

The Historical Context and Rationale for the Development of ts-11 and MS-H

Kevin Whithear

once was mycoplasmaologist

The Vaccines

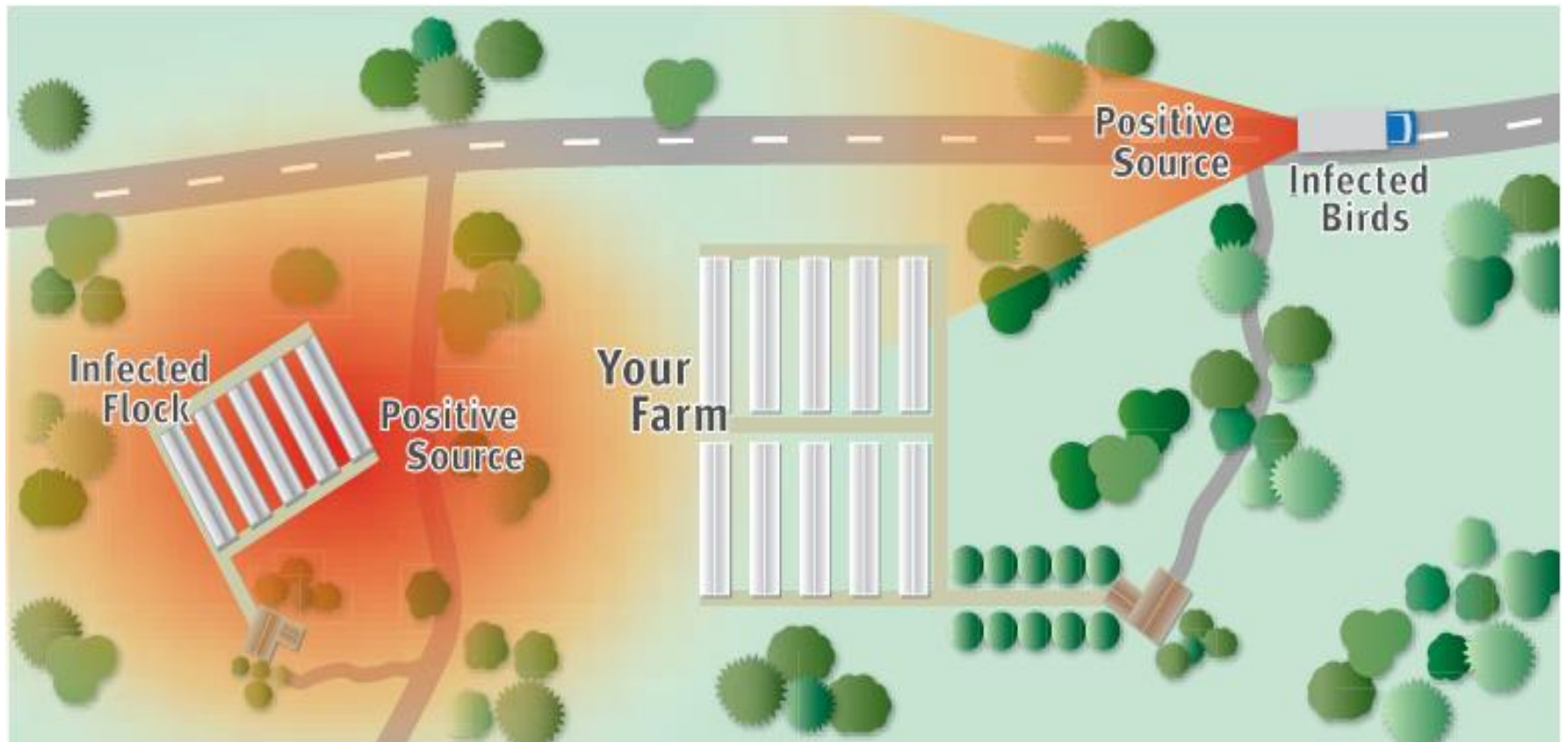
- Australian MG & MS isolates
- Chemical mutagenesis – ts^+ mutants
 - MG ts-11 - 1990
 - MS-H - 1995



Mycoplasma Control in Australia 1970's

- Medicate
 - Particularly with tylosin
- Eradicate
 - Achieved to grandparent breeder level in 1970's
 - Problem maintaining parent breeders free
- Vaccinate
 - Vaccines not used

Potential Airborne Transmission?



