Avian Mycoplasma Myths

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PhD BVSc

Aim until now: Mycoplasma freedom for poultry

• Essential for genetic stock
  – Single age
  – Well isolated
    • 400 yards (=400 metres) between sheds (AA GP manual)
  – Achieved for MG in the 1970s
  – MS?
• Gives options to customers
  – Breaks are a problem (production forecast)
    • No immunity in flock
  – Risk management

Myth 1: MS does nothing.

• Clinically mycoplasmosis can be diagnosed but laboratory testing (culture and speciation or PCR) is need to differentiate MG and MS as the cause
• Anything MG can do MS can also do.
• Chronic infections mean these organisms stay around.
• Antibiotics can make infection status difficult to determine.

Quantification of Pathogen cost

Mycoplasma gallisepticum
• Layers – 10 to 20 eggs per year and FCR
• Egg drop in lay
• Decreased hatchability
• Primary respiratory disease and CRD
  – Mortality and poor FCR in progeny

Mycoplasma synoviae
• Layers – 5-10 eggs per year and FCR
• Infectious synovitis
  – Amyloidosis in brown layers
• Egg drop in lay
• Decreased hatchability
• Increased condemnations
• Respiratory disease in combination other viruses and respiratory vaccines
  • E. coli Peritonitis in layer
  • Glass top eggs
adapted from Stipkovits and Kempf 1996
MS strain effects

- Most strains do not cause Infectious synovitis
  - Often no joint disease
- Some countries’ vets argue that their MS strains do nothing so why worry
  - Because their country describes MS new syndromes every year
  - Their experts say MS is important
- Harder to grow than MG
  - NAD requirement
  - No stationary phase
- Harder to control
  - No official control
  - No monitoring

Airsaculitis after combined MS & IBV (experimental).

Chronic Respiratory Disease

MG or MS

CRD

E.Coli
Salmonella

Respiratory insult
Dust, Respiratory viruses And vaccines

MS involvement in *E. coli* peritonitis coming into lay

- Peritonitis in early lay is the most important cause of mortality in commercial layers in Europe
  - Prophylactic enrofloxacin is used in some areas.
- Experimental evidence from Raviv and Kleven
- Not the same as peritonitis in Broiler breeders

Synovitis due to MS – field case

MS Glass top eggs.
Egg Apical Abnormality
EAA

- This condition is temporarily responsive to OTC or tylosin and MS can be easily isolated from the oviduct.
- May be MS strain associated (Hammond group 3) and IBV may have a big effect.
- The Netherlands, Italy, Germany, UK, Turkey, France, Denmark and Japan. Up to 4-10% of eggs.
- Less dramatic in Broiler breeders (2%).

Second quality eggs (Not EAA)

- Hagan & Bradbury: UK Survey done by ELISA on egg yolk and questionnaire.
- Statistically significant increased second quality eggs in MS infected layer flocks.
- Similar findings in Australia.
  - Low shell breaking strength and shell deformation in seropositive flocks (yolk antibody).

Korean and Japanese belief

- MG ts-11 prevents speckled brown eggs.
- Valuable in these markets.

Airsacculitis in Pips

- Increase late mortality and decreased hatchability.
- Not sure if all strains do this equally but may be useful in low tech monitoring.

MS – does it do anything?

- Often argued that MS does nothing. This is very strain dependent but also salesman dependent.
  - If you can’t fix it technically then feature it.
- Some strains cause more problems than MG strains (Strain s10 in Arkansas, Egg production drop, airsacculitis in broiler).
- Under-diagnosed because of prophylactic antibiotic therapy (products with zero withdrawal times).
Antibiotics

What are antibiotics doing in Asian poultry production

- Limiting MG and MS impact
  - Especially during lay
  - Limiting MG and MS antibody production
- Helping to control *Avibacterium paragallinarum*, *P. multicoda*, *Salmonella*, *E. coli*, *Brachyspira* species
  - Can we control these with other strategies?

Mycoplasmas have airborne transmission!

