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MS-H

Vaccine Eyedrop Suspension

Vaccination against Mycoplasma synoviae using MS-H strain, live temperature sensitive vaccine

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Introduction

- Review of Mycoplasma synoviae and its impact
  - Eggshell Apex Abnormalities (EAA)
- Vaccination strategies & MS-H vaccine development
- Review of MS-Vaccine

> **Mycoplasma Synoviae (MS) can cause disease without any sign of synovitis**

Significance of Ms

- Effects of Ms are strain / pathotype dependent
- Some strains associated with respiratory issues whereas others are associated with egg production loss
- MS can exacerbate problems such as E. coli peritonitis in layers
- Research & experience prove the economic impact of MS infection but widespread use of antibiotics have masked the infection & economic loss
- Expectation of bird performance is now greater, Ms more likely to be a limiting factor.

Ms Clinical Manifestation

Respiratory strains:
- Subclinical
- Airsacculitis (+/- other infections)

Arthropathic strains:
- Lameness & swollen joints

Salpingotropic or oviduct strains:
- Eggshell Apex Abnormalities (EAA)
- Egg production ↓
- Egg breakage ↑

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Qualification of Ms Cost

- Layers – 5-10 eggs per hen per year
- Infectious synovitis.
  - Amyloidosis in brown layers
- Egg production drops in lay
  - Roller coaster performance
- Decreased hatchability in broilers
- Increased condemnations
- Respiratory disease in combination other viruses and respiratory vaccines.
- E.coli Peritonitis in layers.
- Glass top eggs (EAA).

Economic impact

Layer Farm

- EEA production 2-3%
- Egg losses due to breakage 2-3%
- Downgrading 2-3%
- Increased labor (selection & cleaning)

Packing/station

- Increased labor (selection & cleaning)
- Egg losses during transport 10%

Total NL 3.1 million €

Why is Ms Still a Problem

- Survival of Ms
  - Outside host
    - Hair/nose: 1-3 days
    - Feathers/dust/straw/cotton/rubber: 2-4 days
    - Egg material: 6-18 weeks
  - Within host: 'evades' the immune system
- Transmission
  - Lateral:
    - Direct bird contact
- Indirect:
    - Airborne transmission
    - Materials
    - People
    - Equipment including Mobile phones!!
    - Artificial Insemination
- Intracelluar survival
- Reservoir of infection
  - Backyard poultry
  - Multi-age
- Limited vaccine efficacy with traditional vaccines
- Antibiotic resistant strains

Diagnostic Difficulties

- RPA (IgM)
  - Very sensitive, but less specific
  - Cross reactions (false positives)
  - Non specific reactions (false positives)
  - Affected by antigen quality (False negatives)
- HI (IgG)
  - More specific but less sensitive than RPA
  - HI properties of strains vary
- ELISA
  - More sensitive than RPA or HI
  - ? Non specific reactions especially in older birds
  - ? Cross reactions with other mycoplasmas
- PCR
  - Sensitive
  - Specific
  - Can test samples of oviduct

NB: some strains cause very slow seroconversion

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Ms: Current Situation in Europe

- There is a large variation in the pathogenicity of M. synoviae field strains.
- Some more recent strains cause a significant increase in downgrades of table eggs due to egg shell abnormalities as a result of generalised infection and colonisation of the oviduct (EAA).
- M. synoviae was the only major pathogen of chickens for which there has been no effective live vaccine available in Europe.

EEA

- Occurrence
  - first seen in 2000
  - Variable time of onset (usually later in breeders)
  - Brown and white layers affected
  - All types of housing affected

Synergism Ms-IBV EAA

	Ctrl	Ms	IBV D1466 & Ms EAA
Mean egg/ hen/day	0.75	0.49	0.54
% EAA	0	7 (49/685)	14 (96/673)
Strength non-EAA*	31.7	35.6	37.2
Strength EAA*	-	12.7	12.7

**Newton*

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Vaccination Strategies

Inactivated vaccines

Pros
No vaccine transmission possible.
May improve clinical signs & economic return.
Generate plenty of humoral antibody

Cons
Poor efficacy against respiratory disease.
Don't stop vertical transmission.
Cost of multiple doses
Injection site reactions & effects on uniformity.
Practicalities of use if monovalent.

