Combining vaccines to improve *Mycoplasma gallisepticum* (MG) Control

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Mycoplasma infections and the role of vaccines in their control

Mycoplasma gallisepticum (MG) is an economically important pathogen causing respiratory disease and egg production drops in chickens and turkeys worldwide. MG vaccines have been used for several years as tools to control MG; vaccine programs may be implemented to control spread of the infection, to prevent clinical disease and to reduce egg transmission to progeny. MG vaccines may be part of a longer-term eradication program by displacing virulent field strains. Inactivated, live attenuated and recombinant vaccines have been developed for MG and the different vaccines vary in their ability to achieve the specific goals of an MG vaccine program.

The host response to MG infection is characterized by a substantial lymphoproliferative response and the resulting tissue damage appears to be a major part of the pathogenic effects of infection. One of the fundamental first steps in the infectious process is adhesion of the organisms to cells in the upper respiratory tract, and mucosal antibodies have been demonstrated to be of particular importance in the ability to prevent colonization, probably by blocking attachment of the MG cells to host epithelial cell.[1]

Aside from respiratory disease, MG infection may also result in egg production drops and egg transmission, and although the pathogenesis of these processes have not been fully elucidated, a more systemic immune response may play a role in protection of the reproductive system once the respiratory tract has been colonized. Humoral antibody levels do not correlate well with protection against infection, but circulating antibodies appear to be of some benefit to the host[2,3]. Cell-mediated immunity appears to play somewhat of a role in MG clearance and host resistance[4]. MG is able to persist in the host for lengthy periods of time, even in the face of a relatively strong immune response.

As live attenuated vaccines colonize the upper respiratory tract they can result in local MG-specific immunity as well as some degree of systemic immune response. In reducing or preventing infection, these vaccines may also prevent clinical signs and egg transmission. Live attenuated vaccines have been used to displace virulent wild-type MG strains from commercial poultry flocks. F-strain live attenuated vaccine has been used extensively; it is very immunogenic and effective in displacing virulent (field) strains from poultry operations.

Inactivated MG vaccines (bacterins), which appear to primarily produce a systemic antibody response, have been shown to reduce egg production losses and egg transmission of MG, but reports demonstrating the efficacy of bacterins against protection from respiratory disease and protection have been variable. There is some indication that MG bacterins may have a role in reducing colonization in the trachea.[2]

Although F-strain is a highly efficacious MG vaccine, there may be field situations where the efficacy of a live vaccine may be improved upon by the addition of a killed vaccine. Combination programs (live plus killed) have had extensive use in some geographic areas, but there have been few scientific reports on the relative benefits, and so it has been difficult to justify the implementation of these programs in the face of the lack of objective evidence.

Even though one of the few articles evaluating live and killed vaccine combinations for MG found no statistical differences in egg production, it should be noted that the best protection from an egg production drop after challenge was seen in the group that received both live and killed vaccines[5]. In the recent trials, described in this bulletin, we attempted to update the data by examining the relative protection derived from a dual program compared to a single immunization as well as examine the duration of immunity.

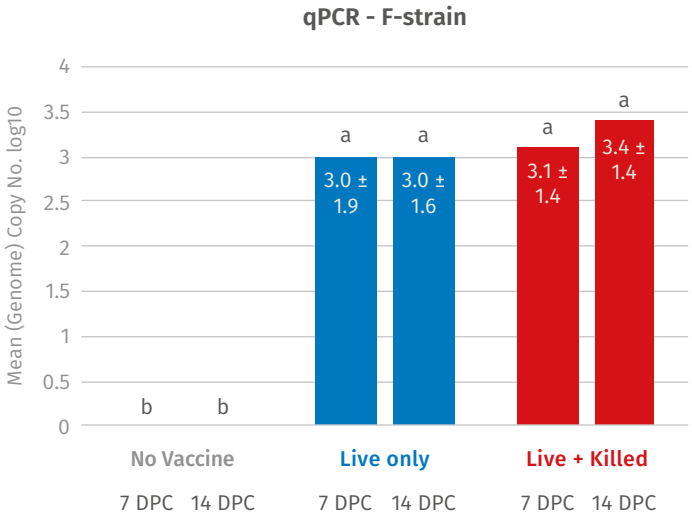
Trial study 1: Comparison of live, inactivated and combination (live + killed) vaccine programs against MG R-strain challenge