Mycoplasma Control in Australia 1980's

- Medicate
 - Tylosin resistance emerges
- Eradicate
 - Frequent breaks at parent breeder level
 - Usually while in production – egg transmission
- Vaccinate
 - Industry demand for a vaccine MG then MS
 - Layer flocks
 - Tool to help control egg transmission in parent breeder flocks

“We felt we had exhausted all available options for preventing infection, so a vaccine was desperately needed.”

Dr Rami Cobb

Former Veterinarian
Inghams Enterprises

First Attempts

- Killed MG and MS Vaccines
 - Parenteral without and with adjuvant
 - Aerosol
- Did not protect

Strains Used to Develop a Live MG Vaccine

- Strain 80083 –
 - Isolated from broiler breeder – slow spreading
 - Parent vaccine strain
 - Pathogenicity and immunogenicity cf F strain
- Strain Ap3AS –
 - Isolated from broiler – tylosin resistant
 - Challenge strain
 - Pathogenicity cf R-strain

Strain 80083 as Live MG Vaccine

- Attenuate by *in vitro* passage
 - 80083 (6)
 - 80083M (50)
 - 80083H (100)
- Produce ts^+ mutants
 - ts-11

Relative Virulence

STRAIN	% BIRDS WITH AS LESIONS	MEAN AS SCR \pm SD
80083	60	1.9 \pm 1.4
80083H	10.5	0.1 \pm 0.2
AP3As	96	3.3 \pm 0.2

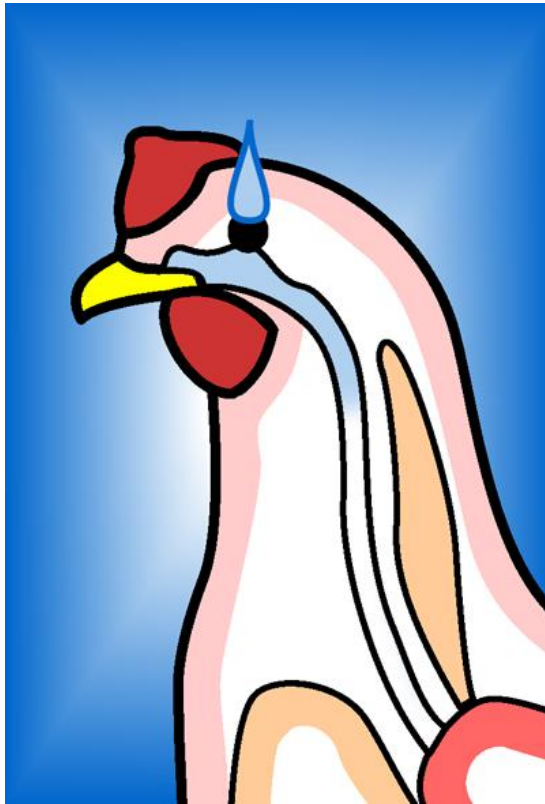
Average of 4 experiments after Soeripto et al 1989

Birds inoculated intra-abdominally with 0.2mL thawed undiluted culture

Important Procedural Innovations in Development of ts-11 and MS-H

- Eye drop route of vaccine administration
- Standardised aerosol challenge method
- Objective method of assessing protection

