At present, control measures in enzootically infected territories aim only at avoiding the epizootic occurrence of *M. bovis* mastitis and the importation of the agent into disease-free territories. A diagnostic surveillance programme involving regular laboratory investigation of milk samples from cows with diseased udders should be established. Strict observation of the measures described for the control of *M. bovis* mastitis outbreaks is essential to minimise economic losses to the farm. Such measures must guarantee that pathogen-free territories are protected.

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**MYCOPLASMA BOVIS, AGENT DE LA MAMMITE, DE LA PNEUMONIE, DE L’ARTHRITE ET DES TROUBLES GÉNITAUX CHEZ LES BOVINS. – H. Pfützner et K. Sachse.**

Résumé : Les maladies des bovins dues à Mycoplasma bovis ont de graves conséquences économiques sur la production animale. Dans les grands élevages laitiers, cet agent pathogène est surtout responsable de mammites résistantes à toute thérapie, tandis que dans les petites exploitations, les mycoplasmoses les plus courantes sont la pneumonie et l’arthrite du veau. Cet agent est également à l’origine de troubles génitaux. On ne peut lutter efficacement contre une infection due à *M. bovis* que si les mesures appropriées sont mises en œuvre dès les toutes premières phases. Comme il a été démontré que la vaccination et les traitements antibiotiques étaient inefficaces, la prophylaxie doit viser à introduire des normes d’hygiène strictes, à limiter les déplacements des animaux en dehors des élevages infectés et à éliminer les animaux cliniquement atteints ainsi que ceux qui excrètent le mycoplasme (cette dernière mesure s’appliquant uniquement en cas de mammite et de troubles génitaux).

Les auteurs décrivent les signes cliniques caractéristiques des différentes maladies dues à *M. bovis*, ainsi que les phases de l’évolution de l’infection. La description des principales propriétés de l’agent pathogène et de ses réservoirs et un rappel des résultats obtenus lors des transmissions expérimentales effectuées sur des organes sensibles permettent aux auteurs de clarifier l’origine et les causes de la propagation de l’infection.

Un diagnostic efficace étant une condition préalable à l’introduction de mesures de prévention précoces, les auteurs présentent en détail les avantages et les inconvénients des méthodes de diagnostic actuellement appliquées. Les examens bactériologiques effectués en routine sur les prélèvements cliniques présentent le défaut majeur de ne pas toujours inclure la recherche des mycoplasmes. De ce fait, certaines infections dues à *M. bovis* ne sont pas détectées et il devient donc impossible de lutter efficacement contre l’apparition de nouveaux foyers. Enfin, les auteurs font des recommandations concrètes en matière de prophylaxie et de lutte, dont notamment la constitution d’élevages exempts de mycoplasmes.


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MYCOPLASMA BOVIS COMO AGENTE DE MASTITIS, NEUMONÍA, ARTRITIS Y TRASTORNOS GENITALES EN EL GANADO VACUNO. – H. Pfützner y K. Sachse.

Resumen: Las enfermedades de los bovinos causadas por Mycoplasma bovis pueden engendrar considerables pérdidas económicas en las actividades de producción pecuaria. Mientras que en el caso de lecherías de gran tamaño este patógeno es responsable sobre todo de mastitis resistentes a cualquier forma de tratamiento, en granjas más pequeñas las enfermedades micoplasmáticas más frecuentes son la artritis y la neumonía en terneros. Por otra parte, este agente también es responsable de trastornos genitales. La única forma de controlar con eficacia la infección por M. bovis reside en la aplicación de medidas adecuadas en una fase lo más temprana posible. Dado que la inmunoprofilaxis y el tratamiento con antibióticos se han demostrado ineficaces, las medidas de control deben comprender la introducción de normas estrictas de higiene, la limitación del movimiento de animales pertenecientes a rebaños infectados y el sacrificio de aquellos ejemplares con signos clínicos de la enfermedad o que excretan el virus (estos últimos sólo en el caso de las mastitis y los trastornos genitales).

Los autores describen los síntomas de las diversas enfermedades causadas por M. bovis así como el curso característico del proceso infectivo. Con el fin de arrojar luz sobre el origen y propagación de la infección, los autores describen asimismo las propiedades principales del agente patógeno y sus reservorios, y resumen los resultados obtenidos experimentalmente sobre los modos de transmisión a los órganos sensibles.

Examinan también en detalle las ventajas e inconvenientes de los métodos de diagnóstico utilizados actualmente, pues la práctica de un diagnóstico efectivo es requisito indispensable para la introducción de medidas precoces de control. La ausencia de pruebas para la detección de micoplasmas en los protocolos de análisis bacteriológico de rutina de muestras clínicas constituye un grave defecto. Ello imposibilita la detección de algunas infecciones por M. bovis y por ende un adecuado control de los posibles brotes. Los autores hacen por último algunas recomendaciones prácticas para la prevención y control, entre ellas la formación de rebaños libres de micoplasmas.


REFERENCES


