Istituto Zooprofilattico Sperimentale delle Venezie

Avian 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 **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. Moreover, mycoplasmas can be detected in several hosts (mammals, avian, reptiles, plants) where they can act as opportunistic agents or as pathogens *in sensu stricto*. Avian mycoplasmosis **cause great economic losses in the poultry industry**, due to prophylaxis, therapy, decreased production performances and high carcass condemnations.

More than 25 Mycoplasma species, with variable pathogenic role, have been detected in birds up to now. The scientific literature widely demonstrated the pathogenic role of *Mycoplasma gallispeticum* (MG), *Mycoplasma synoviae* (MS), *Mycoplasma iowae* (MI) and *Mycoplasma meleagridis* (MM) in the different avian categories and consequently **prophylaxis (direct and indirect) and monitoring programs are routinely applied in the field**.

As these pathogens are **horizontally and vertically transmitted**, the development and maintenance of mycoplasma-free breeder groups, associated to the application of strict biosecurity measures, are key factors in containing the spread of these bacteria. Besides high morbidity, the mortality rate is low but basing on pathogen-host-environment conditions **variable mortality can be reported** (secondary bacterial and/or viral diseases, age of birds, animal density, ventilation and litter quality).

The affected birds develop a chronic disease and show **decreased food consumption, higher food conversion rate, and temporary drop of eggs production** and in layer sector eggs disorder classified as EAA (*Egg Shell Apex abnormalities*). Then high carcass condemnations can occur after the onset of secondary bacterial infection (*E.coli*).

The correct **strategy for control and management of avian mycoplasmosis** should be based on epidemiological situation, farm characteristics and producer standards and needs, and can include several actions such as birds' depopulation, compartment zones, strict biosecurity, vaccination program and antibiotic therapy.

IZSVe activities and services

The Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe, www.izsvenezie.com) developed through years several **laboratory techniques for the direct and indirect diagnosis** of avian mycoplasmosis.

Intra vitam diagnosis

The Avian Medicine Laboratory (SCT1 - Verona and Vicenza) perform *Mycoplasma synoviae* **Real-Time PCR and an end-point *Mycoplasma gallisepticum* PCR from different specimens**. These laboratory techniques

are useful for a rapid detection in monitoring plan (as routinely applied in the breeders' sector) or for confirmation in symptomatic flocks.

The SCS6 Virology Laboratory provides indirect diagnosis of *Mycoplasma synoviae*, *Mycoplasma gallisepticum* and *Mycoplasma meleagridis* via both **ELISA and RSA (Rapid Serum Agglutination)**. Serology for mycoplasmas can be a useful tool to point out presence and/or prevalence of infection in the poultry industry and for the evaluation of vaccination and/or eradication programs.

Post portem diagnosis

The Avian Medicine Laboratory (SCT1 Verona and Vicenza) provides a **service of gross-pathology examination** for confirmation of avian mycoplasmosis performing *Mycoplasma synoviae* real-time PCR and an end-point *Mycoplasma gallisepticum* PCR from different specimens. Moreover, for the confirmation of MG, MS, MI, MM infection and/or other species of relevant mycoplasmas (for which specific PCRs are not available), the SCT1 O.U. Mycoplasma (located in Legnaro. Padova) offers a panel of diagnostic tests for *Mycoplasma spp.* diagnosis from different samples, which can be collected during field or necropsy activities.

- Different diagnostic samples such as animal swabs (tracheal, nasal, sinus, cloacal, etc.), joint fluid, eggs and embryonated eggs, environmental specimens can be submitted to the O.U. Mycoplasma for the **microbiological cultivation** of Mollicutes. (MYCMIC/MYCA).
- In case of positivity, the **species identification** 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. Co-infections of different species of avian mycoplasma has been established in backward birds, in the layer sector, and in minor avian species (like in gamebirds and in ducks and goose). Moreover, the application of DGGE together with 16S rRNA and sequencing can be useful in the possible recognition of novel mycoplasmas for poultry (emerging pathogens).
- Then, the O.U. Mycoplasma provide **genotyping analysis** for *Mycoplasma synoviae* (vlhA gene) and *Mycoplasma gallisepticum* (mgC2) and a MLST analysis (Multilocus Sequence Typing) for *Mycoplasma synoviae* has actually been implemented.

Antimicrobial susceptibility and resistance

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

These 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 performing deeper analysis (i.e. genotyping, experimental infection, autologous vaccine, etc.).

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