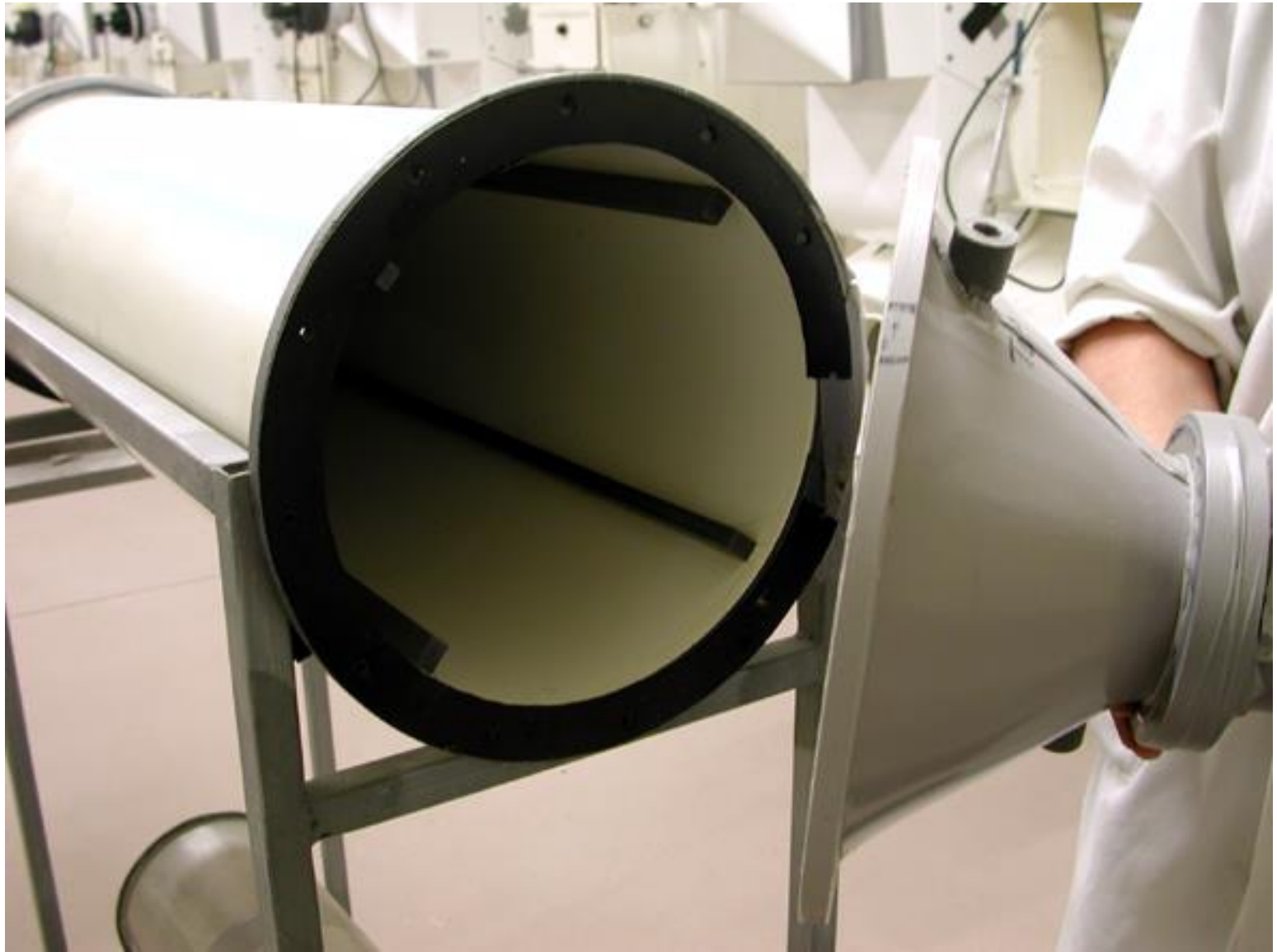
Why Use Eye Drop?