- MS can travel 2km in the wind in temperate climates. The reason why some areas can’t control MS.
- Unknown distance in tropical climates

Risk factors for mycoplasma transmission

- Size of flock
- Antibiotic treatment
- Stress
- Time since infection
What have we got

- Multiage GP and PS farms
  - Sheds separated by 400 m
- Layer farms
  - Multiage with or without rearing on site

Biosecurity only for Mycoplasma freedom

- If you choose freedom then you are maintaining large populations with no protection from infection.
- You must keep the bird separated totally from the mycoplasma.

Multiage layer sites

- Current control in many areas is routine treatment with Tiamulin, tylosin or CTC/OTC often in feed (Zero withdrawal).
- Residues
- Resistance (gradual loss of efficacy)
- Other bacterial infections may also be being controlled.
  - Salmonella (vaccinate)
  - Brachyspira (acidify water)

Is ts-11 strong enough?

- Scientific studies to date are not relevant to the field situation
  - Based on pen trials
  - In contact challenge is too strong
- Practical experience is that ts-11 is strong enough.
Do you need to eradicate with F strain first before using ts-11?

  - “Field experience and unpublished studies…”
  - Contrast to Australian and overseas experience where persistent use of ts-11 has been more successful (K. Whithear pers comm).
- It can be done (Turner and Kleven 1998 Avian Dis 42, 404-407)
  - At a cost (layers, residual virulence, -7 eggs/HH)
- Pen studies results may not be appropriate for extrapolation to the field.

**Antibiotics**

- Control only
  - Decrease mycoplasma numbers
  - Prevent clinical disease but decreases antibody response
- Resistance development can be a problem
- Residues and withholding periods

**Vaccination aims/claims**

- To prevent clinical disease
  - Respiratory
  - Reproductive
  - Synovitis
- To prevent exacerbation of other infections
- To prevent vertical transmission
- To prevent subclinical losses
- To prevent wild strains from infecting birds (horizontal transmission)
- To decrease antibiotic dependence

**Avian mycoplasma vaccination**

<table>
<thead>
<tr>
<th>Controlled exposure</th>
<th>1960s</th>
<th>First generation live vaccine</th>
<th>Loss of 5 to 20 eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Killed Bacterins</td>
<td>Late</td>
<td>Injection(s)</td>
<td>Limited DOI and limited protection</td>
</tr>
<tr>
<td>Mild strains</td>
<td>1970s</td>
<td>Second generation</td>
<td>F strain 6/85</td>
</tr>
<tr>
<td>Attenuated strains</td>
<td>1980s</td>
<td>Third generation</td>
<td>ts-11 MSH</td>
</tr>
<tr>
<td>Pox vectored</td>
<td>1990s</td>
<td>Fourth generation</td>
<td>Limited success</td>
</tr>
</tbody>
</table>

**Controlled exposure**

- Based on the observation that natural infection provides protection against subsequent challenge
- Make sure hens were positive for MG before production
- Prevents egg production drops
- Still have losses from sub-clinical infection.
Killed bacterins and mild strains

Plenty of humoral antibody but.....

Immunogenicity appears correlated with residual pathogenicity

**ts** vaccines

- Core temperature of the chicken (>41.5°C) is too high for the vaccine strain to survive so infection is limited to the upper respiratory tract.

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Dose Response: Air sac lesions 2 weeks after challenge

5 week old SPF birds vaccinated with MS-H
Challenge 6 weeks after vaccination with wild-type MS
Neg. = non-vaccinated non-challenged
Pos. = non-vaccinated + challenged

Onset of immunity: Air sac lesion incidence

Duration of immunity: Air sac lesion incidence (combined onset and duration of immunity results)
Protein Profile of MSH & recent MS strains from Europe. Each strain is from a different Hammond group – genetically diverse.

Proteins probed with sera from (A) unvaccinated birds and (B) vaccinated birds. Shows multiple antigens recognized from eyedrop vaccination. Predicts that protection will be broad (especially compared to subunit vaccines).

Economic benefits: Australia

“The introduction of MS-H into the broiler-breeder parent population has meant that clinical disease related to MS is essentially no longer recognised” Dr. P. Scott (2002) Merial newsletter ‘Vaccination at work in Broiler Breeders’.

