BIOPROPERTIES
The Vaccine Innovators

# Avian Mycoplasma Myths

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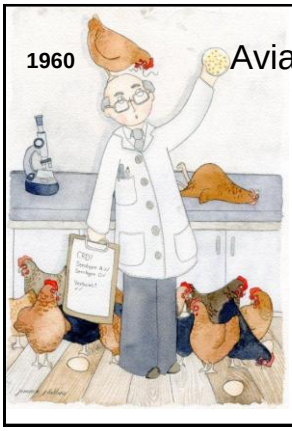

## Avian Mycoplasmosis

- Hard to grow "simple" bacteria
- No cell wall
- Chronic infections
- The idea of mycoplasma freedom

## 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 I

## *MYCOPLASMA SYNOVIAE* IS NOT IMPORTANT.



## 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

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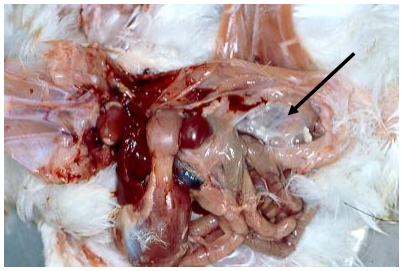
## Airsaculitis after combined MS & IBV (experimental).



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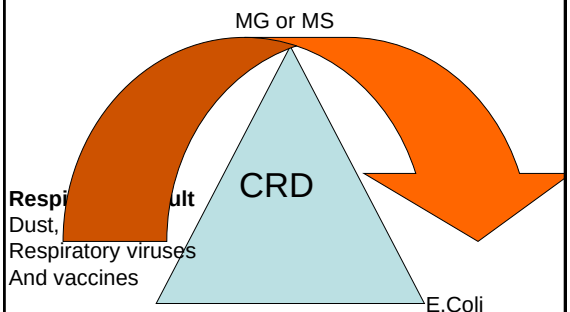
## Chronic Respiratory Disease

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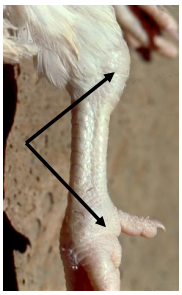
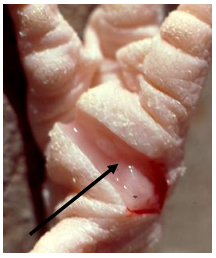
## Synovitis due to MS – field case





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## 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

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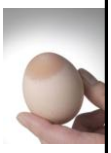
## MS Glass top eggs. Egg Apical Abnormality



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Feberwee and others Avian Path Nov 2008

## EAA

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## 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%)

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## 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)
    - Gole, K.K. Chousalker, J. Lievaart and J. R. Roberts APSS 2012

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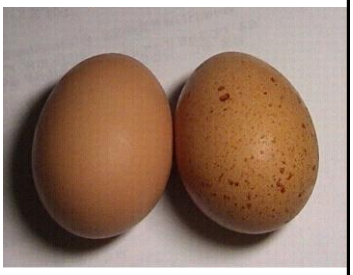
## Korean and Japanese belief

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



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## 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

Pfizer Animal Health Global Poultry

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## MS –does it do anything?

- Often argued that MS does nothing. This is very strain dependent but also salesman dependent.
  - If you cant 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).

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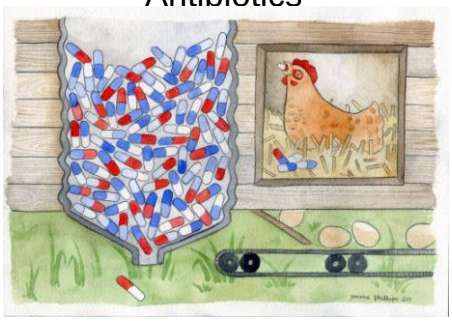
## Antibiotics



**Antibiotics in – eggs out!!!**

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## 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?