- Every bird vaccinated
- Reliable method of getting an effective dose at the target site
- Effective way of stimulating mucosal immunity
 - Harderian gland
 - MALT
- Uniform flock immunity

Standardised Aerosol Challenge

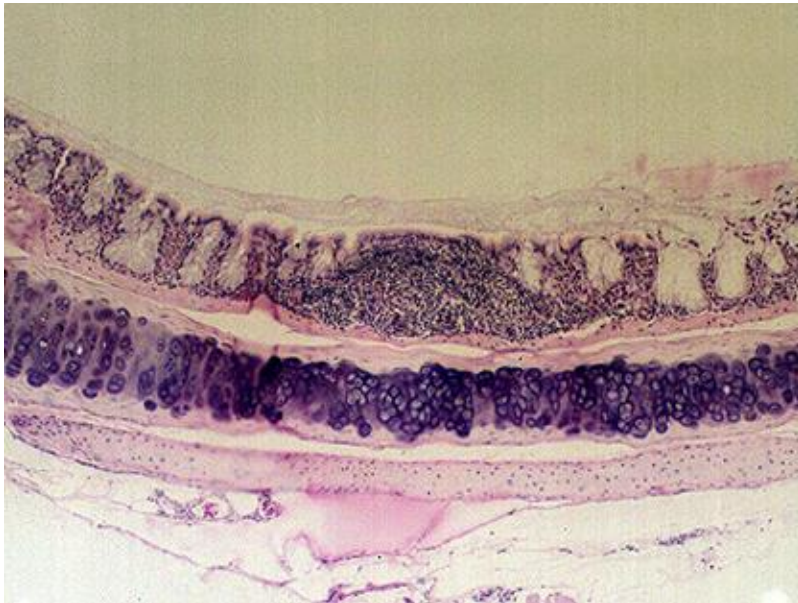




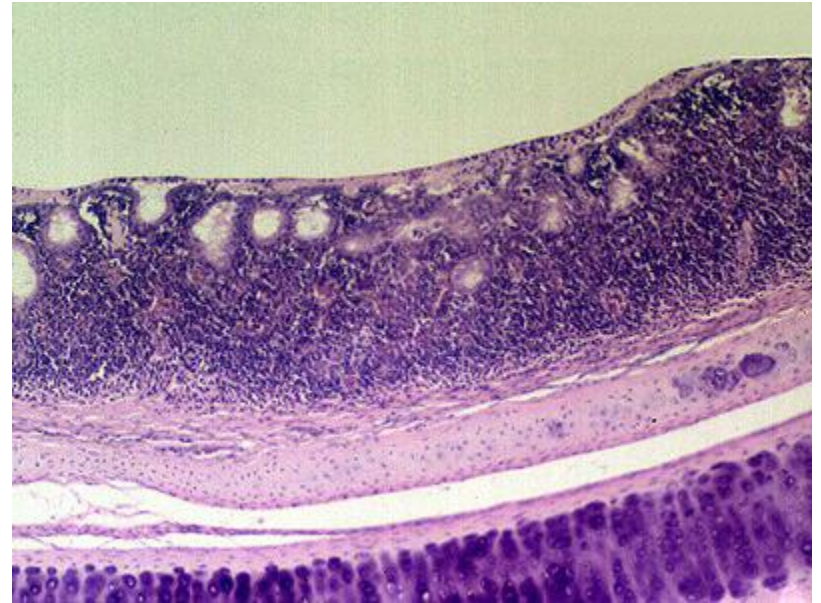


Objective Measure of Protection

Challenge 40 w After Vaccination



ts-11 Vaccinated
= 73.2 μm



Unvaccinated
= 238.0 μm

Prolonged Immunity in Trachea

Challenge 40 w After Vaccination with ts-11

Group	n	RSA Score	Mucosa μm
ts-11*	13	1.3±1.0	73.2 ^a
Unvaccinated*	10	0	238.6 ^b
Not challenged	9	0	50.6 ^a

