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A Cattle Pathogen *Mycoplasma bovis* : A Review

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Abstract

Mycoplasma bovis was first isolated in the USA from the milk of a mastitic cow in 1961. It is the most important pathogenic bovine mycoplasma mainly causing pneumonia, arthritis, mastitis. The organism is usually transmitted by close and repeated contact over short distances; untreated infected milk can also be a source of infection to calves. Transmission may also be via shedding of the pathogen through external mucosal surfaces of an infected animal. They are quite resistant to environmental conditions thus, can transmit through fomites and milking equipments. Classically diagnosis was depended on the use of selective culture media and prolonged incubation in an environment enriched with CO_2. Recently, some alternative to culture methods, like, PCR based techniques have evolved. Disease associated with *M. bovis* is often chronic, enfeebling, and poorly responding to antimicrobial therapy. Thus this cattle born disease causes significant economic loss. Till now, vaccines for *M. bovis* are unavailable. So, the remaining approaches to control these infections are sanitary control measures and antimicrobial treatment. The aim of this review is to summarize the current knowledge regarding the clinical signs of the diseases caused by *M. bovis* . Various diagnostic approaches used in detection of *M. bovis*, the prevention and the controlled measures are discussed.

Keywords:

Mycoplasma bovis;
Cattle;
Diagnosis;
PCR;
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1. Introduction

Mycoplasma bovis is a member of the Mycoplasmataceae family in the class *Mollicutes.* They are bounded by the plasma membrane but lack cell wall. Thus they are pleomorphic and vary in shape from cocci to pear-shaped to helical or branched filament. Their average cell volume is about 5% of that of common bacillus. They have a low G+C content [23±40%] and small genome size [0.58±1.4Mbp].They may be considered as the simplest and smallest self-replicating free-living life-form. They can be cultivated in artificial media, with some special requirements. Their colonies are very small in size (less than 1mm in diameter).

Mycoplasma bovis is the most important pathogenic bovine mycoplasma. In older cattle, *Mycoplasma bovis* can cause arthritis, mastitis pneumonia, keratoconjunctivitis. In female cattle vaginitis and abortion in

late pregnancy may be associated with *M. bovis*. They can also cause respiratory disease in calves. Otitis media, a disease in calves are often seen, with a noticeable head tilt, dropped ears [24].
M. bovis generate phospholipases, hydrogen peroxide, and superoxide radicals that can damage host cells [21]. They are able to change the surface proteins [5], thus can evade the host's immune response. They can form biofilm also [26], which provide them a temporary protection from both the immune system and antibiotics. This review covers the general information about *Mycoplasma bovis*, the diseases it causes, the diagnosis of *M. bovis* and possible treatment of resulting diseases.

2. Diseases

Diseases caused by *M. bovis* are, mastitis, pneumonia, arthritis, otitis media, keratoconjunctivitis, decubita absecesses, meningitis, endometritis, salpingitis, oophoritis, seminovesiculitis, infertility, vaginititis and abortion in late pregnancy [22, 25]. Among then the first three have high mortality rate.

2.1 Clinical signs

In case of mastitis, initially, affected cows were normal. Within one week or more, they may have acute mastitis; which may be followed by chronic mastitis. Subclinical infection or acute flare-ups may occur within acute and chronic mastitis [12, 15]. A drastic decline in milk production may occur, where the milk yields decrease down to a few milliliters, even some cows may cease lactating. Initially, the drawn milk shows normal, but rapidly separates in a clear supernatant and a floccular deposit [3]. Clinical signs of mastitis are mild fever, hyperpnoea, dyspnoea, and poor appetite, with or without nasal discharge and coughing. Cattle of any age can be affected by *M. bovis* arthritis. Calves and young cattle can be affected when mycoplasmal mastitis is already present in mother cattle. Main symptoms of arthritis are acute severe of lameness (especially in carpal and tarsal joints). Capsule of affected joints are warm, painful, swollen, distended and fluctuant on palpation. Affected joint capsules are distended by opaque, fibrin containing cream colored exudates. Tendon sheaths and periarticular soft tissues may also be affected [1,18, 32]. Mycoplasmal pneumonia is generally connected with arthritis. The symptoms are slight tachypnea, mild fever, depression and stress or movement induced coughing [8]. Secondary infections with pasteurellae, staphylococci or streptococci are common in mycoplasmal pneumonia. The severity of pneumonia increases with those secondary infections [4,7,16, 37]. In these conditions, pneumonia becomes therapy resistant. Clinical signs of otitis media are ear droop, ptosis, head shaking, rubbing ears, head tilt and, circling [24]. All of these occur due to ear pain and facial nerve deficits. Meningitis can develop with advanced otitis media-interna [32]. Clinical signs of keratoconjunctivitis are mucopurulent ocular discharge, severe eyelid and conjunctival swelling, and corneal oedema and ulceration [23,33].