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Myth II

## MS TRANSMISSION NEEDS DIRECT CONTACT!

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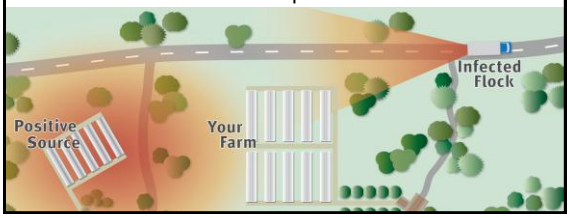
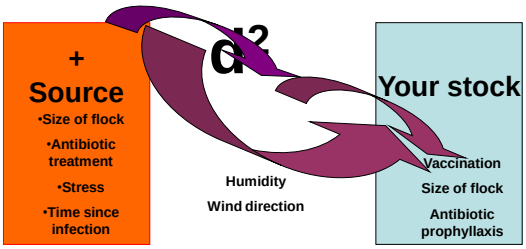
## Mycoplasmas have airborne transmission!

- MS can travel 2km in the wind in temperate climates. The reason why some areas cant control MS.
- Unknown distance in tropical climates




## Risk factors for mycoplasma transmission

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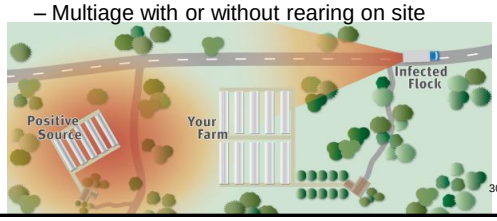
## What have we got

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

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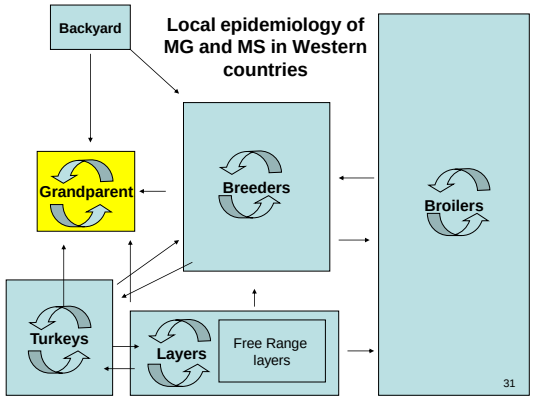


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## Biosecurity only for Mycoplasma freedom

- 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.

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## 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)

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Myth III-

## ts-11 IS NOT STRONG ENOUGH

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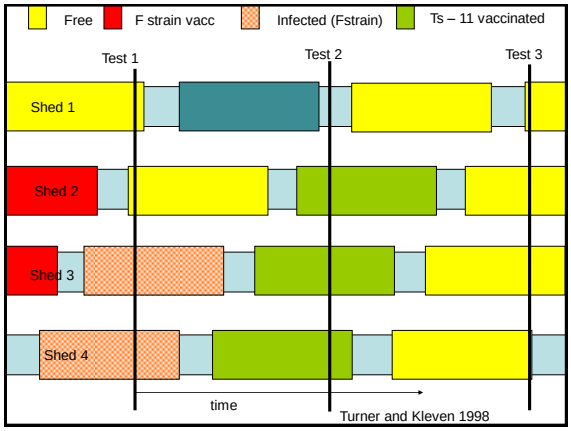
## Is ts-11 strong enough?

- 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?

- Levishon and Kleven 2000 (Rev Sci Tech OIE 19(2): 425-442)
  - *"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

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## 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

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

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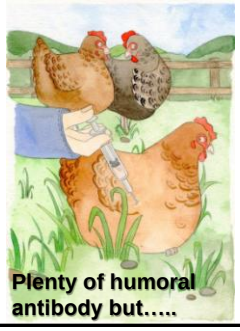
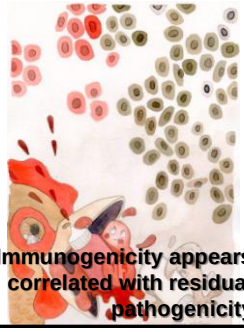
## Controlled exposure

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

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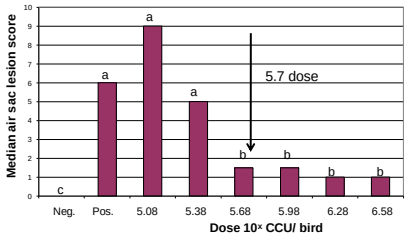
## Dose Response: Air sac lesions 2 weeks after challenge




