BIOPROPERTIES
The Vaccine Innovators

TECH bulletin

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Control of Avian Mycoplasma by Live Vaccination

MG ts-11 and MS MSH vaccines offer protection to poultry producers against the effects of field strain challenge. From experience, these vaccine strains colonize the chicken in vaccinated flocks for life (similar to field strains) and provide an increased resistance of the flock to wild strain challenge or disease, pathogenic effects and production effects. This protection is for life and protection is associated with continued presence of the vaccine strain.

These vaccines are sometimes considered expensive to buy (compared to other poultry vaccines on a 'per dose' basis), expensive to administer (the labour cost of eye drop), difficult to store and transport but they are the best protection against avian pathogenic Mycoplasma when used correctly (especially with the increased availability of Mycoplasma free replacement stock). Really the best control is the most cost effective total programme. They offer an alternative to antibiotic based prophylaxis programmes (which are more expensive and make chicken production a potential source of AMR organisms – a threat to human health).

The protection these vaccines generate lasts for life after successful vaccination with rapid colonization of the bird and persistent upper respiratory tract populations thereafter. With MG ts-11 these populations may start to decrease after peaking at 4 weeks after vaccination but then build up again after laying begins and then become easy to demonstrate for the life of the vaccinated bird. The mechanism(s) of immunity to resist infection and the effects of infection have not been elucidated yet but does not seem to be humoral antibody (which is the only response to commercial killed vaccines that has been described). It is also unlikely to be competitive exclusion as after peaking the tracheal populations stabilize at a lower level for the life of the bird (surely this suggests that many potential attachment sites are unoccupied).

Immunity to development of disease and infection can be demonstrated in bird experiments after challenge (prevention or reduction of airsacculitis and/or prevention of tracheal mucosal thickening) and this protection is probably local immunity with vaccine immunoglobulin and/or CMI; local or systemic immunity (like coccidiosis immunity). The immune response to MG and MS field strains seems to be a major part of the pathology seen and biological cost of infection. That said the protection is susceptible to immunosuppression (notably by IBD and CAV).

Recommended worldwide programme for use with Mycoplasma-free replacement stock

- No anti-mycoplasmal antibiotics for 4 days before vaccination
- No LaSota or stronger NDV vaccine within 14 days of live Mycoplasma vaccination
- Eyedrop with MG ts-11 and MS MSH together after 21 days to 6 weeks
- No prophylactic anti-mycoplasmal antibiotics after vaccination
    - Only treat flocks that are clinically sick
        - For necrotic enteritis use amoxycillin or other penicillin.
    - If you have CRD after this time:
        - Reassess the status of your replacement stock
        - Look at possible sources of potent early horizontal challenge (try to implement biosecurity till 5 weeks after vaccination.
- No need for antibiotics, killed Mycoplasma vaccines, or serology after live vaccination (for cost and potential interference reasons)

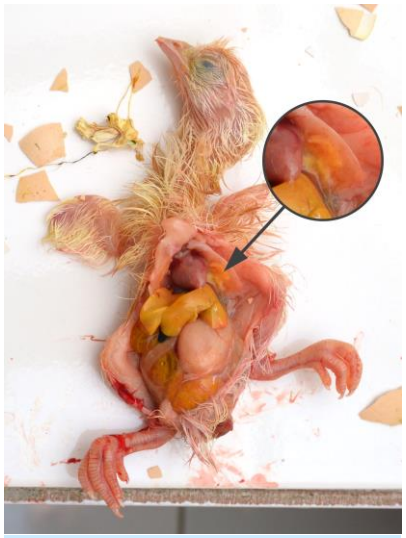
This programme has been very successful everywhere. Vaccination of vertically infected flocks can have benefits for pullet rearers with slight modification of the recommended programme. Vaccination at transfer has been more successful than initially expected.

The maintenance of protective immunity is dependent on the maintenance of vaccine infection. In a recent experiment the continuous feeding of tylosin to birds (this decreased the vaccine population) that had been vaccinated was able to abolish the vaccine immunity after 20 weeks. This is longer than one might have expected but the experiments perhaps underestimate the cumulative effects of immunosuppression from other infections, nutrition, physiological stressers that birds may be faced with in the field.

The chronic infection state that wild strain avian Mycoplasmas establish could be the reason why there is such a big biological cost to being infected. For an organism to stay in high levels on these mucosal sites it needs to evade the host immune system. Mechanisms that have been described to date include epitope switching, Fc receptors, intracellular invasion, and biofilm formation. There are probably more mechanisms.

The immune system is actively chasing these parasites and under some conditions (typically respiratory infections) it can flare up into clinical disease. At sub-clinical levels it costs nutrients that need to be diverted from growth or egg production to the immune pursuit.

The estimates of these biological (and financial) costs can be made by comparing infected flocks to uninfected flocks. For MG this was estimated as 10-20 eggs per hen per year in infected hens. The impact of MS was estimated to be about half the number of eggs lost due to MG infection (Stipkovits & Kempf, 1996).



