Mycoplasma ts vaccines – 20 years field experience, pen trials and myths

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The recognition of Mycoplasma gallisepticum (MG) as a major cause of sickness in intensively farmed chickens led initially to two main approaches for control.

First was antibiotics but the high failure rate of these chemicals to consistently eradicate the organism was the initial major problem soon followed with acquired resistance and residue control.

Secondly was the idea that mycoplasmas could be universally eradicated. This later idea was successfully done at the elite breeding level providing industry with free stock but horizontal transmission and fragmentation of the effort to eradicate resulted in less successful results at production levels.

One reason for this was mycoplasmas transmission can be airborne between farms in areas with dense poultry populations (Fig. 1). No matter how well you upgraded your own biosecurity, breaks could still occur with contamination coming from your neighbours and other sources outside your control.

The introduction of vaccines was the next attempt to control avian mycoplasmas (see Table 1).

Each successive vaccine generation tried to solve perceived problems in previous products. Initially these were disease problems but more recently the focus has been on eradication of wild strains.

The third generation MG vaccine ts-11 was the first commercial MG vaccine that was specifically attenuated (selected for temperature sensitivity (ts)) and selected to combat the effects of wild MG challenge.

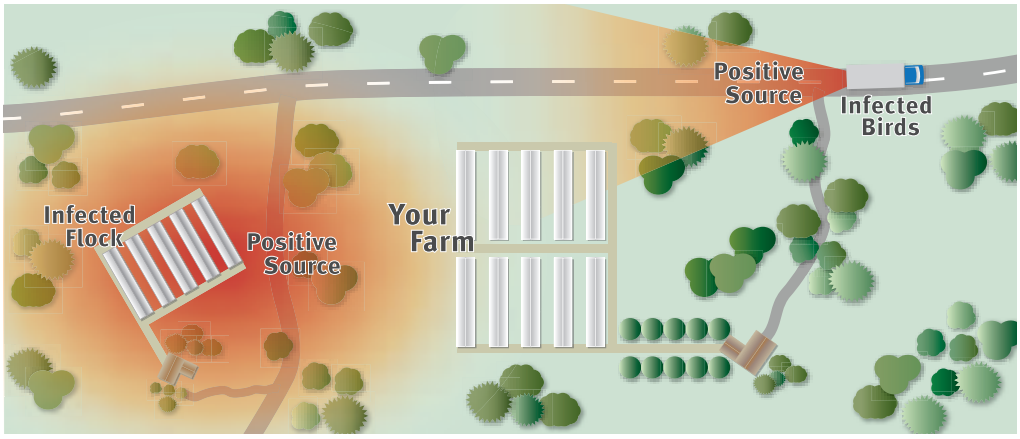

Fig. 1. Risk factors for transmission of MG and MS between flocks. Distance is the most important factor for decreasing risk and the basis of good biosecurity.

Australian field studies with ts-11 showed no production penalty from vaccination compared to unvaccinated, unchallenged paired flocks and a gain of eight eggs per hen when placed on MG infected multi-age farms.

Ts-11 was introduced into the field in Australia about 20 years ago and its success prompted the development of MS-H vaccine. Perhaps this is a good time to reflect on our subsequent experience with these vaccines.

Table 1 summarises the history of avian mycoplasma vaccination. The big advantage of the ts vaccines is the attenuation makes them very safe but they still are immunogenic (protective; not necessarily causing serum antibodies).

Table 1. History of the development of avian mycoplasma vaccines.