In our first trial, specific-pathogen-free (SPF) chickens were vaccinated with live F-vaccine (5 weeks of age), inactivated MG bacterin (9 and 13 weeks of age), or a combination of both vaccines. Groups of vaccinated birds, along with controls, were challenged with virulent MG R-strain at 22 weeks of age and 41 weeks of age. The serological results, F-strain and R-strain colonization, air sac lesions, tracheal lesions and ovarian regression results in this study correlate with previous reports on the response to and efficacy of F-strain and inactivated bacterins in laying hens.

The F-strain vaccination, bacterin vaccination, and a combination of the two types of vaccines, all resulted in significant protection of the respiratory and reproductive systems following both the early challenge (22 weeks) and late challenge (41 weeks) ($P \leq 0.05$).

The F-strain vaccination resulted in significantly better protection than the bacterin ($P \leq 0.05$); and the combined program (live and killed vaccinations) improved on the live vaccine with respect to egg production and airsacculitis.

Figure 1: Colonization of F-strain following vaccine administration at 5 weeks of age and challenge with R-strain at 17 weeks of age[6]



Values with a different lower case superscript are significantly different ($P \leq 0.05$)

Table 1: Egg production from vaccinated and non-vaccinated chickens pre- and post- inoculation with *M. gallispeticum* (R-strain) at 41 weeks of age[6]

Treatment	F-Vaccination	Bacterin Vaccination	Challenge (R-strain)	Average Daily Egg Production (%)[B]		
				Pre-Challenge	Post-Challenge	Difference
A	None	None	No	86.3	89.4	+3.1
B	None	None	Yes	95.6	75.6	-20.0
C	Yes	Yes	Yes	87.9	95.7	+7.9
D	None	Yes	Yes	78.1	79.4	+1.3
E	Yes	None	Yes	89.4	90.6	+1.3

[B]Average daily egg production for 10 day pre-challenge and 10 days post-challenge

Trial study 2: Comparison of live and combination (live + killed) vaccine programs against a range of different doses of the challenge strain (MG R-strain)

In a second trial, we challenged groups of vaccinated birds with a range of doses of the challenge strain to investigate the minimum challenge dose necessary to overcome the protection generated by the different vaccine programs. SPF chickens were vaccinated as in the earlier trial and groups of vaccinated birds, along with controls, were challenged via aerosol with different doses of virulent MG R-strain at 17 weeks of age. We used strain differentiating qPCR to evaluate the ability of the vaccine programs to prevent infection with the virulent challenge strain. The vaccination programs resulted in significant protection against infection as well as airsacculitis compared to the controls, and there were also significant differences between the vaccine programs, with the combination program coming out as superior to the live vaccine only program ($P \leq 0.05$). The birds vaccinated with the combined program were significantly more resistant to colonization with even the highest doses of challenge compared to the birds that received only the live vaccine ($P \leq 0.05$). There was also significantly less airsacculitis in the group that receive the live plus killed program ($P \leq 0.05$). We did not evaluate egg production in this trial (these birds were too young).

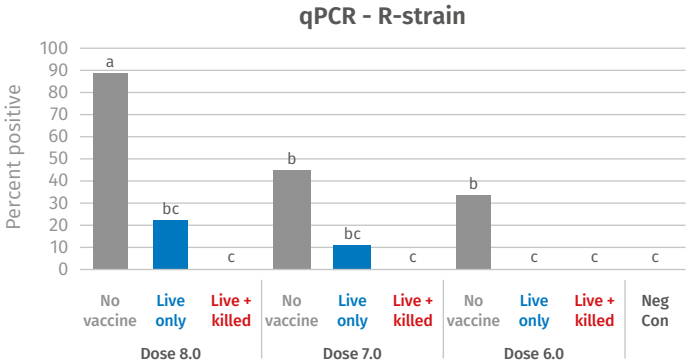
Discussion: Combination (live + killed) vaccine programs

One of the concerns raised about combination programs is that high levels of antibody may interfere with the replication and persistence of the live vaccines and therefore interfere with the long-term efficacy of the live vaccine. In the trials that we conducted there were no significant differences in colonization or persistence of the F-strain vaccine regardless of whether the birds also received the killed vaccine ($P \leq 0.05$).

