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Avian Mycoplasmosis-recent updates

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Mycoplasma gallisepticum (Mg) and Mycoplasma synoviae (Ms) are the most important avian mycoplasma species for the commercial poultry industry. Both mycoplasma species are known to be of clinical and economic relevance to the poultry industry. The control of both mycoplasma species contributes to more profitable poultry production however, control measures to achieve this depend on the prevalence, housing type (single versus multi-age) and geographical area (or integration). Mg is regarded as a risk for commercial poultry, especially in the presence of other respiratory agents, and is responsible for respiratory disease, increased mortality, and in broilers, increased condemnation rates at slaughter. The clinical and economic relevance of Mg for the commercial poultry industry was recognized 70 years ago, and the control programmes have been running since then.

On the other hand, Ms has always been present in commercial poultry but its clinical and economic relevance has been debatable.. Meta-analysis of recently published prevalence data for India shows a marked increase in the prevalence of Ms (Ramasamy et al. 2021; Yadav et al. 2022; Giram et al. 2022). Information from these studies and recently developed diagnostic tests provide insight into the clinical and economic relevance of Ms for the poultry industry. New diagnostic tests and research evidenced the presence of Ms strains related to respiratory disease, infectious synovitis, eggshell abnormalities, and egg production losses. Subclinical infections are still frequently reported. Due to the increasing reports on primary pathogenic Ms strains, the clinical and economic relevance has become less debatable. The latter has also led to more focus on the control of Ms in the poultry industry (Feberwee et al. 2022).

The understanding of reservoirs and survival of both Mg and Ms outside the host has improved over the years. Both mycoplasma species are transmitted from parent to progeny through the egg (vertical transmission) and by horizontal transmission (direct, indirect contact), and lifelong carriership after recovery (Feberwee et al. 2022). Both mycoplasma species can survive outside the host but only for a few (1-4) days. However, survival is low on most materials except for those contaminated with egg materials, in which both species can survive for several months. This makes materials contaminated with egg debris a major risk factor in the horizontal transmission of Mg and Ms (Christensen et al. 1994; Abolnik & Gouws, 2014). Artificial Insemination, multi-age housing and poor hygiene management are risk factors for introducing and transmitting Mg and Ms (Buntz et al. 1986). Vertical transmission is responsible for continuing the mycoplasma cycle in poultry production. The highest vertical transmission rate occurs in the acute phase of the infection. Vertical transmission usually accounts for 2-3% of infected chicks. It is in the progeny growing phase that the horizontal spread occurs exponentially. This highlights the importance of hatchery hygiene, biosecurity, and hygiene of the farms, and most importantly, controlling Mycoplasma in breeders is a prerequisite for interrupting vertical transmission.

The control approach is dependent on the prevalence level. Slaughter of infected parent stock and biosecurity measurements are important in controlling vertical and horizontal transmission of Mg and Ms in a low-prevalence situation. However, slaughtering of infected parent stock to cut down the vertical transmission route is not economically sustainable in a situation of high prevalence. In a situation of high prevalence, besides good biosecurity, antimicrobial treatment and vaccination programmes using live vaccines are essential tools in controlling vertical and horizontal transmission (Feberwee et al 2022). In geographical areas with a high poultry density and the presence of multi-age sites, Mg and Ms control will be a even more significant challenge. The approach in the control of Ms and Mg has to be tailor-made depending on the feasible goals under the geographical or integration prevalences (ter Veen et al. 2020). In an infected flock, an antimycoplasma antibiotic treatment given at a defined time point before a live vaccine will ensure that the vaccine strain may develop to its optimal protective effect during the flocks life. In this way, the live vaccine will establish itself in the flock without interference by an already present field strain. An antibiotic treatment can also be given when proven by laboratory diagnosis and the onset of clinical signs that a field strain has taken hold in the flock (Schonewille & Uriarte, 2023). Channelling clean chicks to all-in-all-out premises and, chicks from known infected parents to previously infected premises, and compulsory vaccination of chicks in multi-age infected premises with live mycoplasma vaccines is known to reduce the prevalence of mycoplasmosis and spread of the bacteria (ter Veen et al. 2020).

