VACCINE STRAINS AND RECENT EXPERIENCES WITH SEROLOGICAL TESTING FOLLOWING FIELD VACCINATION WITH TS-11 AND MS-H IN A BROILER BREEDER OPERATION IN AUSTRALIA

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Vaccination is becoming increasingly accepted as an effective tool for controlling mycoplasma infections in breeder and layer flocks(Whithear, 1996). Strain ts-11 (Whithear *et al*., 1990) and strain MS-H (Morrow *et al*., 1998) are attenuated vaccine strains that are used to control *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS), respectively.

Development of *Mycoplasma gallisepticum* strain ts-11 and *M. synoviae* strain MS-H

The parent of ts-11 is an Australian MG field strain (80083) (Soeripto *et al*., 1989; Soeripto *et al*., 1989) which was originally chosen as a potential vaccine candidate because it was moderately pathogenic and elicited a strong protective immune response. In this sense it was similar to F strain. However, it was considered that this level of pathogenicity was undesirable in a commercial live vaccine, so an attempt was made to attenuate strain 80083. This was done by exposure of an actively growing, low passage culture to the chemical mutagen N-methyl-N-nitro-N-nitrosoguanidine (NTG) and then selecting for variants that expressed a temperature sensitive (ts^+) phenotype. The ts^+ phenotype is a useful marker for attenuation of vaccine strains. NTG causes multiple linked mutations at the point of DNA replication. This means that besides acquiring a ts^+ phenotype other mutations that contributed to attenuation were also likely to occur. A number of ts^+ mutants were evaluated but the one called ts-11 had the most desirable characteristics of lack of pathogenicity combined with a capacity to stimulate a protective immune response.

The success of using NTG mutagenesis to select the ts-11 strain was repeated in the development of the MS-H strain from an Australian field isolate (strain 86079/7NS) of MS (Morrow, *et al*., 1998).

Both ts-11 and MS-H were developed to be administered by eye-drop to individual birds between 4- and 16-weeks of age and at least 4 weeks before anticipated exposure to field infection with MG or MS. Protective immunity is dose dependent, so it is important that each bird receives the recommended dose. Both vaccines can be administered concurrently. Only one dose of each vaccine is required. The vaccine strains appear to persist for long periods and stimulate effective life-long immunity.

The serological response to ts-11 and MS-H as detected by the rapid serum agglutination (RSA) test

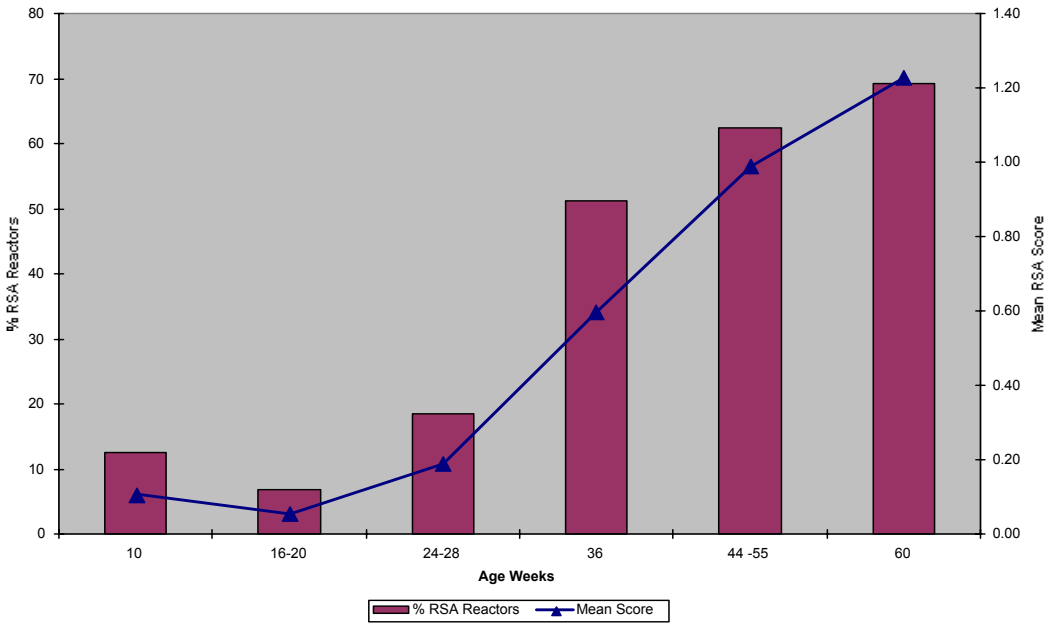
Detection of serum antibodies has traditionally been used to determine whether flocks have been exposed to MG or MS. The rapid serum agglutination (RSA) test or serum plate test is commonly used for this purpose. However, serum agglutinin responses to attenuated

vaccine strains are usually relatively weak and more difficult to interpret (Markham *et al*., 1998; Whithear *et al*., 1997; Whithear *et al*., 1990) than the response occurring after a flock is infected with a wild-type strain or after vaccination with a bacterin or F-strain MG. Nevertheless, producers expect some evidence of a serological response as assurance of a successful vaccine 'take' with attenuated vaccines.

