
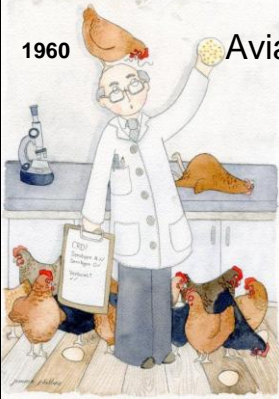




Avian Mycoplasma Myths

Chris Morrow
Global Technical Manager
PhD BVSc


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

4



Myth I

MYCOPLASMA SYNOVIAE IS NOT IMPORTANT.



5

Myth 1: MS does nothing.

<ul style="list-style-type: none"> • Clinically mycoplasmosis can be diagnosed but laboratory testing (culture and speciation or PCR) is need to differentiate MG and MS as the cause 	<ul style="list-style-type: none"> • Anything MG can do MS can also do. • Chronic infections mean these organisms stay around. • Antibiotics can make infection status difficult to determine.
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6

Quantification of Pathogen cost

<p><i>Mycoplasma gallisepticum</i></p> <ul style="list-style-type: none"> • Layers – 10 to 20 eggs per year and FCR • Egg drop in lay • Decreased hatchability • Primary respiratory disease and CRD <ul style="list-style-type: none"> – Mortality and poor FCR in progeny 	<p><i>Mycoplasma synoviae</i></p> <ul style="list-style-type: none"> • Layers – 5-10 eggs per year and FCR • Infectious synovitis <ul style="list-style-type: none"> – Amyloidosis in brown layers • Egg drop in lay • Decreased hatchability • Increased condemnations • Respiratory disease in combination other viruses and respiratory vaccines • <i>E. coli</i> Peritonitis in layer • Glass top eggs <p style="text-align: right; font-size: small;">adapted from Stipkovits and Kempf 1996</p>
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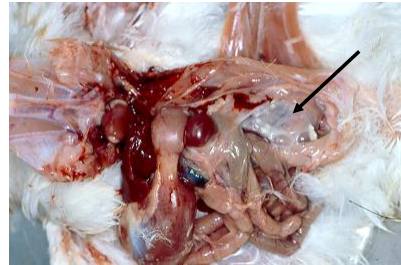
8

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

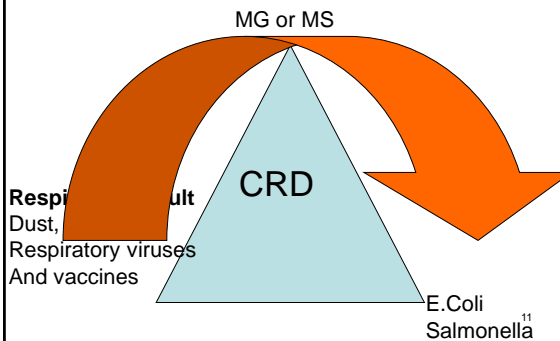
9

Airsacculitis after combined MS & IBV (experimental).



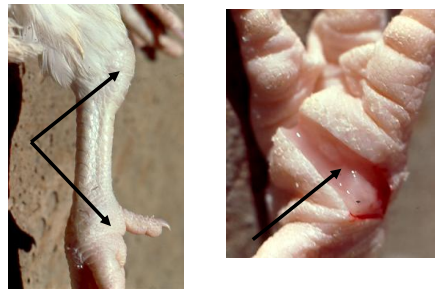
10

Chronic Respiratory Disease



11

Synovitis due to MS – field case



12

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

14

MS Glass top eggs. Egg Apical Abnormality



Febenwee and others Avian Path Nov 2008

15

EAA



17

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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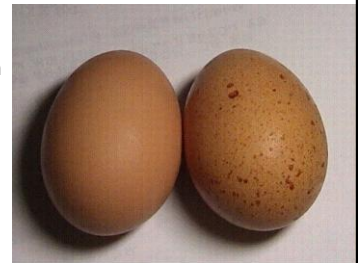
Second quality eggs (Not EAA)

- 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

20

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

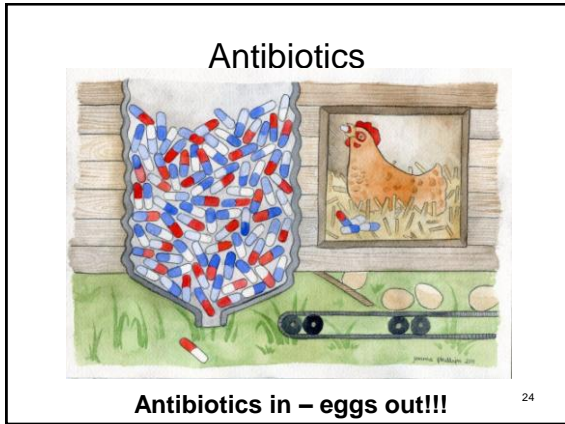
 Pfizer Animal Health
Global Poultry

22

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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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. multocoda*, *Salmonella*, *E. coli*, *Brachyspira* species
 - Can we control these with other strategies?

