

# TECH bulletin

NUMBER 2015-02

## Serology after ts-11 and MSH mycoplasma vaccination

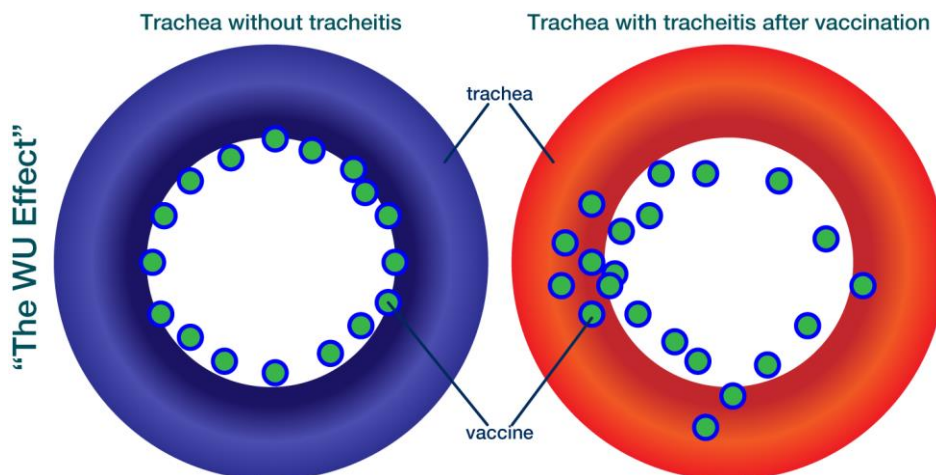
Live ts mycoplasma vaccines were created to be safe but still immunogenic. So how can we assess this immunogenicity in the field? This bulletin condenses the experience of 20 years trying to monitor vaccine response and differentiate problems from normal responses. Various approaches have been taken to improve serological monitoring (for example, cloned homologous antigens) and understand problems but the conclusion is that humoral antibody is not the mechanism of protection of these vaccines and is not even correlated with protection. The results are disappointing with very little being offered by serological testing of vaccinated flocks. Indeed it has been suggested that serological testing of these flocks is confusing and a waste of money and resources.

### Serological responses to ts-11 and MSH vaccination

The serological responses to these vaccines are variable and of limited use to monitor field challenge or vaccine administration. It could be argued that serological testing of vaccinated flocks is a waste of money. The idea that titre cut-offs can be used for differentiating vaccinated from infected animals does not work in practice. Even in Newcastle disease infection this has had to be discarded especially as vaccines have been improved. In mycoplasma serology in vaccinated animals it is even more unreliable.

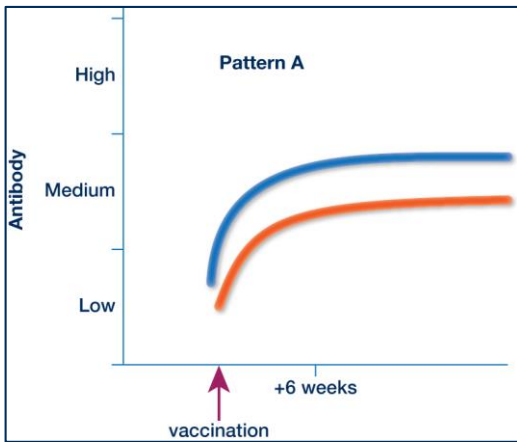
In contrast to infection with most mycoplasma field strains (rapid and to high levels of antibody) the serological response to ts-11 and MSH vaccination is slow and may not be strong. In the laboratory, vaccination of SPF white leghorns by eye drop at three weeks of age will see the development of mild agglutinins after 4 weeks. In the field this is even more complicated. This variation may be associated with the amount of tracheitis (possibly increasing antigen seen by systemic immune system) the birds are experiencing at the time of vaccination. It has been suggested that perhaps the tracheitis is a more important determinant of the variation in serological response than the vaccine.

### Location of Vaccine in Upper Respiratory Tract after Vaccination



*It is hypothesised that in birds with minimal tracheitis that the vaccine strain is entirely on the mucosal epithelial surface (this stimulates the protective local immune response) and the systemic immune processes are not stimulated. But with tracheitis the antigens go deeper, interact and stimulate the systemic immune response. The lack of humoral serological response does not allow one to conclude that the vaccine has not induced immunity.*

In the field one of four basic serological patterns are seen (Patterns A – D). Usually ts-11 will produce less agglutinins and lower ELISA mean titres than MSH. The response of broiler breeders is often less than seen with layer strains. The responses and patterns are tabulated in Table 1.