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

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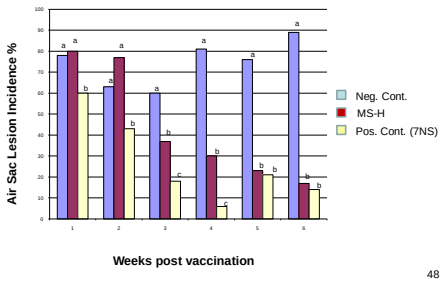
## Onset of immunity: Air sac lesion incidence

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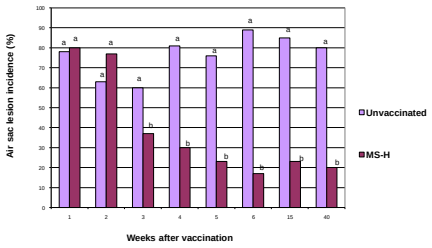
## Duration of immunity: Air sac lesion incidence

(combined onset and duration of immunity results)

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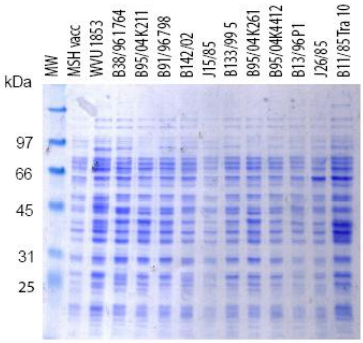
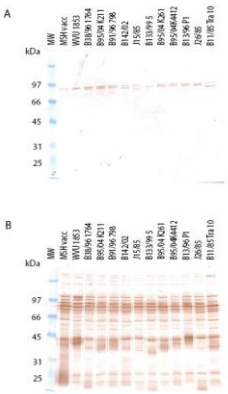
## Protein Profile of MSH & recent MS strains from Europe.




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$^0$C about 9 minutes)
- Keep cool
- Use within 2-3 hrs



## Antibiotic usage in vaccinated flocks

- Amoxycillin (& ceftifour and phosphomycin)
- For gut problems you can use antibiotics not absorbed from the gut
  - Colistin
  - Neomycin, streptomycin, spectinomycin
- Erythromycin (MS inately 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 wont 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)

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## Asian multi-layered MG control programmes.

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

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## Vaxsafe MG (Australian origin)

| | Distributor | Registration date |
|---|---|---|
| Australia | Bioproperties | 1990 |
| Japan | NBI | 30 Aug 95 |
| South Korea | Merial | 30 Jun 98 |
| Thailand | Pfizer | 28 Dec 99 |
| China | Sinder | 1999 |
| Malaysia | Pfizer | 6 Nov 01 |
| Philipines | Pfizer | 14 Mar 01 |
| India | Pfizer | 1 Apr 03 |
| Indonesia | Merial | 2 Mar 06 |
| Iran | Austral Medi | 2010 |
| Turkey | RTA | 2011 |

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## Vaxsafe MG (manufactured under license from Bioproperties)

| Country | Site of manufacture | Date of registration |
|---|---|---|
| USA | USA | 8 Feb 1994 |
| South America | USA | From 1999 |
| Brazil | Brazil | 2 June 2005 |
| South Africa | USA | Sept 1995 |
| Pakistan | USA | 2006 |
| Italy | Italy | 1996 |
| Hungary, Romania, Egypt, Bulgaria, Poland | | Saudi, Lebanon Others |

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## Vaxsafe MS (MSH)

| | Company | Registration |
|---|---|---|
| Australia | Bioproperties | 1996 |
| Mexico | Avimex | 16 Jun 1997 |
| South Africa | Protectachick | August 2005 |
| Japan | NBI | 7 Dec 05 |
| Argentina | Merial | 7 Mar 06 |
| Iran | Austral Medi Vet | 29 Oct 07 |
| Brazil | Merial | 16 Sept 08 |
| Indonesia | Merial | Feb 2011 |
| EU 27 | Pharmasure | June 2011 |
| Philippines | Fort Dodge | June 2011 |

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## 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

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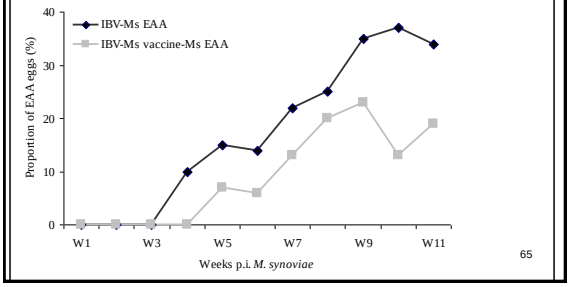
## Experimental challenge of vaccinated birds

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## 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.