Economic benefits: Mexico

“The use of bacterins and medication programs has not been effective in MS control. The introduction of MS-H vaccine strain has allowed recovery of productive performance in breeders and their progeny, plus substantial savings due to medication in both. Likewise, its usage in laying hens has allowed the recovery of egg viability and production (4.5 to 13 eggs per bird per cycle)” Dr. E. Soto. 2002. Proceedings of ANAECA meeting, Puerto Vallarta, Mexico.

Administration of ts+ mycoplasma vaccines

- Eye drop (1x) between 3 and 6 weeks of age
- Administer prior to exposure to wild-type mycoplasma!!
- Dose 30 µL or ≥ 5 x 10^7 viable organisms
- Thaw quickly (~35°C about 9 minutes)
- Keep cool
- Use within 2-3 hrs

Antibiotic usage in vaccinated flocks

- Amoxyccilin (& cefitofur and phosphomycin)
- For gut problems you can use antibiotics not absorbed from the gut
  - Colistin
  - Neomycin, streptomycin, spectinomycin
- Erythromycin (MS irritately resistant).
- Don’t use two weeks before and for 4 weeks after vaccination.
- If you must use a product with antimycoplasmal activity use in short high doses.
- You won’t need it.
Where do these vaccines fit in

- **Eradication**
  - ts-11 is particularly suitable this application with its low horizontal spread
  - Suitable after a random break has occurred
- **Continuous vaccination**
  - Continuous protection
  - If the underlying risk of breaking has not changed then keep vaccinating
  - Discuss with production managers what risk they will accept (insurance)

Asian multi-layered MG control programmes.

- Live MG vaccine (4 weeks)
- Pox vectored MG vaccine (8 weeks)
- $2 \times$ killed MG vaccine
- Tylosin one week per month at .
- Is this any different from Tylosin alone in biological efficacy?
- Tylosin resistance has been noted.

### Vaxsafe MG (Australian origin)

<table>
<thead>
<tr>
<th>Country</th>
<th>Distributor</th>
<th>Registration date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>Bioproperties</td>
<td>1990</td>
</tr>
<tr>
<td>Japan</td>
<td>NBI</td>
<td>30 Aug 95</td>
</tr>
<tr>
<td>South Korea</td>
<td>Merial</td>
<td>30 Jun 98</td>
</tr>
<tr>
<td>Thailand</td>
<td>Pfizer</td>
<td>28 Dec 99</td>
</tr>
<tr>
<td>China</td>
<td>Sinder</td>
<td>1999</td>
</tr>
<tr>
<td>Malaysia</td>
<td>Pfizer</td>
<td>6 Nov 01</td>
</tr>
<tr>
<td>Philippines</td>
<td>Pfizer</td>
<td>14 Mar 01</td>
</tr>
<tr>
<td>India</td>
<td>Pfizer</td>
<td>1 Apr 03</td>
</tr>
<tr>
<td>Indonesia</td>
<td>Merial</td>
<td>2 Mar 06</td>
</tr>
<tr>
<td>Iran</td>
<td>Austral Medi</td>
<td>2010</td>
</tr>
<tr>
<td>Turkey</td>
<td>RTA</td>
<td>2011</td>
</tr>
</tbody>
</table>

### Vaxsafe MG (manufactured under license from Bioproperties)

<table>
<thead>
<tr>
<th>Country</th>
<th>Site of manufacture</th>
<th>Date of registration</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>USA</td>
<td>8 Feb 1994</td>
</tr>
<tr>
<td>South America</td>
<td>USA</td>
<td>From 1999</td>
</tr>
<tr>
<td>Brazil</td>
<td>Brazil</td>
<td>2 June 2005</td>
</tr>
<tr>
<td>South Africa</td>
<td>USA</td>
<td>Sept 1995</td>
</tr>
<tr>
<td>Pakistan</td>
<td>USA</td>
<td>2006</td>
</tr>
<tr>
<td>Italy</td>
<td>Italy</td>
<td>1996</td>
</tr>
<tr>
<td>Hungary, Romania, Egypt, Bulgaria, Poland</td>
<td>Saudi, Lebanon Others</td>
<td></td>
</tr>
</tbody>
</table>

