

Bovine mycoplasmosis

The microorganisms described within the *genus Mycoplasma spp.*, family Mycoplasmataceae, class Mollicutes, are characterized by the lack of a cell wall and by the little size (0.2-0.5 µm). They are defined as **fastidious microorganisms in *in vitro* cultivation**, as they require specific and selective *media* for the growth, which appears slower if compared to other common bacteria. Mycoplasmas can be detected in several hosts (mammals, avian, reptiles, plants) where they can act as opportunistic agents or as pathogens *sensu stricto*.

Several *Mycoplasma* species have been described in the **bovine sector**, which have been detected from different tissues and have been associated to variable kind of gross-pathology lesions. Moreover, likewise in other zoo-technical areas, *Mycoplasma spp.* infection is related to **high morbidity, low mortality and to the chronicization of the disease**. Bovine Mycoplasmosis can affect meat and dairy animals of different ages, leading to great economic losses due to the need of prophylactic/metaphylactic measures, therapy and to decreased production.

The new **holistic approach to multifactorial or chronic diseases** of human and veterinary interest has led to a greater attention also to *Mycoplasma spp.*, which despite their elementary characteristics are considered fast-evolving microorganisms. These kind of bacteria, historically considered of secondary importance apart for some species, are assuming nowadays a greater importance in the various zoo-technical fields with increased diagnostic request in veterinary medicine. Also *Acholeplasma spp.* and *Ureaplasma spp.* are described as commensal or pathogen in the bovine sector. Because of the phylogenetic similarity and the common metabolic activities, they can grow in the same selective media or can be detected through biomolecular techniques targeting *Mycoplasma spp.*.

The **main diseases** related to *Mycoplasma spp.*, *Acholeplasma spp.* and *Ureaplasma spp.* are briefly described below:

- CBPP (Contagious Bovine Pleuro Pneumonia): *M. mycoides subsp. Mycoides small colonies (SC)*, OIE-listed disease with immediate notification required, actually considered exotic in the European countries (Chapter 2.4.8. OIE Terrestrial Manual, 2014);
- BRD Complex (Bovine Respiratory Disease Complex): *M. bovis*, *M. dispar*, *M. bovirhinis*, *M. alkalescens*, *M. arginini*, *M. bovigentialium*, *Ureaplasma spp.*;
- Otitis media and interna (*M. bovis*, *M. alkalescens*, *M. bovirhinis* e *M. agalactiae*) ;
- Ocular disease like kerato-conjunctivitis (*M. bovis*, *M. bovoculi*, *M. arginini*), associated or not to *Moraxella spp.*;
- Arthritis and teno-synovitis (*M. bovis*, *M. alkalescens* e *M. arginini*);
- Mastitis (*M. bovis*, *M. canadense*, *M. californicum*, *M. alkalescens*, *M. bovirhinis*, *M. bovigentialium*, *Acholeplasma laidlawi*);
- Uro-genital disease associated to ipofertility (*M. bovigentialium*, *Ureaplasma spp.*) and abortion (*M. bovis*, *M. bovigentialium*);
- Anemia associated to *M. wenyonii (Ex-Eperythrozoon wenyonii)*.

IZSve activities and services

The Istituto Zooprofilattico Sperimentale delle Venezie (IZSve, www.izsvenezie.com) developed several **techniques for *intra vitam* and/or *post mortem* diagnosis** of bovine micoplasmosis.

Intra vitam diagnosis of Mycoplasma bovis

The SCS3 and SCT3 Departments in Legnaro (Padova) provides a **real-time PCR** that can be applied to different biological matrix (milk included) for the confirmation of disease in symptomatic animals. Then, an **ELISA test is available** for *Mycoplasma bovis* antibody detection from different specimens (i.e animal sera and milk); this test is available by the SCT3 Serology Laboratory and can be a useful tool to point out the presence and or the prevalence of infection in a herd.

Post mortem diagnosis of Mycoplasma bovis

In addition to the real-time PCR for *M. bovis*, this pathogen can be investigated *via immunohistochemistry in histological sections of affected tissues* collected during post-mortem examination, this analysis is performed at the SCS3 Histopathology Laboratory.

Moreover, for the confirmation of *M. bovis* infection and/or other species of relevant mycoplasmas (for which specific PCRs are not available), the O.U. Mycoplasma (located in Legnaro, Padova) offers a **panel of diagnostic tests**, which can be applied to different specimens, collected in the field or during the necropsy activities. Thus animal swabs (from nose, lung, bronchi, ear, eye), joint fluid, lavage of the respiratory tracts (tracheal lavage, broncho-alveolar lavage) or of the reproductive tracts and milk, can be submitted to the O.U. Mycoplasma for the microbiological cultivation of Mollicutes (MYCMIC/MYCM).

In case of positivity, the identification of mycoplasma isolates can be achieved through the 16S-PCR-DGGE; this **biomolecular technique** allows the detection of different species and genera of Mollicutes (i.e *Mycoplasma spp.*, *Acholeplasma spp.* e *Ureaplasma spp.*) and is capable to point out single or multiple infections in the same sample. Moreover, the application of DGGE together with further analysis, such as the amplification of target genes followed by sequencing, can be useful in the possible recognition of novel (emerging pathogens) or exotic (i.e. CBPP) species of Mollicutes.

The **combination of microbiological and biomolecular tests** allows to improve the information concerning the presence of relevant *Mycoplasma spp.* in the bovine sector, achieving deeper knowledges to better understand epidemiology and pathology of mycoplasmas in cattle. Then, this laboratory is performing research activities on MLST- *Multi Locus Sequence Typing*- for *M. bovis*.

Antimicrobial susceptibility and resistance

In addition the O.U. Mycoplasma have a long experience in AST (Antimicrobial Susceptibility Test) applied to mycoplasmas and in detail phenotypic characterization of drug susceptibility and/or resistance in veterinarian mycoplasma species via **MIC (Minimum Inhibitory Concentration)** using commercial or home made plates.

This results have been collected in an **internal database**, which could be useful for the assessment of a drug therapy in the field and especially for a fast detection of any critical antibiotic profile of microbial populations and/or antibiotics' molecules in micro- and macro-areas ("preparedness"). As the MIC is

consider the gold standard technique to monitor the development of antibiotic resistance mechanism, the results provided from this technique could be a useful tool to promote a **conscious use of antibiotics also in veterinary practice**.

Finally, *in vitro* cultivation and strain collection of field isolates allow to perform deeper analysis (i.e. genotyping, experimental infection, autologous vaccine, etc.).

Publications

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