Recent field data on RSA response to ts-11

Data collected between 1999 and 2002 from a broiler breeder operation in Australia have been collated to provide some recent information about serum agglutinating antibodies as detected by the MG rapid serum agglutination (RSA) test using stained antigen (Intervet). Serum samples (6159) were tested from 15 meat parent breeder farms (~ 250,000 Ross breeders) between 10 weeks and 60 weeks of age. Flocks were vaccinated at 6 weeks of age. The percentage RSA reactions and the mean RSA score of different age groups are shown in Figure 1. Both the number of reactors and the RSA score were low during the growing period and progressively increased from the commencement of production until 60 weeks of age.

Figure 1. Percentage of MG RSA test reactors and mean RSA scores** of serums collected at different flock ages from 15 ts-11 vaccinated broiler breeder farms in Australia from 1999 to 2002*

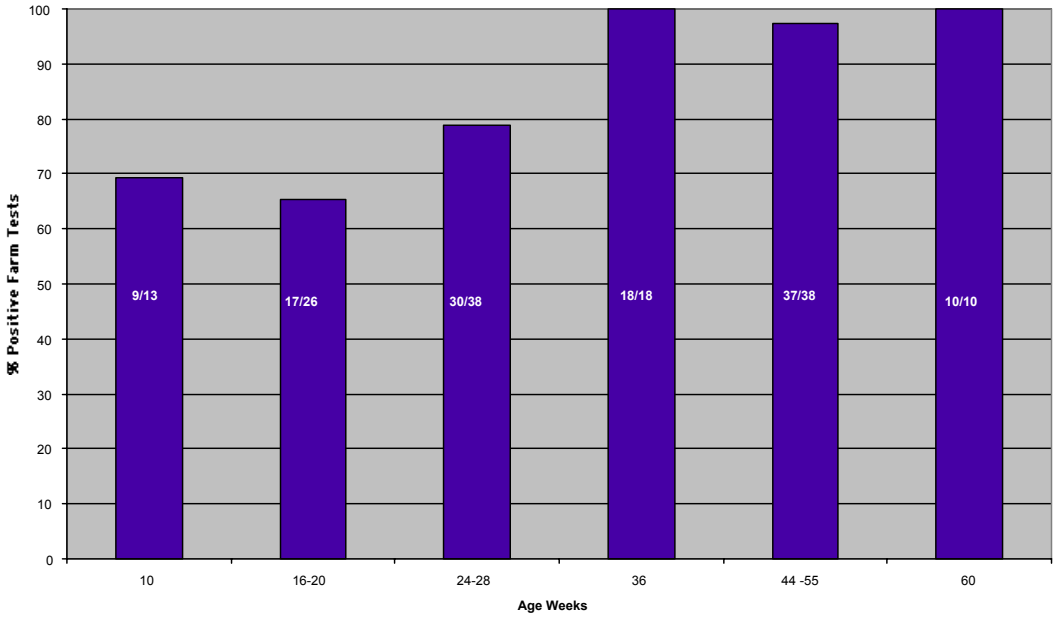


*Rapid serum agglutination test (serum plate test) using Intervet stained MG antigen

**Serums were scored from 0 (no reaction) to 4+ (large agglutination clumps)

The percentage of serologically positive flocks (farms) detected over the same ages are shown in Figure 2. Overall, 85% of farms tested gave positive results, although of those tested between 10 weeks and 20 weeks, one third failed to show any serum with a detectable RSA reaction.

Figure 2. Percentage of ts-11 vaccinated broiler breeder farms which gave positive RSA reactions at different flock ages from 1999 to 2002.



Figures within columns are the number of positive farm tests/number of farms tested at that age.

Recent field data on RSA response to MS-H

Flocks from the broiler breeder operation described above were also vaccinated with MS-H at the same time as ts-11. Results of RSA testing using MS stained antigen (Intervet) are given in Table 1. Only 7 of the 15 farms were tested for MS serum antibodies (1838 serums) and testing was confined to the period from 10 to 20 weeks of age.

Table 1. MS RSA responses in broiler breeders after vaccination with MS-H

Flock Age Weeks	% RSA Reactors (Range)	Mean RSA Score ±SD	RSA Positive Farms	Farms Tested	% RSA Positive Farms
10	40.6 (1.7-88.3)	0.48±0.30	13	13	100
16-20	43.2 (0.0-96.6)	0.50±0.38	24	25	96

Flocks from all but one farm (tested at 10 to 20 weeks of age) gave detectable RSA reactions although the percentage range of reactors was variable (Table 1). The intensity of agglutination reactions was also low with most being scored as trace (0.5) or 1+.