Airsacculitis in pipped embryos can be monitored to confirm effective Mycoplasma control. When >20% then diagnostic investigation is warranted.

Proxy estimations have been made for MG of the effect on feed conversion by comparing infected flocks on continuous antibiotics to infected flocks (Ose et al, 1979). Here the efficiency of converting feed into eggs was 12% better in the treated birds. No estimates of the effects on efficacy of egg production or meat production for MS are published. Proxy estimate for layers for the effects of MS infection are available from comparisons of vaccinated flocks versus infected flocks. More than 4% better feed efficiency in the conversion of feed into eggs can be demonstrated regularly. If you consider that feed is 70% of the cost of production of eggs in all parts of the world this is a 3% decrease in the cost of production (and feed costs are an ongoing cost every week independent of the price of eggs). No estimates of the effects on the production efficiency of MS infected broilers are available. Barbour et al (2000) showed that field MG infection in comparison to vaccinated flocks resulted in 20% poorer FCR. This reinforces the need for control of MG and MS field infections in layers as well as breeders.

MS infection on arrival at multiage farms seems to trigger *E.coli* perionitis at the beginning of lay and a mortality spike.

Finally, avian Mycoplasma infection control has made the poultry industries in some areas very dependent on antibiotics. In laying birds this is seen with programmes where antibiotics are used every 4 to 8 weeks throughout lay and broilers where antibiotics are routinely used around 18-22 days of age. ■

Controlling Mycoplasma infections in a cost-efficient way

Although Mycoplasma freedom has long been considered the ultimate goal of the poultry industries, it is risky and thus a plan B should be in place for flocks that become infected. Usually the flock gets culled if the producer is agreeable but when it comes to a second flock to be culled then a think tank comes up with the suggestion to use antibiotics and streaming of progeny from the infected flock with early culling of infected flocks as a concession. On multi-age sites (that we have often inherited) antibiotics are suggested even earlier.

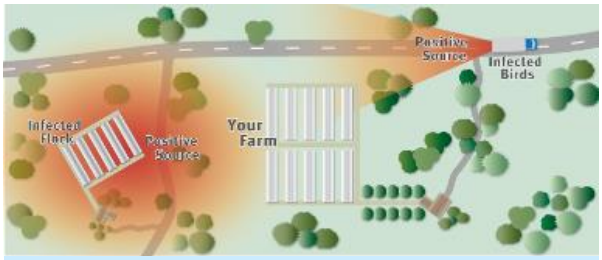
The problem with Mycoplasma freedom as a strategy is that the Mycoplasma free flocks are totally susceptible to getting infected. One way of looking at live Mycoplasma vaccination is that you are increasing the resistance of the birds to field Mycoplasma challenge. Broiler breeders aim to be free from field Mycoplasma while layer producers have a variable attitude. This means layers who do not control MS can be a reservoir for broiler industry. Some people only control MG and this is problematic as MS will have an impact. Vaccination as a strategy only works if all the expected challenges are covered. If a region has good MG control with freedom then perhaps MG vaccination is not needed but this is rare and becoming rarer.

Some people want to use vaccination on the way to Mycoplasma freedom. This only has very limited application as the risks for reinfection may still be too much for the farm where it is being considered. The neighbouring farms may still be a potential reservoir

It makes sense to control MS as well as MG and to use the same strategy for both Mycoplasmas. MS is important – its importance becomes more apparent as MG is effectively controlled. MS can mimic anything MG can do. The variation in pathogenicity of MG or MS strains is greater than the variation between MG and MS strains.

that cannot be moved away. The protection needed from a vaccine are those to resist the local challenge.

The costs of Mycoplasma control strategies and effectiveness need to be compared in choosing which Mycoplasma control strategy to adopt. It is important that the components of the control strategy do not negatively interfere with the efficacy of other components of control. For example, antibiotics after



Protect against airborne challenge by vaccination

vaccination can interfere with vaccine immunity as mentioned above. Antibiotics can also interfere with serological monitoring in freedom programmes. Serological monitoring in vaccination-based programmes is non-informative especially as vaccinated flocks with MSH and ts-11 may seroconvert as they reach peak production (but with no sign of wild strains and no clinical signs) (Zavala et al, 2015) and the large variation in serological response seen between flocks in the field. Indeed this late seroconversion is probably the origin of the myth at ts-11 only works to 40 weeks post vaccination (Serology bulletin, www.bioproperties.com.au).

The total costs of Mycoplasma control by vaccination are the cost of a single dose of MSH and ts-11 and the cost of administration. This compares favourably with antibiotic programmes where antibiotics are given one day a week or one week a month in lay. Antibiotics for control is under pressure as there is a need to phase out antibiotic prophylaxis in animal production systems for human health reasons. It is cheaper than using killed vaccines including autogenous multivalent vaccines without even considering the poor control killed vaccines give and the possibility that they could interfere with the efficacy of live vaccines (Glisson & Kleven, 1984). There is a tendency for consultants to finesse the programmes recommended by the vaccine supplier but this is unnecessary and could be counter-productive.