Phylogenetic analysis of *mgc2* and *vlhA* gene sequences of Indian Mg and Ms isolates showed a close clustering of these with that of *mgc2* and *vlhA* gene sequences of Australian vaccine strains Mg ts-11 for Mg and MS-H for Ms, respectively (sequences retrieved from NCBI GenBank)(Giram et al 2022; Ramadass et al. 2006). This is very encouraging as it supports the use of these two vaccines to control Mycoplasma in India. MG-F falls in a separate clade. There were some closeness reported 6/85 in one sample as well (Giram et al. 2022). It all comes down to considering the safety and efficacy of the vaccines when considering which vaccine to choose for the purpose. There is no benefit in using a very safe and low efficacious vaccine when it does not provide any control. At the same time, a less safe, highly efficacious vaccine used in a place where challenge is minimal is also a waste. So, to get the maximum benefit, one needs to balance safety and efficacy. Live vaccines are the best option when you have a serious mycoplasma situation and the aim is to control the clinical effects and eradicate Mycoplasma. Killed vaccines provide some control against clinical signs but none against infection so, it is of no use in eradication programs. Egg production drops have also been recorded if given near or during production. Recombinant vaccines are best used in very low to no challenge areas where the birds are unlikely to be affected by a virulent mycoplasma. Similarly it has no place in eradication programs.

While Vaxsafe MS-H remains the most efficacious live vaccine for Ms control, research into finding a better live Mg vaccine is continuing. Recently, Vaxsafe Mg ts-304 was registered by the APVMA in Australia after demonstrating safety and efficacy in chickens vaccinated by eye drops at three weeks of age. Laboratory studies have demonstrated that a single vaccine application of this novel freeze-dried product provides protection to at least 60 weeks of age (Condello et al. 2020b). This is similar to the persistence shown by Mg ts-11 vaccine everywhere, including India (Morrow & Achari, 2020). Application on-farm during rearing is becoming more difficult for producers due to lack of skilled labour. There is also a significant biosecurity risk of mobilising

vaccination crews between farms. Applying vaccines in the hatchery, ideally using mass administration methods, can improve animal welfare, reduce labour costs and improve biosecurity associated with handling each bird in the field. A suite of studies has been recently conducted with Vaxsafe Mg ts-304 to assess colonisation, immunogenicity and protection in day-old SPF and commercial chickens using gel-spray, coarse-spray and eye-drop methods. A study was also conducted to evaluate the protection induced by Vaxsafe Mg ts-304 after administration to a- day-old SPF chicks and challenge with a virulent strain of Mg strainAp3AS in isolators at APCAH, University of Melbourne. Mg ts-304 vaccine was delivered as a single application either at a high dose ($10^{7.0}$ CCU) or a low dose ($10^{5.7}$ CCU) via eye-drop, coarse-spray in water, and gel-spray in Vaxsafe Live Gel. Vaccine efficacy was assessed after two weeks after a challenge by nebulisation with a virulent *Mg.* strain Ap3AS at seven weeks of age. Vaxsafe Mg ts-304 could be detected in palatine cleft swabs by qPCR after vaccination by eye drop and both spray application methods (only at the higher dose when applied by spray). Similar to earlier reports for Mg ts-11 and MS-H (Morrow et al. 2023), seroconversion (immunogenicity) at six weeks of age correlated well with colonisation rates (Condello et al 2020a). Vaxsafe Mg ts-304 was shown to be safe at the higher dose, and by all administration methods. Protective immunity (as measured by tracheal mucosal thickness and air sac lesion scores) was evident after vaccination by eye-drop and both spray methods but only after vaccination at the higher dose. At the lower doses delivered by spray, individual birds were protected within each group. Vaxsafe Mg ts-304 was safe when applied to day-old SPF chicks by eye-drop and various spray methods. The vaccine was also shown to be effective after day-old application by eye-drop, but it was more dose-dependent after mass vaccination methods. These results suggest that Vaxsafe Mg ts-304 can be applied by spray to day-old chicks; however, the lower efficiency of vaccine uptake associated with mass administration methods was correlated with lower protection of the group at lower doses (Condello et al, 2020a, Arachchige et al. 2021).