Interpreting RSA responses in vaccinated flocks

The RSA responses in ts-11 and MS-H vaccinated flocks are relatively weak and even very fine (trace) agglutination reactions should be considered significant. However, some flocks may fail to give any agglutination reaction in the weeks after vaccination, particularly

following use of ts-11 vaccine. It is of interest that serums from most ts-11 vaccinated flocks showed progressively stronger reactivity in the RSA test after the flocks commenced production (Figure 1). It is not known whether this is because the vaccine strain becomes more active and/or whether the flocks were challenged with wild-type MG. In several attempts to isolate MG from flocks which have shown a sudden increase in serum antibodies, the vaccine strain, but no wild-type MG, have been recovered. This may indicate that activation of the vaccine strain is responsible for the increased antibody response, although it is also possible that ts-11 rapidly displaces the field challenge strain from the respiratory tract, so the latter cannot be recovered.

A poor RSA response in vaccinated flock does not mean lack of protection

While the desire to see a strong serological response as an indicator of successful vaccination is understandable, it should be pointed out that there is no correlation between levels of serum antibody and protection against MG. Provided flocks have been correctly vaccinated with a full dose of ts-11, they should be protected, regardless of whether there are detectable agglutinins in the serum. Protection against experimental aerosol challenge with virulent MG has been demonstrated in laboratory vaccinated birds that had no serum antibody detectable by RSA test (Whithear, et al., 1997). More recently, Noormohammadi *et al*. (2002c) investigated protective immunity in birds from 2 field vaccinated broiler breeder flocks which gave no or weak RSA responses (at 11 or 14 weeks after ts-11 vaccination). There were no significant differences in microscopic tracheal lesions or mucosal thicknesses between vaccinated groups whether they were challenged or unchallenged (Noormohammadi, *et al*., 2002c). An unvaccinated challenged control group had significantly greater microscopic tracheal lesions and mucosal thicknesses than the vaccinated groups 2 weeks after challenge. The conclusion made was that broiler breeders vaccinated in the field showed significant protection against virulent MG challenge even when no serum antibody was detected by RSA test (Noormohammadi, *et al*., 2002c). Seroconversion detected by RSA test following ts-11 vaccination is thus not a reliable predictor of protection against MG infection.

With MS-H there is some evidence of a correlation between serum antibody levels and protection (Markham *et al*., 1998) although this does not mean that serum antibody is responsible for protection. Challenge studies, similar to those described for ts-11 field vaccinated birds by Noormohammadi, *et al*. (2002c), are required to confirm that protective immunity is demonstrable in MS-H vaccinated flocks that show weak RSA serological responses.

Alternatives to the RSA test

It is possible that negative RSA results are due to a lack of sensitivity of the RSA antigen for ts-11 specific antibodies produced in the first weeks after vaccination. An indirect ELISA using affinity purified pMGA antigen derived from ts-11 has been developed to address this potential sensitivity problem (Noormohammadi *et al*., 2002a). pMGA is the immunodominant surface protein of MG. Of 953 serum samples from vaccinated flocks 26.5% gave RSA reactions and 32.8% were ELISA positive. All flocks so far tested which have given no RSA reactions following vaccination have been identified as positive using this ELISA.

Noormohammadi *et al*., (1999) identified the most antigenic region of one of the major species-specific surface proteins (MSPB) of MS, and the purified recombinant protein was shown to be a useful serodiagnostic reagent in an indirect ELISA. However, sera from MS-H

vaccinated SPF chickens produced lower optical densities in the assay than those infected with field strains (Noormohammadi, *et al*., 1999). Subsequent work established that there were some sequence differences between MS strains in the gene encoding MSPB and that the autologous recombinant MSPB was more sensitive for detecting antibodies to MS-H vaccine (Noormohammadi *et al*., 2002b). Of 330 serum samples from vaccinated flocks, 54.2% gave RSA reactions and 82.1% were MS-H ELISA positive (Noormohammadi, *et al*., 2002b). Of significance was the ability of the MS-H ELISA to detect antibodies sooner after vaccination (at 40 days) than the RSA test. Prior to this, antibodies were barely detectable with any test (Noormohammadi, *et al*., 2002b).

The ability of attenuated vaccine strains of MG and MS to elicit serum antibodies is predictably, relatively weak. However, it would also appear that the sensitivity of the antigens used to detect that response is also a factor, and that autologous antigens may give superior results. We recommend use of these autologous antigen tests when RSA reactions are equivocal.

Acknowledgements

Mr Peter Cowling and Ms Denise O'Rourke are thanked for doing the serological testing.

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