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Myth II

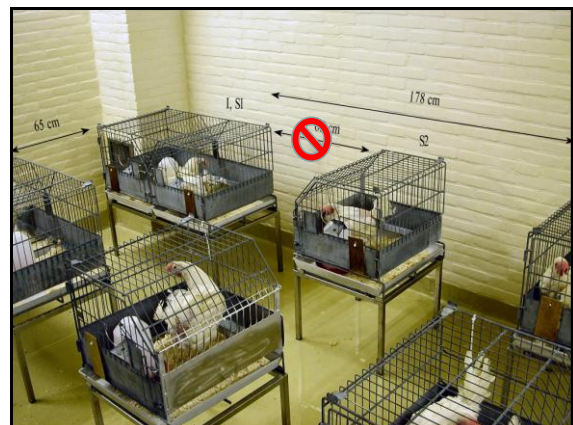
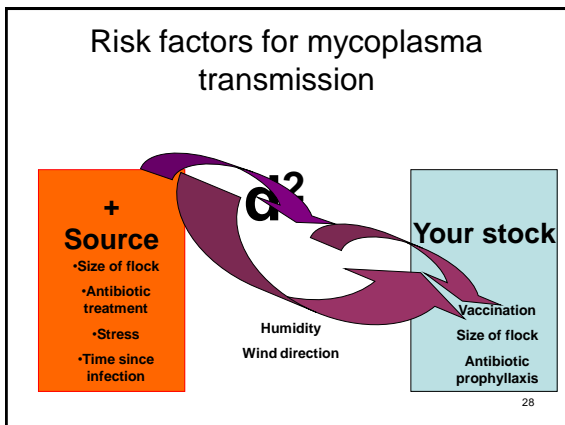
MS TRANSMISSION NEEDS DIRECT CONTACT!

26

Mycoplasmas have airborne transmission!

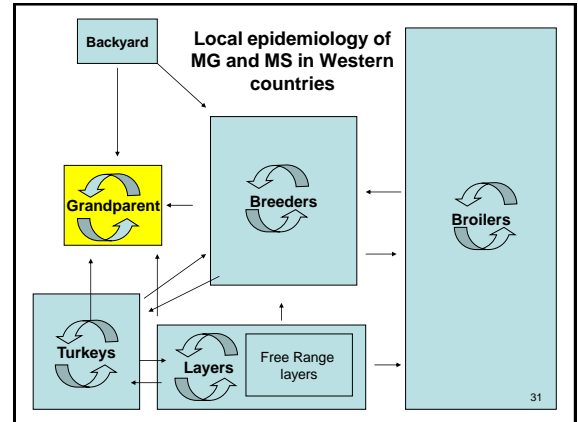
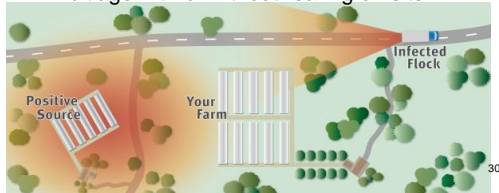
- MS can travel 2km in the wind in temperate climates. The reason why some areas cant control MS.
- Unknown distance in tropical climates

25



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.

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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)

35



Myth III-

ts-11 IS NOT STRONG ENOUGH



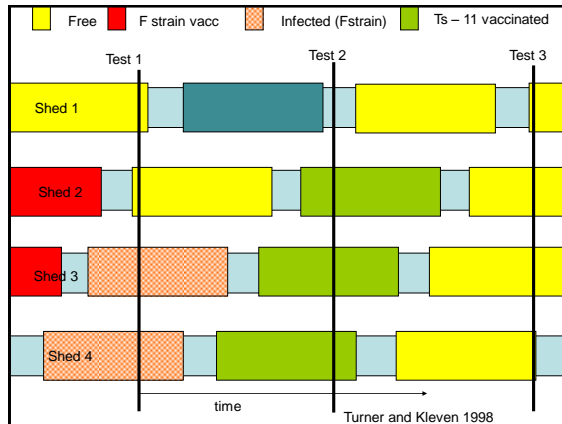
36

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

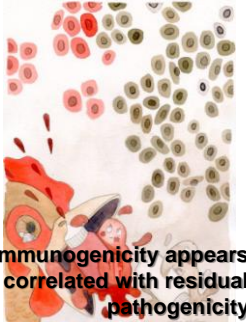


- Based on the observation that natural infection provides protection against subsequent challenge
- Make sure hens were positive for MG before production
- Prevents egg production drops
- Still have losses from sub-clinical infection.

Killed bacterins and mild strains



Plenty of humoral antibody but.....