*Aerosol challenge

^{a,b} P<0.05

Prolonged Immunity in Reproductive Tract

Group	n	Ovarian activity	MG isolated
ts-11*	13	77%	0%
Unvaccinated*	10	30%	80%
Not challenged	9	100%	0%

*Aerosol challenge 40w after vaccination

Improved Egg Production

Commercial Cage Layers - Australia

Trial groups	*Mean HH eggs to 65 weeks
Unvaccinated	211.9^a
Vaxsafe MG (ts-11)	219.6^b

***Mean of 6 trials ^{a,b} P<0.01**

Improved Egg Production

*Commercial Cage Layers - Japan**

Trial groups	Mean HH eggs to 76 weeks
MG-BAC (Solvay)	315.3
Vaxsafe MG (ts-11)	338.4

*Data courtesy Nippon Biologicals Inc

Developing a Live MS Vaccine

- Strain 86709/7NS – parent vaccine strain
 - ts^+ mutant MS-H
- Strain 88064FP + T strain IBV – challenge strain

Stability of ts^+ Mutant Vaccines

- 10^{-4} back mutation rate to ts^-
- ts^- variants retain lack of virulence of ts^+ vaccine strains
- Slight increase in virulence of ts^- variants isolated from Australian flocks vaccinated for some time – no clinical disease
- Georgia USA - ts^- variants of ts-11 – clinical disease

No Correlation Serum Antibody and Protection

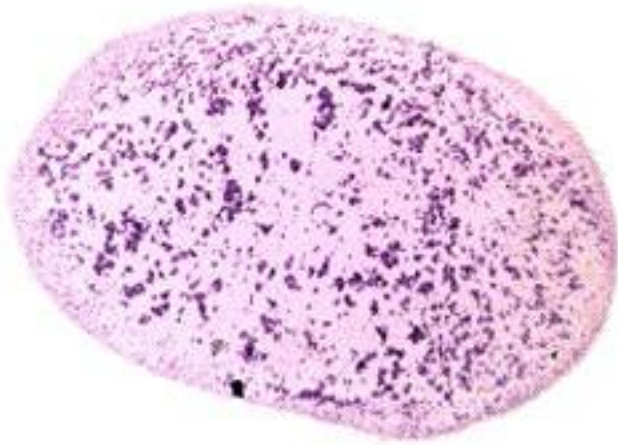
Comparison of ts-11 and a Bacterin

Group	n	RSA Score	Mucosa µm
ts-11*	10	1.8±1.1	71.4 ^b
Bacterin*	10	3.7±0.5	251.1 ^c
Unvaccinated*	10	0	253.6 ^c
Not challenged	10	0	44.3 ^a

*Aerosol challenge

a,b,c P<0.05

Reliability of Serum Antibody as Indicator of Protection



- ts-11 vaccinated
 - Positive predictive value = 74%
 - Negative predictive value = 44%
- Unvaccinated
 - Negative predictive value = 100%

Serum Antibody and Protection

Group	Age Vac weeks	RSA Reactors (scr range)	Tracheal Mucosa μm
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

After Noormohammadi et al (2002)

Interpreting Serology

- Never been easy
- Bioproperties Tech Bulletin – *Serology after ts-11 and MS-H mycoplasma vaccination*
- Four serological patterns
- Pattern B – low to no serum antibody response. The vaccine is doing its job!

Benefits of Vaccination

- MG and MS no longer cause significant disease in commercial layer and meat chicken flocks in Australia
- Disease freedom can be largely attributed to the continued use of ts-11 and MS-H vaccine
- With continued use of the vaccines the prevalence of wild-type MG and MS seems to have diminished

Benefits of Vaccination(2)

- Greatly reduced need for antibiotics particularly tylosin and other macrolides
- Anecdotal, less coryza and fowl cholera
- A better night's sleep for farm managers

Acknowledgements

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