Vaccine strategy	Examples	Advantages	Disadvantages
Killed bacterins 1960s	Numerous	No live organisms so reversion to virulence (RTV) cannot occur	Limited duration of immunity and delay of onset of egg production. Does not stop wild type infection or vertical transmission
First generation live (controlled exposure) 1960s	Local wild strains and strains arriving vertically from breeding programmes	Prevented egg production drops in lay by making sure infection occurred before lay	Loss of up to 20 eggs per hen and increased FCR etc from subclinical effects of infection
Second generation live (mild strains) 1970s	F strain 6/85 K5831 Others	Do not have to worry about RTV issues during registration, (the strain is mild)	Appears to be direct relationship between immunogenicity and residual pathogenicity
Third generation live (attenuated strains) 1980s	ts-11 MS-H	Dissociation of the relationship between immunogenicity and pathogenicity	No transfer of protection to progeny
Vectored subunit vaccines 1990s	MG pox vectored virus GMO	No serological response	Limited duration of infection by vaccine and limited MG antigens will provide limited immunity

Transmission myths

There are many myths associated with avian mycoplasma. Poultry production managers in the USA and UK have often thought that MS transmission is not airborne and this myth comes from emphasis by mycoplasma experts on transmission routes that the production manager can influence and less emphasis on those (perceived) that a manager can not influence.

The reasoning is farm layout (even with 400 yards between sheds) and proximity of other potential sources of infection can not be altered so concentrate on portals of entry we can influence (like movement on staff). This needs re-evaluation because live vaccines offer protection against these sources of contamination like taking out insurance.

Mycoplasma eradication as the final goal at all levels of poultry production has been deeply indoctrinated into poultry managers but this idea was developed before effective live vaccines were available. Why

Continued on page 15

Group	Antibody at time of challenge	Assessment of immunity by measurement of tracheal thickening after challenge
Non-vaccinated controls	No demonstrable antibody	No protection against tracheal thickening with virulent MG challenge
Birds selected from vaccinated flocks with no measurable antibody	No demonstrable antibody	Protection against tracheal thickening
Pen trials with birds vaccinated with bacterin	Nearly all birds had 4+ MG RSA antibody reactions	No protection against tracheal thickening with virulent MG challenge

Table 2. Summary of trials looking at humoral antibody and protection from MG challenge in birds from the field.

Continued from page 13

have large populations of totally naïve stock constantly at risk of becoming infected when you can increase their resistance to infection with vaccines and decrease the risk?

Are the vaccines we currently have up to the task? The idea that MG field strains can overwhelm the immunity generated by ts-11 is anecdotal or perhaps mythical and has been confounded by results from pen trials. Airborne transmission of mycoplasma to uninfected flocks is by two stages. The first stage is probably inocula travelling by air and once one or more birds are infected in a flock then direct contact (second stage) is order of magnitudes more efficient leading to rapid horizontal spread of infection.

Pen trials by Kleven and Feberwee have looked at direct transmission of MG with commingling birds and this simulates the second stage of transmission. The challenge under these circumstances is a lot greater than in situations without direct contact (as evidence by the control birds in Feberwee's experiments where no transmission from infected birds was observed between cages 65cm apart). Dose response experiments in the laboratory using challenges not propagated in media are probably needed to confirm this field experience.

Field challenge protection

The ts vaccines provide enough protection against field challenge from other farms and infected birds in other sheds on a farm and indeed may even provide enough against the strongest field strains for birds as long as they are not in direct contact with infected birds.

Our experience around the world is that there is a cumulative effect from vaccination on multiage farms where the longer the vaccine is used then the greater the effect (and usually a decrease in serological response which could be a loss of field challenge). Persistence is the key in this case. Health and production benefits come early and serological flat lining later. Antibody rise does not equal vaccine failure – it might just indicate challenge and successful resistance. There is still a need for biosecurity to keep the challenge low.

Another myth is that humoral serology is protective against infection. The low serological response seen in vaccinated birds in the field to ts-11 and MSH vaccination often worries veterinarians and production people. There is no evidence that maternal antibody protects hatched progeny from infection (it may improve embryo survival in birds infected with wild strains improving the efficacy of vertical transmission). The useful immunity generated by live vaccines is at the mucosal surface of the respiratory tract and this is not necessarily proportional to humoral antibody. In a series of experiments birds that were vaccinated with ts-11 and selected with low serological responses were taken from the field and challenged in the laboratory. They were significantly protected.