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## Japanese Vaxsafe® MSH trials

| Parameter to 57 weeks | Trial 1 82K sonia grey | Trial 2 Lohmann |
|---|---|---|
| Total eggs | +11.4 eggs/HD | +13.4 eggs/HD |
| Normal eggs | +1.4% | +2.9% |
| Egg mass | +795 g | +787g |
| FCR | -0.12 | -0.07 |
| | More eggs earlier | More eggs earlier |

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## Pathology IS MS

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## 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

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## 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

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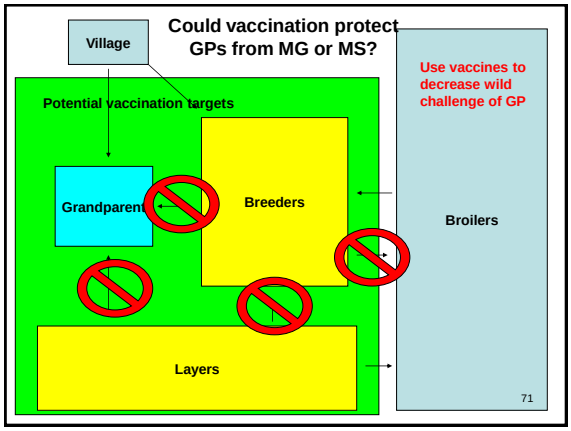


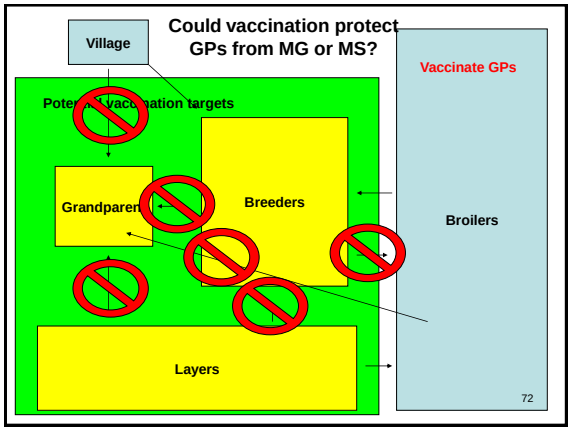


Myth IV

## SEROLOGY IN VACCINATED FLOCKS

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## 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.

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## Serological result after vaccination

| Antibody Response | Vaccine (6+ weeks) | Alternative explanation | Follow up |
|---|---|---|---|
| High | Field challenge vaccine worked | Field challenge vaccination failure | PCR and assessment of protection |
| Med | Usual | Early field challenge | PCR Rebled |
| Low to zero | Can happen especially before lay | Poor vaccination | PCR Rebled |

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## Predictive value of MG RSA test in broiler breeders

| Group | Age vac wk | RSA reactors (score range)* |
|---|---|---|
| ts-11/C | 3 | 0% (0-0) |
| ts-11/NC | 3 | 0% (0-0) |
| ts-11/C | 6 | 40% (0-1) |
| ts-11/NC | 6 | 20% (0-0.5) |
| NV/C | NV | 0% (0-0) |

*Tested at 17 wk, immediately before challenge

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## Predictive value of MG RSA test in broiler breeders

| Group | Age vac wk | RSA reactors (score range)* | Tracheal mucosa µm† |
|---|---|---|---|
| ts-11/C | 3 | 0% (0-0) | 101±5[a] |
| ts-11/NC | 3 | 0% (0-0) | 98±5[a] |
| ts-11/C | 6 | 40% (0-1) | 105±5[a] |
| ts-11/NC | 6 | 20% (0-0.5) | 105±6[a] |
| NV/C | NV | 0% (0-0) | 273±44[b] |

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

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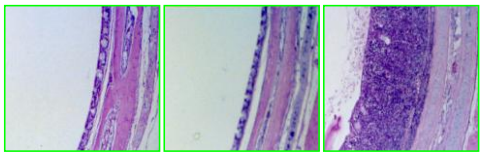
## Does negative RSA response to ts-11 mean poor protection?