Immunogenicity appears correlated with residual pathogenicity

ts⁺ vaccines



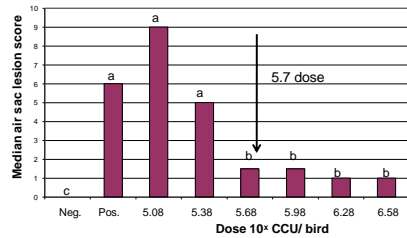
- Core temperature of the chicken (>41.5C) is too high for the vaccine strain to survive so infection is limited to the upper respiratory tract.

45



15 11 2005

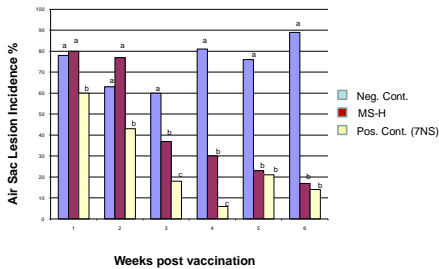
Dose Response: Air sac lesions 2 weeks after challenge



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

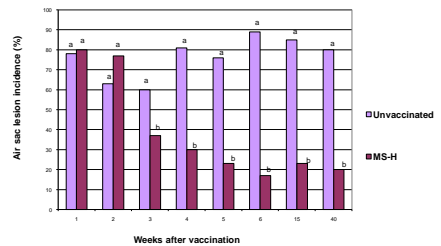
47

Onset of immunity: Air sac lesion incidence

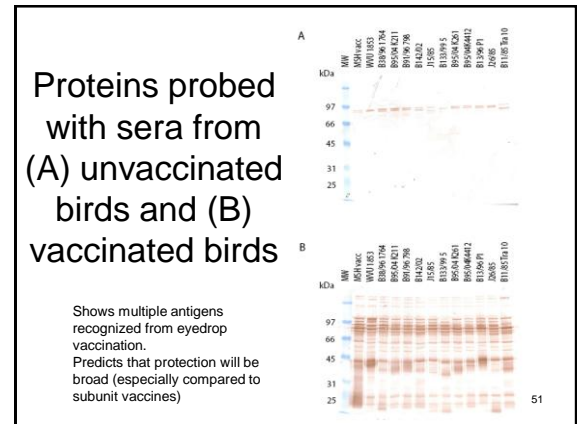
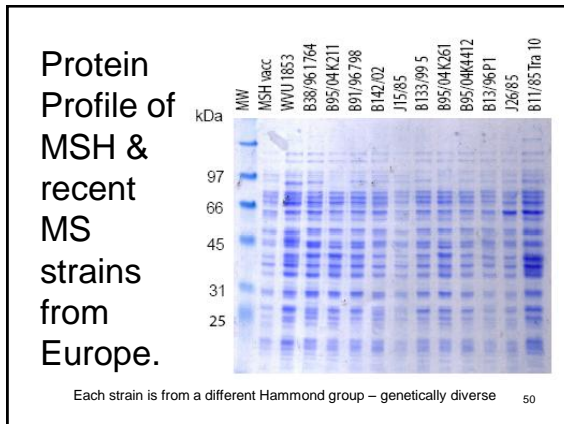


48

Duration of immunity: Air sac lesion incidence (combined onset and duration of immunity results)



49



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’.

52

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

53

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 μ L or \geq 5 x 10⁷ viable organisms
- Thaw quickly (\sim 35°C about 9 minutes)
- Keep cool
- Use within 2-3 hrs

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Antibiotic usage in vaccinated flocks

- Amoxicillin (& ceftifour and fosfomycin)
- 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 x 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
Philippines	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

59

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

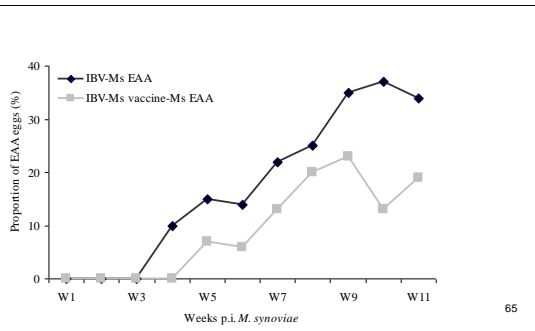
60

Vaccination to prevent EAA

- Done at Deventer laboratory using their challenge model
- IB intratracheal 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	Trial 1	Trial 2
to 57 weeks	82K sonia grey	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