2.2 Transmission

Mycoplasma bovis is commonly transmitted by close, repeated direct contact with infected animals. They colonize mucosal surfaces such as the mammary gland, nose, respiratory tract, eye, ears, vagina and prepuce. Infected cattle sporadically shed *M. bovis* through milk and secretions of respiratory and genital tracts. Thus, disease may be transmitted via those secretions. Transmission can also occur by aerosolisation of nasal secretions. The risk of airborne transmission between farms depends on many other factors such as temperature, humidity, dust density, ventilation types and structural or geographic obstructions in herd [7]. There are possibilities for horizontal transmission at all stages of the development of cattle. Vertical transmission of *M. bovis* through genital organs and amnion to the foetus and finally, to the newborn calf have been recorded [28]. The artificial insemination of infected cattle semen can transmit *M.bovis* to female cattle [9, 30]. A mother cow infected by *M. bovis* mastitis sheds between 10^5 and 10^8 CFU per ml of milk. Considerable shedding ($1O^3$ to $1O^6$ CFU/ml) also occurs before the onset of the clinical stage. Thus, milking equipments, cloths that is used to wash the udder, and reflux of milk (from neighbouring cows) within the pipeline are also the cause of spreading [28].

2.3 Diagnosis

Mycoplasma bovis infections could be diagnosed by bacteriological culture [15, 25,34]. Milk, tracheal swab, joints exudates and blood form affected or suspected cattle are usually used as sample. All samples were transported to the laboratory in cold chain and stored at –20ºC. Inoculums' size was restricted to ≤0.2ml, when a broth is inoculated with a sample. Larger inoculum size may cause acidification of the media which can be easily contaminated. After 2-5 days of subsequent incubation, *M. bovis* can be isolated on modified solid media. Selective and highly enriched media such as Friis's or Hayflick's mycoplasma media are recommended for culture. Culture must be kept at 37 ^oC in 5 % CO_2 and incubated for 7-10 days [13, 27]. The colonies have a distinctive 'fried egg' appearance when observed under magnification. *Acholeplasma* also have a similar morphological 'fried egg' appearance that can result in false mycoplasma-positive samples. An additional biochemical step (digitonin sensitivity) can be used to distinguish *Mycoplasma*

(digitonin sensitive) from *Acholeplasma* (digitonin resistant). 1.5 % digitonin saturated paper disc give a large zone of inhibition for *Mycoplasma*, but a small to non-existent zone of inhibition for *Acholeplasma*.
Serological methods are useful for screening tests but its main limitation is 'long window period' (sero-conversion usually occurred in 10 -14 days after the onset of infection) [35]. *M. bovis* antigen detection by a monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (sELISA) is very promising.
Due to the presence of large amounts of proteins and other inhibitor of PCR, milk sample is not suitable for PCR. But PCR is much promising for the direct detection of *M. bovis* from tracheal swab. Swab samples can be analyzed by PCR. Amplification of mb-mp gene of *M. bovis* (mb-mp1F: 5-TAT TGG ATC AAC TGC TGG AT-3; mb-mp1R: 5-AGA TGC TCC ACT TAT CTT AG-3) which is 447 bp long can be performed by using *M. bovis* specific primers. Usually the reaction cycles are: DNA denaturing at 94°C for 1 min, DNA annealing at 54°C for 1 min, extension at 72°C for 1 min for 30 cycles and final extension step at 72°C for 10 min. After electrophoresis at 80 V for 2 h in 2% agarose gel the amplified products can be detected by staining with 10 mg/ml Ethidium Bromide [15]. Realtime PCR (RT-PCR) is highly sensitive and specific for the detection of *M. bovis* in clinical samples [31]. The oppD gene encodes an oligopeptide permease and the uvr C gene encodes deoxyribodipyrimidine photolyase. Both genes can be used as a probe for identifying *M. bovis* using RT-PCR. PCR with denaturing gradient gel electrophoresis (PCR/DGGE), DNA sequencing, matrix associated laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) methods are recently used.