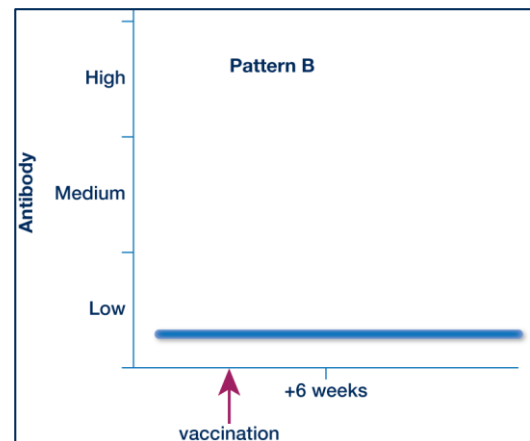


May vary flock to flock as indicated by orange and blue lines.

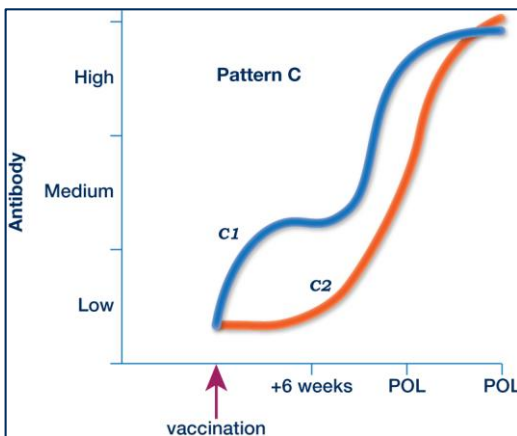
**Pattern A:** Moderate serological response after 6 weeks post vaccination. In ELISA testing this is often seen with the mean titre being somewhere between 800 and 4000 ELISA units and this can be consistent flock after flock for a few years but then it may change. In some areas the response is higher and the antibody may persist for life at these levels.

ELISA technology is improving but it is not a DIVA (Differentiating Infected from Vaccinated Animals) test. The use of homologous antigens in the ELISA including cloned antigens from the vaccines can improve detection of humoral serological responses but it still does not always prove that a bird has been vaccinated and is not infected with a field strain. One suggestion has been to use two different MS ELISAs on sera after vaccination with MSH – one containing the cloned vaccine antigen and the other with a whole cell antigen and with this system it was possible to detect an earlier response to the cloned antigen by testing at 4 weeks post vaccination (Todte 2014) confirming vaccination.

**Pattern B:** Low to no antibody response. This will often occur after a couple of years of vaccinating all birds on a site. Perhaps this is with minimal tracheitis and after the field strain has been completely displaced from the farm. This is more common with ts-11 but can also happen with MSH.



Can be normal especially after long use (See Pattern D)

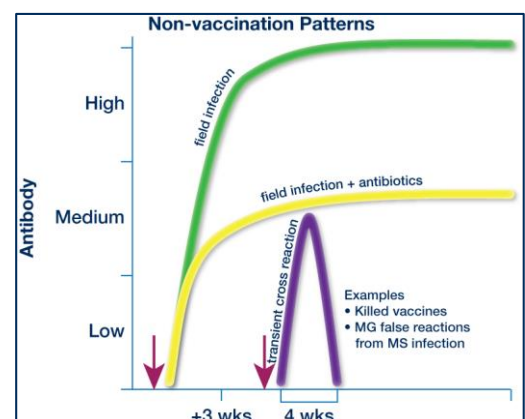


POL – Point of Lay      POL – Peak of Lay

No field strains have been found in this situation by PCR, only vaccine strains. Pattern C1 begins by looking like Pattern A and Pattern C2 begins by looking like Pattern B.

**Pattern C:** This is a rise in antibody titre anytime from the beginning of lay to 40 weeks of age in vaccinated flocks. When this has been investigated, only vaccine strains have been found (Zavala and others 2015). The techniques used (culture and PCR) are not sensitive for finding a second strain if it is only present as a small proportion of the population but perhaps this is a more useful answer than being told two strains are present if the minority strain does not readily transmit to other flocks and does not cause a problem.