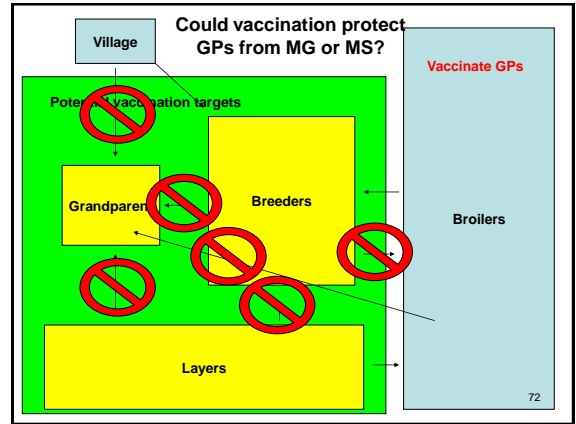
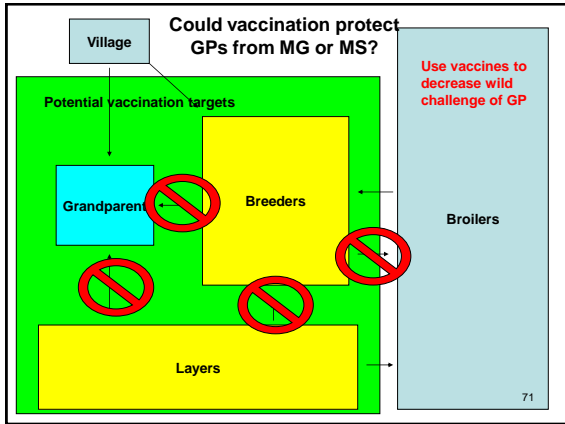
- MSH Vaccination on infected sites
 - Increased 8-12 eggs per hen housed
 - Reduced *E. coli* and less culling needed
 - Less runting and better uniformity
 - Less dramatic production drops when challenged with other diseases or stresses and better recovery
 - Total reduction in infeed medication and 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

73

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

76

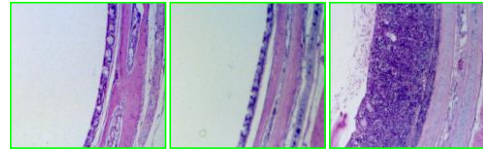
Predictive value of MG RSA test in broiler breeders

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

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

77

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



Vaccinated 3w
Challenged 17w

Vaccinated 3w
Not challenged

Not Vaccinated
Challenged 17w

78

Local Immunity in Airways

Comparison of ts-11 and a killed MG vaccine

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

RSA= rapid serum agglutination test (serology)
79

Local Immunity in Airways

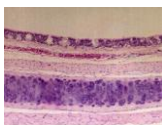
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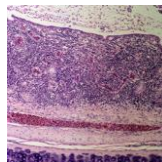
*Aerosol challenge ^{a,b,c} P<0.05
 RSA= rapid serum agglutination test (serology)
80

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

82

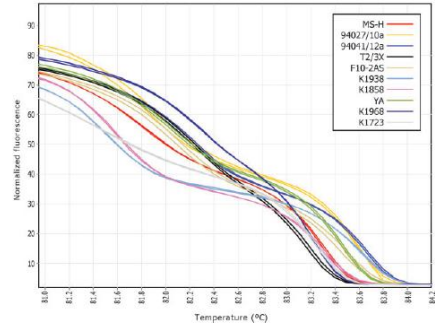
Strain ID



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

83

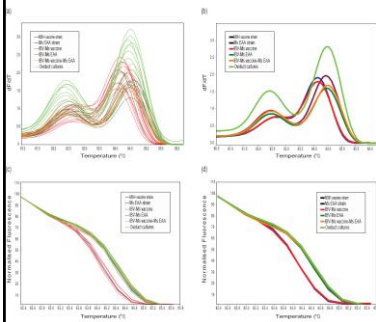
Strain ID of MS by HRMP analysis



Jeffrey et al. Microbiology 2007 153:2679-2688

84

Strain identification of MS



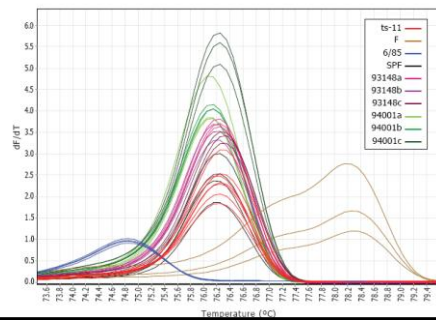
By strain ID method we could show that challenge strain only was associated with EAA eggs

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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Total Mycoplasma Control

Freedom, vaccination, biosecurity and good diagnostics





The End

THANK YOU



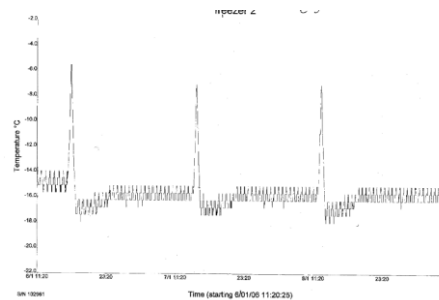
Investigating ts-11 problems

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