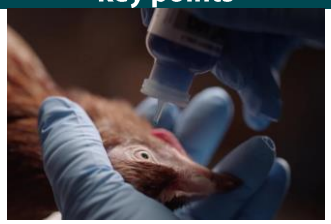
You still need biosecurity but these vaccines are able to protect from the challenges flocks are faced today in our modern production systems. With the procurement of Mycoplasma free replacement stock and use of both MS and MG live vaccines, protection against challenge from neighbouring flocks (even on the same site) and production of Mycoplasma free progeny will be achievable. No immunity is passed to the progeny (maternal antibody is considered ineffective in protecting progeny) but horizontal challenge in broilers is not a big problem with a little biosecurity and long-lived progeny (layers and breeders) can be vaccinated.

Even if availability of Mycoplasma free replacement stock is not possible, there are benefits of using these vaccines. There are differences between live Mycoplasma vaccines for chickens and indeed some vaccines seem to have changed over time. There are differences in efficacy but sometimes the differences in presentation seem to be more important in some areas of the world. If choosing a less efficacious vaccine programme purely based on cost or convenience does not guarantee the protection and benefits needed, then you should consider MSH and ts-11. The synergistic effects of Mycoplasma field strain infection on other poultry infections will also see benefits from effective Mycoplasma control in the control of other viral and bacterial diseases. ■

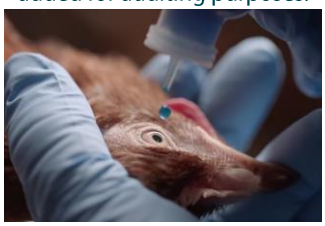
BENEFITS FROM LIVE MYCOPLASMA VACCINATION – trial results

Trial Location	MSH	ts-11	Type	Advantage	FCR Advantage	Comments
Australia 1996		●	Layers	+ 8 eggs / HH	Not measured	Whithear et al, 1996
USA 1996	●		Layers	+ 4 eggs / HH	4% less / egg	Merial Select internal trial results 1997
Japan 2007	●		Layers	+ 11.4 eggs / HH	6% less / egg	Ouchi et al, 2009
	●		Layers	+ 13.4 eggs / HH	3.5% less / egg	
Indonesia 2014	●	●	Breeders	+ 22 chicks	12% less feed / chick	International Hatchery Practice 2015
France 2016	●		Layers	+ 8 eggs / HH	4% less / egg	DuBord et al, 2017
Argentina 2020	●		Layers	+ 14 eggs / HH	8% less / egg	Boehringer Ingelheim trial results
Croatia 2020	●		Layers	+ 14 eggs / HH	14% less / egg	L. Korosi - unpublished trial

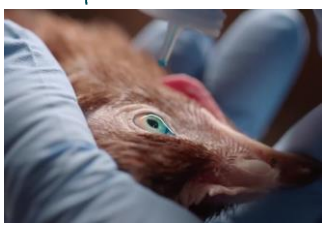
Successful vaccination key points



The tip should be perpendicular to the eye to deliver 30µl of the vaccine. A blue dye can be added for auditing purposes.



Allow the drop to form and fall, i.e. the drop should not be touching the eye. In this way 30µl is administered.



After 3 weeks of age, a birds eye is capable of absorbing 45µl and the tip is designed to deliver 30µl.

Birds should blink before release for successful uptake of the vaccine.

Successfully vaccinated birds should show tongue and palate cleft coloration with the dye within a minute of vaccination.

Abbreviations

AMR	antimicrobial resistance
CAV	Chicken anaemia virus
CMI	Cell mediated immunity
CRD	Chronic respiratory disease
DIVA	Differentiation of infected from vaccinated animals
FCR	Feed conversion ratio
Fc receptor	bacterial protein binding fragment C of the antibody molecule.
IBD	Infectious bursal disease
MG	*Mycoplasma gallisepticum*
MS	*Mycoplasma synoviae*
NDV	Newcastle disease virus
PCR	polymerase chain reaction

Monitoring vaccinated flocks

1. Audit the vaccination process and make sure the cold chain is maintained
    a. Do not store vaccine for greater than 4 weeks at -20°C (use chest freezer)
    b. Follow the thawing instructions
    c. Administer by eye-drop
    d. Use approved dye for training, increasing drop accuracy in low lighting and assessment of application
2. Monitor the health and production of the flock
3. In breeders you can also routinely monitor progeny
    a. Pipped embryo monitoring of hatch debris (over 20% means Mycoplasma is not being controlled)
    b. Monitor status of progeny (serology at 42 days – off sexes)
4. Investigate problems with DIVA PCR technology
5. Remember purchasing hatching eggs, spiking with vaccinated males (will cause seroconversion of unvaccinated free flocks but no disease impact) and other "biosecurity suspect" management interventions are risks
6. Serology results are irrelevant without clinical signs.

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BIOPROPERTIES
The Vaccine Innovators

Bioproperties Pty Ltd
ABN 49 007 303 728

36 Charter Street
Ringwood Vic 3134
Australia

T: +61 3 9871 2000
F: +61 3 9876 0556
W: www.bioproperties.com.au

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