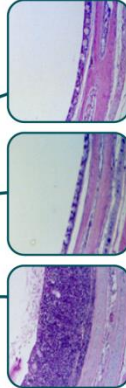
**Pattern D:** A change in pattern. Usually moderate serological responses (Pattern A) becoming Pattern B. This may be due to a loss of wild challenge of vaccinated birds (Morrow & Whithear 2011).



# Laboratory investigations of the protective role of humoral antibody in vaccinated birds

## Predictive value of MG RSA test in field vaccinated broiler breeders

Group	Age vac wk	RSA reactors (score range)*	Tracheal mucosa $\mu\text{m}^\dagger$
ts-11/C	3	0% (0-0)	101 $\pm$ 5 <sup>a</sup>
ts-11/NC	3	0% (0-0)	98 $\pm$ 5 <sup>a</sup>
ts-11/C	6	40% (0-1)	105 $\pm$ 5 <sup>a</sup>
ts-11/NC	6	20% (0-0.5)	105 $\pm$ 6 <sup>a</sup>
NV/C	NV	0% (0-0)	273 $\pm$ 44 <sup>b</sup>



\*Tested at 17 wk, immediately before challenge - scored 0-4  
 $^\dagger$ Tested 2 weeks after challenge  
 NV = Not Vaccinated, C = Challenged, NC = Not Challenged

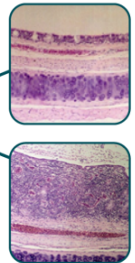
*No antibody does not mean that the vaccinated birds are unprotected!*

The observation of some vaccinated flocks not developing humoral antibody raised the question of "Were these flocks protected?"

In a series of experiments the link between humoral antibody and protection in vaccinated birds was investigated. Birds from flocks with very low serological responses to ts-11 vaccination were taken back into the laboratory at 17 weeks (and tested again) and then challenged by aerosol. Protection was assessed by tracheal thickness two weeks after challenge. All flocks were protected. (Noormohammadi and others 2002b).

## Protection by MG vaccines in the laboratory

Group	n	RSA score (mean)	Tracheal Mucosa $\mu\text{m}$
ts-11*	10	1.8 $\pm$ 1.1	71.4 <sup>b</sup>
Bacterin*	10	3.7 $\pm$ 0.5	251.1 <sup>c</sup>
Unvaccinated*	10	0	253.6 <sup>c</sup>
Not challenged	10	0	44.3 <sup>a</sup>



\*Aerosol challenge  
<sup>a, b, c</sup> P < 0.05  
 RSA = rapid serum agglutination test (serology) - scale 0-4

*Birds with high humoral antibody after killed vaccines are not protected from infection or CRD.*

Birds were vaccinated with ts-11 or killed vaccine and challenged with virulent MG by aerosol. Protection was measured by tracheal mucosal thickness. In this experiment, at the time of challenge massive amounts of humoral antibody was generated by killed MG vaccine. This did not stop the birds from developing tracheal mycoplasmosis.

Antibody Response	Vaccine (6+ weeks)	Alternative explanation	Follow up
High (Pattern C)	+/- Field challenge? No problem	Field challenge: Problem	PCR History
Medium (Pattern A)	Usual	Early field challenge	PCR Rebleed
Low to zero (Pattern B)	Can happen especially before lay	Poor vaccination	PCR Rebleed

Table 1: Summary of possible causes of serological responses in vaccinated flocks.  
 Conclusion: Irrespective of the serological pattern, no decision can be made without further testing.

# How to know what is happening in a ts-11 and/or MSH vaccinated flock

Live vaccines. Routine auditing of vaccination is the best way to ensure that flocks are vaccinated properly and this should include appraisal of:

1. Cold chain
2. Vaccine application
  - dye studies
3. Antibiotic use in the flock immediately before and after vaccination and long term antibiotic use after application
4. Immunocompetence
5. Biosecurity and expected challenge timing.

Trouble-shooting is easy by investigating with a Strain ID PCR test. The vaccine strain should be able to be detected by PCR (with Strain ID) if the flock has been properly vaccinated. This is best done at 6 weeks after vaccination.

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