Vaccinated 3w Challenged 17w

Vaccinated 3w Not challenged

Not Vaccinated Challenged 17w

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## Local Immunity in Airways

*Comparison of ts-11 and a killed MG vaccine*

| Group | n | RSA Score |
|---|---|---|
| ts-11* | 10 | 1.8±1.1 |
| Bacterin* | 10 | 3.7±0.5 |
| Unvaccinated* | 10 | 0 |
| Not challenged | 10 | 0 |

RSA= rapid serum agglutination test (serology)

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## Local Immunity in Airways

*Comparison of ts-11 and a killed MG vaccine*

| Group | n | RSA Score |
|---|---|---|
| ts-11* | 10 | 1.8±1.1 |
| Bacterin* | 10 | 3.7±0.5 |
| Unvaccinated* | 10 | 0 |
| Not challenged | 10 | 0 |

*Aerosol challenge [a,b,c] P<0.05
RSA= rapid serum agglutination test (serology)

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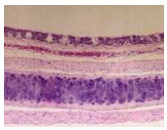
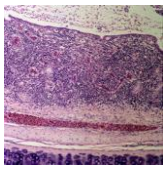
## Protection by MG vaccines

**Ts-11**



**Killed MG vaccine**



High level of serum antibody does not provide protection !

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## 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

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## Strain ID

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

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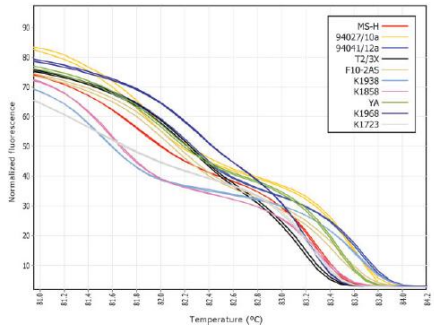
## Strain ID of MS by HRMP analysis




Jeffrey *et al.* Microbiology 2007 **153:**2679-2688

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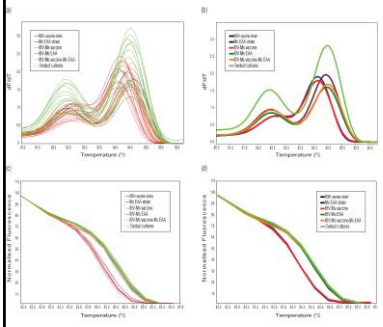
## Strain identification of MS



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

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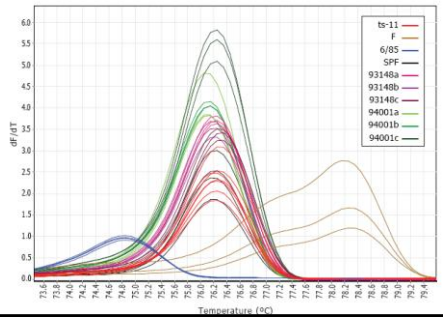
## Strain ID of MG – ts-11

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## 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.

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BIOPROPERTIES
The Vaccine Innovators

## Total Mycoplasma Control

Freedom, vaccination, biosecurity and good diagnostics

The End

## THANK YOU

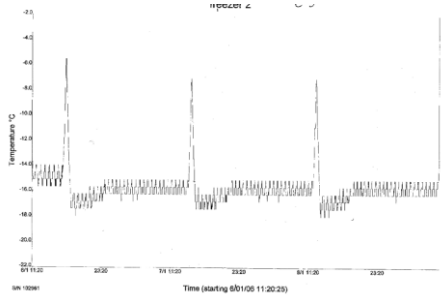
## Investigating ts-11 problems

Chris Morrow
Bioproperties

## 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