2.4 Treatment

Mycoplasmas are cell wall less bacteria and do not synthesize folic acid, which making them resistant to beta-lactam group of antibiotics and sulfonamides, trimethoprim respectively [29,36]. *M. bovis* is generally susceptible to protein or DNA synthesis inhibitors (certain fluoroquinolones and macrolides). *In vitro*, tulathromycin, florfenicol, gamithromycin, oxytetracycline, tilmicosin, tylosin, lyncomycin, spectinomycin and enrofloxacin are effective [25]. But those antibiotics have limited efficacy *in vivo*, they cannot penetrate and restore organs that have been affected by severe chronic disease. Therefore, antibiotic treatment must be done early in the course of the disease even prophylactically, to prevent the establishment of Mycoplasmal disease. Arthritis and mastitis caused by *M. bovis*, usually have a poor response to antibiotics. Beside antibiotic therapy, symptom associated treatments are also recommended. In arthritis, affected joints should be lavaged by a through flushing method which provides easy access to feed and water to prevent starvation and dehydration and is an effective supportive treatment for arthritic claves. For the treatment of otitis media in calves, myringotomy with irrigation of the middle ear is effective [38].

3. Prevention and Control

Vaccines have been reported to be available in developed nations, but have not proved to be protective [2,11]. Maintaining a closed herd is the best way to prevent entry of *M. bovis*, if that is not possible, to screen and quarantine newly purchased animals is recommended. A comprehensive diagnostic programme, both serological and genomic, should be conducted that can form the basis for effective control. As culture and PCR techniques for detecting infected bulls are limited by variable shedding, care should be taken on the purchase of bulls(from herds with no history of Mycoplasma associated disease is recommended). The use of separate place for infected cattle and good sectioning of different age groups of calves and young animals are very important to prevent outbreaks. Feeding of waste milk to calves should be avoided where *M. bovis* has been identified. But pasteurized or aseptically acidified or UV treated milk is safe for feeding [6,14]. Colostrums may be heat treated at 60^0 for 60 minutes to reduce the risk of transmission [18]. Calf health records should be examined if *M. bovis*-associated diseases such as mastitis, otitis media have been observed. [21]. 1% hydrogen peroxide, 1% chlorine dioxide and 1% to 0.5% iodophor should be used as a teat dip compounds in milking method [10]. It was found that 0.5% sodium hypochlorite or 2% chlorhexidine can effectively eliminate *Mycoplasma spp*. from contaminated bedding sand [19], and thus those components can be used for disinfection. Hygiene of veterinary vehicles, clothing, artificial insemination equipments and milking equipments should properly be maintained. The milking system should be thoroughly sanitized following milking infected cows. Floor, walls and other working equipments of an infected herd should be disinfected with the help of formalin or peracetic acid solutions. Cattles that recover from mastitis should be treated as persistently infected. Thus, all animals currently or previously infected with *Mycoplasma bovis* need culling.

Early detection of disease, improved husbandry conditions, and treatment with effective antimicrobial with proper dose are the best approaches in the control of the disease [36].

4. Conclusion

Infection of cattle with *M. bovis* currently plays a significant role in animal husbandry. Over the last decade, the frequency and severity of *M. bovis* infection has been increasing rapidly. Presence of frequent animal movements contaminated feeding and overcrowding were associated with the outbreak of clinical *M. bovis* disease. Due to its ability to vary the expression of some specific membrane surface proteins, *M. bovis* becomes enabling to evade the host's immune response. Ability to formation of biofilms, may also account for chronicity and the lack of chemotherapeutic response. As mycoplasmas lack a cell wall the β-lactam antibiotics are not effective. Studies show the increasing antibiotic resistance of *M. bovis, in vivo*. Thus, *M. bovis* becomes notorious over time, emphasizing the need for a reliable method of molecular typing for outbreak investigation and epidemiological surveillance.

Symptoms associated diagnosis is helpful only to some extent. As asymptomatic or subclinical phase is common in *M. bovis* infection, culturing techniques with molecular methods (e.g., ELISA, PCR, RT-PCR, DNA sequencing) are effectively useful for detection of *M. bovis.*

Development of vaccines to control *M. bovis* infections is required. More research is needed to investigate the pathogenesis and virulence factors of *M. bovis*, which is necessary to rejuvenate therapeutic and prophylactic measures to combat and to control the impact of *M. bovis* infection.

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