### Vaxsafe MS (MSH)

<table>
<thead>
<tr>
<th>Company</th>
<th>Registration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>Bioproperties</td>
</tr>
<tr>
<td>Mexico</td>
<td>Avimex</td>
</tr>
<tr>
<td>South Africa</td>
<td>Protectachick</td>
</tr>
<tr>
<td>Japan</td>
<td>NBI</td>
</tr>
<tr>
<td>Argentina</td>
<td>Merial</td>
</tr>
<tr>
<td>Iran</td>
<td>Austral Medi Vet</td>
</tr>
<tr>
<td>Brazil</td>
<td>Merial</td>
</tr>
<tr>
<td>Indonesia</td>
<td>Merial</td>
</tr>
<tr>
<td>EU 27</td>
<td>Pharmasure</td>
</tr>
<tr>
<td>Philippines</td>
<td>Fort Dodge</td>
</tr>
</tbody>
</table>

### Vaccination to prevent EAA

- Done at Deventer laboratory using their challenge model
- IB intratrachael and IM all groups.
- Groups
  - No MS vaccine no MS challenge (IB only)
  - MS vaccine no MS challenge
- MS vaccine and MS challenge
- No Ms vaccine but MS challenge
Experimental challenge of vaccinated birds

Vaxsafe® MSH, Japan
• Commercial layers
• Comparison of flocks vaccinated with MSH and ts-11 to ts-11 vaccinated flocks only
• Japanese management – moult flocks when they fall to 77% HD production
• Vaccinated flocks did not have glass top eggs compared to previous flocks
  – Up to 4% glass top and 10% total second quality eggs before MSH vaccination.

Japanese Vaxsafe® MSH trials

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>to 57 weeks</td>
<td>82K sonia grey</td>
<td>Lohmann</td>
</tr>
<tr>
<td>Total eggs</td>
<td>+11.4 eggs/HD</td>
<td>+13.4 eggs/HD</td>
</tr>
<tr>
<td>Normal eggs</td>
<td>+1.4%</td>
<td>+2.9%</td>
</tr>
<tr>
<td>Egg mass</td>
<td>+795 g</td>
<td>+787g</td>
</tr>
<tr>
<td>FCR</td>
<td>-0.12</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

Pathology
IS MS

Infectious Synovitis in South Africa in brown layers
• MSH Vaccination on infected sites
  – Increased 8-12 eggs per hen housed
  – Reduced E.coli and less culling needed
  – Less runting and better uniformity
  – Less dramatic production drops when challenged with other diseases or stresses and better recovery
  – Total reduction in infeed medication and 80% decrease in water medication

Other experiences
• Have been able to repeat Barbour et al (2000) in China and some other places
  – Vaxsafe® ts-11 Vaccination of breeders leading to MG free broilers
  – Similar results with MSH in Mexico
• In Australia clinicians report less non mycoplasmal bacterial problems after mycoplasma vaccination
  – Less total dependence on antibiotics
Could vaccination protect GPs from MG or MS?

Use vaccines to decrease wild challenge of GP

Potential vaccination targets

Grandparent BB Broilers Breeders Layers Village

Grandparent BB Broilers Breeders Layers Village

Could vaccination protect GPs from MG or MS?

Vaccinate GPs

Diagnostic problems

• Serology (RSA and ELISA)
  – False positives
  – Equivocal results during early infection
  – Decreased antibody response after antibiotic treatment
  – Not definitive if the birds have been vaccinated
  – No good for determining Day old chick status.