The primary immune escape strategies employed by Mycoplasma include invasion, biofilm formation and regulation of immune response (through the suppression of immune cell activity and function as immunomodulatory molecule modulation). Bacterial biofilm plays an important role in the bacterial disease process, allowing bacteria to evade the host's immune defences, inducing drug resistance and increasing toxin accumulation (Sorci, 2013). The ability to form biofilms suggests survival advantage and increased resistance to disinfectants hence a risk factor for horizontal transmission leading to persistent infections and making the eradication programs challenging (Feng et al. 2020; Wang et al. 2017). This biofilm formation also increases the survival potency inside the host, thus increasing the potency for vertical transmission (Chen et al. 2021). Strains Nobilis Mg 6/85, S6 (P5 and P20), D9604, and SU15 were reported to be strong biofilm producers. Strains Rlow (P10 and P100), NCL, CG5, YL4, and F were weak biofilm producers. Strains Vaxsafe Mg ts-11 and F36 did not produce biofilm as verified using a crystal violet staining assay (Chen et al. 2012). Compared with the planktonic Mycoplasma, these biofilm-grown cultures were more resistant to tetracycline, gentamicin, and Triton X-100 treatments. The results indicated that the transcriptions of some genes in the biofilm-grown cells were markedly decreased, including vlhA3.03, csmC, hatA, gapA, neuraminidase, and mgc2 (Chen et al. 2012). Even though Vaxsafe ts-11 is reported not to produce biofilms, it has been shown to be able to tolerate up to 7ppm of chlorine in drinking water when tested for up to 240 minutes (Achari et al. 2023a).

Additionally, Mycoplasma employs antigenic variation strategies both at genomic level and post transcriptionally. Molecular mimicry is also involved in mycoplasma immune escape.

Immunosuppression has been shown to increase the pathogenicity of mycoplasmas (Bao et al. 2020; Prezotto et al. 2016) and negatively impact the efficacy of vaccination in Mg infections (Arachchige et al. 2021). Chicken anaemia virus (CAV) and infectious bursal disease are common causes of immunosuppression in chickens. Mycoplasma immunity highly depends on local cell-mediated immunity in the trachea. This is highly dependent on the health of the bursa of Fabricius and the thymus for B and T cells, respectively (Omotainse et al. 2023). Vaccinating birds for Mycoplasma before known infection time points with CAV and IBD agent is known to provide relief and increase the protection capabilities of the vaccines. The proper use of live vaccines for CAV and IBD will need to be considered when formulating vaccination programs in mycoplasma infected premises. Similar considerations must be given when infectious laryngotracheitis (ILT) is circulating.

Decreased antibiotic susceptibility in several Mycoplasma species are also known to be associated with mutations in topoisomerase and ribosomal genes that result in (i) Altering the cellular permeability to avoid entry of antibiotics into the cell, (ii) modifying the targets of the antibiotics so that they can no longer act on them, (iii) enzymatic modification of antibiotics to render them inactive but other strategies such as (iv) expression of active efflux pump mechanisms that actively pump out antibiotics from cell interior are also described (Nagy et al 2023; Schonewille & Uriarte, 2023; Sharma et al 2019). Currently, in India, similar to other Asian countries, the poultry growers have realised dosage creep associated with common anti mycoplasma antimicrobials. Minimum inhibitory concentrations (MIC) test results were recently discussed, where Mg isolates from Vietnam were found to be resistant to seven frequently prescribed antimicrobials with anti-mycoplasma activity. This is quite possibly a result of the extensive repeated use of the same antibiotics over several years (Achari et al. 2023b; Morrow et al. 2020). A similar survey is currently underway for Indian strains.

First of all, in diseases with few distinct pathognomonic clinical signs such as Mg and Ms lab support is needed to confirm clinical suspects and to develop further action plans in control strategies (Schonewille & Uriarte, 2023; Feberwee et al. 2022). Commercial serological tests (RPA and ELISA-tests) and commercial PCR tests, including PCR tests can differentiate between vaccine and field strain (DIVA) are available nowadays. Serological tests are the most commonly used tests as serology is quick, inexpensive and best for screening purposes in flocks not vaccinated with live vaccines. Live vaccines have been shown to generate a serological response, which can complicate interpretations of the ELISA test results. Although more expensive than serology, PCR tests have become important in the control of Mg and Ms. Furthermore, sequence-based strain typing techniques can help monitor the persistence or the introduction of new infections which can help monitor the effectiveness of control strategies. PCR and molecular typing tests are not always available on location so sampling on FTA cards to perform a PCR or molecular typing test can be regarded as a good and valuable alternative.

In a situation of low prevalence, the focus will be on detecting absence or low level of infection. In a situation of high prevalence, diagnostic tests play an important role in monitoring the effect of measurements implemented to control Ms and Mg, to monitor Ms or Mg status before vaccination

or to monitor the status of parent stock through the day-old chicks. Sample frequency and size and test- characteristics play a role in the efficiency of the monitoring programmes. Also other factors such as circulating Mycoplasma species, vaccination programme, and immunity etc, can influence the performance of the tests. These factors can even differ per geographical area or integration. Also the application of Ms and Mg vaccines and use of antimicrobial treatments will influence the results of diagnostic tests.

Live Vaccines work!

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