Indeed in these experiments comparison to birds vaccinated with MG bacterins with enormous amounts of humoral antibody were shown to be not protected against respiratory challenge.

This focus on humoral antibody stems in part from the successful pullorum eradication programmes where antibody identified individual infected birds. Coupled with experience monitoring AE vaccination success by looking for anti-AEV antibodies before lay and knowing that demonstrating antibody would mean complete protection against AE could be expected in lay led many flock managers to try to use MG serology for the same purpose.

These are the wrong models for mycoplasma vaccination monitoring as mycoplasma infections are chronic and mucosal immunity is important not humoral antibody (unlike AEV). There are many commonly used poultry vaccines where vaccination application can not be assessed by simple demonstration of humoral antibody, including Marek and coccidiosis vaccination. Indeed we quality control vaccine administration with these later vaccines by training programmes, audits and monitoring disease occurrence.

Conclusion

It is contended in the light of this discussion that what we need from avian mycoplasma vaccines is protection against low intensity challenges and the dampening down of multiplication and transmission of field strains. Both these aims can be met with ts vaccines and many producers around the world are reaping the benefits of these vaccines with none of the downsides of other older vaccines. Do we need serological responses to monitor vaccination or field challenges? – who cares as long as the birds are protected.

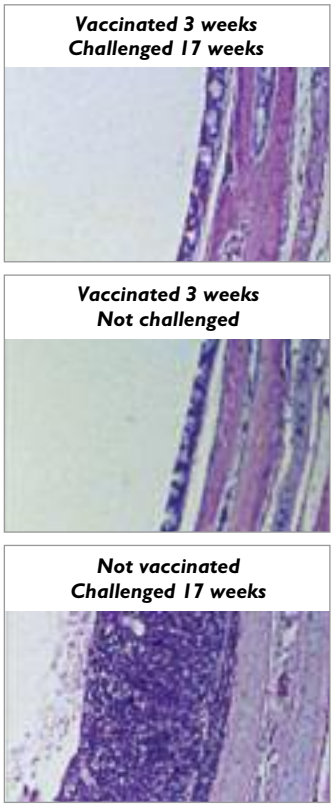

Fig. 2. Tracheal thickening after MG challenge. All birds had no measurable antibody at challenge (see Table 2). Note typical mycoplasma pathology in the bottom panel above.

We have now developed sophisticated strain identification methods using genomic tests to analyse field problems. These vaccines have allowed routine production of eggs and poultry meat in Australia and elsewhere without the need for regular antibiotic treatment. One drop of each vaccine in the eye provides immunity for the total productive life of the bird. ■

Table 3. Investigating apparent vaccine failures.

Presenting history	Possible causes	Comment and action
Birds given ts-11 still snoring/coughing	• Check that MS is not the cause • Vaccination failure	MS can mimic any syndrome that MG produce
Clinical disease still present and wild strains demonstrated	• Cold chain problems • Poor vaccination • Challenge too soon after vaccination • Antibiotic administration around time of vaccination	Vaccine must be handled correctly and given by eyedrop. Must be given three weeks before wild strain challenge.
No difference between vaccinated and unvaccinated birds in trial	• No challenge • Antibiotic administration of control group during trial or in response to challenge	Current measures in control flocks (antibiotics in feed each month) may interfere or obscure benefits.
No serological response	• Commonly seen in flocks that receive no subsequent challenge • Vaccination failure is a possibility	Always keep some sera aliquoted from positive, negative and low positive for thawing and using to internally compare batches of ELISA kits. PCR can demonstrate vaccine strain in birds.
Strong serological response	• Wild challenge but this does not mean vaccination failure.	Vaccination failure is the occurrence of clinical signs not serological response
Serological response in subsequent batches disappears	• Wild strain challenge disappearing	Perhaps a sign of success rather than failure