We have confirmed that the killed vaccine is efficacious against virulent MG challenge, that the F-strain live vaccine is more efficacious than the bacterin, and that a combination program is even more efficacious, specifically with respect to resistance to colonization with a virulent MG strain (so that a higher challenge dose is necessary to infect birds) as well as better protection against mycoplasma associated effects such as airsacculitis and egg production drops.

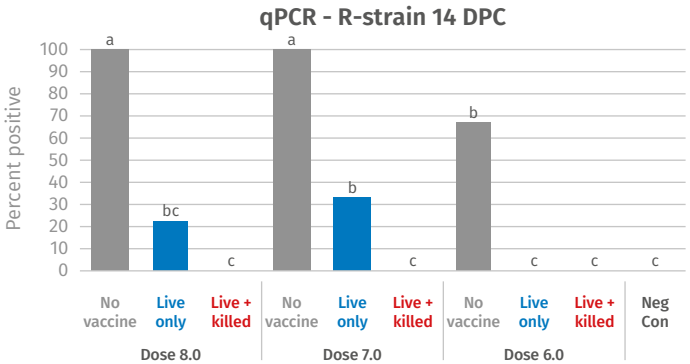
The present studies confirm the opportunity for poultry veterinarians to improve the control of *Mycoplasma gallisepticum* through the use of a combined mycoplasma vaccination program to achieve a stronger protection against the disease and improve production and welfare parameters.

Figure 2: Colonization of R-strain at 7 DPC* (challenged at 17 weeks of age)[7]



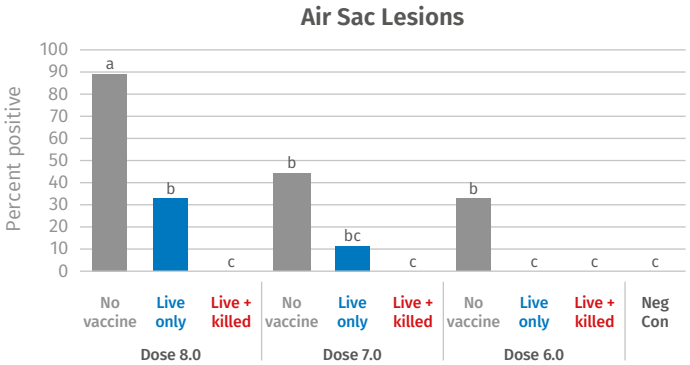
Values with a different lower case superscript are significantly different ($P \leq 0.05$)

Figure 3: Colonization of R-strain at 14 DPC* (challenged at 17 weeks of age)[7]



Values with a different lower case superscript are significantly different ($P \leq 0.05$)

Figure 4: Percentage of birds with air sac lesions at 14 DPC* (R-strain challenge at 17 weeks of age)[7]



Values with a different lower case superscript are significantly different ($P < 0.05$)

*DPC = Days post challenge

References:

1. Avakian, A.P. and D.H. Ley, Protective immune response to *Mycoplasma gallisepticum* demonstrated in respiratory-tract washings from *M. gallisepticum*-infected chickens. Avian Dis, 1993. 37(3): p. 697-705. **2.** Talkington, F.D. and S.H. Kleven, Evaluation of protection against colonization of the chicken trachea following administration of *Mycoplasma gallisepticum* bacterin. Avian Dis, 1985. 29(4): p. 998-1003. **3.** Chhabra, P.C. and M.C. Goel, Immunological response of chickens to *Mycoplasma gallisepticum* infection. Avian Dis, 1981. 25(2): p. 279-93. **4.** Gaunson, J.E., *et al.*, The cellular immune response in the tracheal mucosa to *Mycoplasma gallisepticum* in vaccinated and unvaccinated chickens in the acute and chronic stages of disease. Vaccine, 2006. 24(14): p. 2627-33. **5.** Glisson, J.R. and S.H. Kleven., *Mycoplasma gallisepticum* vaccination: effects on egg transmission and egg production. Avian Dis, 1984. 28(2): p. 406-15. **6.** Elanco Data on file. **7.** Elanco Data on file.

Think Twice

LIVE VACCINE + INACTIVATED VACCINE