Serological result after vaccination

<table>
<thead>
<tr>
<th>Antibody Response</th>
<th>Vaccine (6+ weeks)</th>
<th>Alternative explanation</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Field challenge vaccine worked</td>
<td>Field challenge vaccination failure</td>
<td>PCR and assessment of protection</td>
</tr>
<tr>
<td>Med</td>
<td>Usual</td>
<td>Early field challenge</td>
<td>PCR Rebled</td>
</tr>
<tr>
<td>Low to zero</td>
<td>Can happen especially before lay</td>
<td>Poor vaccination</td>
<td>PCR Rebled</td>
</tr>
</tbody>
</table>

Predictive value of MG RSA test in broiler breeders

<table>
<thead>
<tr>
<th>Group</th>
<th>Age vac wk</th>
<th>RSA reactors (score range)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ts-11/C</td>
<td>3</td>
<td>0% (0-0)</td>
</tr>
<tr>
<td>ts-11/NC</td>
<td>3</td>
<td>0% (0-0)</td>
</tr>
<tr>
<td>ts-11/C</td>
<td>6</td>
<td>40% (0-1)</td>
</tr>
<tr>
<td>ts-11/NC</td>
<td>6</td>
<td>20% (0-0.5)</td>
</tr>
<tr>
<td>NV/C</td>
<td>NV</td>
<td>0% (0-0)</td>
</tr>
</tbody>
</table>

*Tested at 17 wk, immediately before challenge
Predictive value of MG RSA test in broiler breeders

<table>
<thead>
<tr>
<th>Group</th>
<th>Age vac wk</th>
<th>RSA reactors (score range)*</th>
<th>Tracheal mucosa µm†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ts-11/C</td>
<td>3</td>
<td>0% (0-0)</td>
<td>101±5a</td>
</tr>
<tr>
<td>ts-11/NC</td>
<td>3</td>
<td>0% (0-0)</td>
<td>98±5a</td>
</tr>
<tr>
<td>ts-11/C</td>
<td>6</td>
<td>40% (0-1)</td>
<td>105±5a</td>
</tr>
<tr>
<td>ts-11/NC</td>
<td>6</td>
<td>20% (0-0.5)</td>
<td>105±6a</td>
</tr>
<tr>
<td>NV/C</td>
<td>NV</td>
<td>0% (0-0)</td>
<td>273±44b</td>
</tr>
</tbody>
</table>

*Tested at 17 wk, immediately before challenge
†Tested 2 weeks after challenge

Does negative RSA response to ts-11 mean poor protection?

Local Immunity in Airways

Comparison of ts-11 and a killed MG vaccine

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>RSA Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>ts-11*</td>
<td>10</td>
<td>1.8±1.1</td>
</tr>
<tr>
<td>Bacterin*</td>
<td>10</td>
<td>3.7±0.5</td>
</tr>
<tr>
<td>Unvaccinated*</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Not challenged</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

RSA= rapid serum agglutination test (serology)

Protection by MG vaccines

Ts-11

Killed MG vaccine

High level of serum antibody does not provide protection!

Strong Mucosal Immunity Requires:

- Antigenic stimulation at the mucosal surface
  - Live vaccines better than killed
- Persistent antigenic stimulation
  - Immunological memory for mucosal immunity tends to be short
- MS-H persists on the mucosal surface of the bird and stimulates protection for life
Strain ID

- Sequence based
  - Direct sequencing
  - HRM
- Not RAPD
- Not ts phenotype

Strain identification of MS

By strain ID method we could show that challenge strain only was associated with EAA eggs. Can be used in field studies to identify vaccine. 2hr result from tracheal swab.

Strain ID of MS by HRM analysis

Jeffrey et al. Microbiology 2007 153:2679-2688

Strain ID of MG – ts-11

Time is right

- With MS and MG vaccination the need for antibiotics is greatly reduced.
- Export markets are sensitive to antibiotic residues.
- Supermarkets may lead rather than government.

Total Mycoplasma Control

Freedom, vaccination, biosecurity and good diagnostics
Investigating ts-11 problems

Chris Morrow
Bioproperties

The End
THANK YOU

ts-11 failure to protect

- Cold chain problems
- Administration problems
- Overwhelming challenge
- Birds already infected
- Birds challenged before immunity develops
- Antibiotics
- Immunosuppression
- MS problem – not MG

Frost free freezers are bad

Dye stains eye and mouth

- Expect 100% of mouths to be stained rapidly after application
- Only use dyes recommended by Bioproperties