MS-H live vaccines

Pros
Apathogenic.
Persistent stimulation of mucosal immunity
Generates lifelong protection.
No vertical transmission.
Very limited horizontal transmission.
Single dose required.
No reversion to virulence.
Can be differentiated from field strains.

Cons
Eye drop administration.
Frozen vaccine- training required for use.
May elicit an antibody response.

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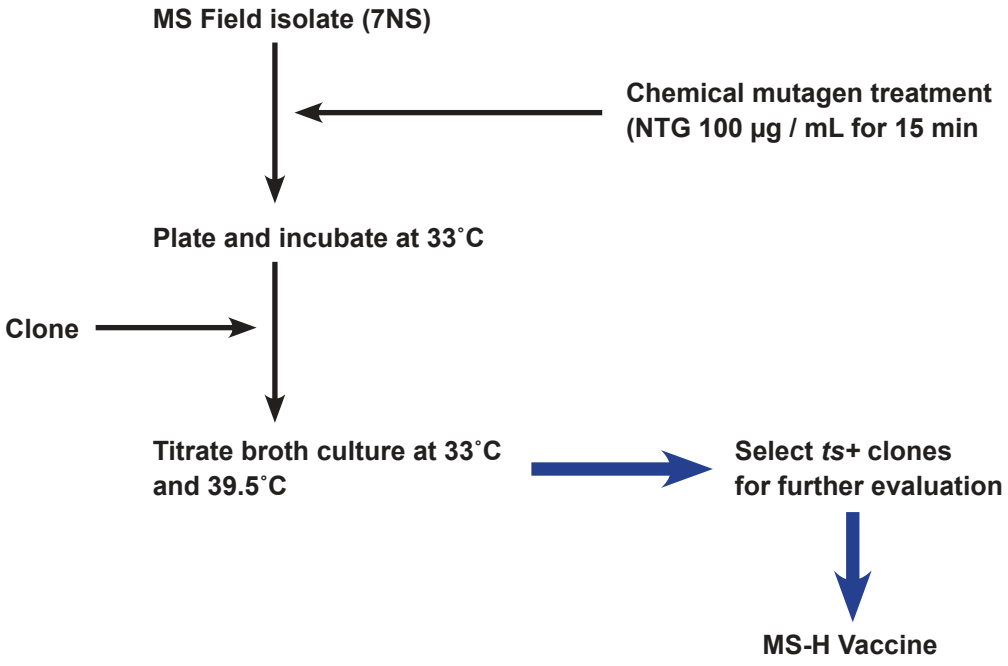
Ms-H Development Process



The field strain was isolated from a ts-11 vaccinated Australian layer flock that still had respiratory disease during lay. The mutagen NTG was used to mutate the field strain.
After mutagenesis the survivors were plated out and grown at the permissive temperature. Individual colonies were then cloned/selected and grown 33˚C (the permissive temperature) and then characterize for temperature sensitivity. This was defined as have a greater than 2 log10 reduction in titre when grown at the non permissive temperature. 39.5˚C. This temperature is lower than the core temperature of the chicken (41.5˚C).
A small number of clones displayed the temperature sensitive property and these were selected to see if they would be suitable as a vaccine. Some would not infect chickens etc but MSH was selected as a potential vaccine candidate.

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Creating an Ms Vaccine Candidate

- Temperature sensitivity (*ts*+) limits colonization of the bird in the cooler upper respiratory tract.
  - Growth is reduced at the bird's core temperature
- NTG mutagenesis creates multiple mutations.
  - Creation of multiple mutations offer protection against reversion to virulence.

Auxotrophs of mycoplasmas are nearly impossible to make as the media is very complex containing at least 10% animal sera (thus it is very hard to make it deficient in something like Histidine or Arginine). The temperature of the respiratory tract varies from the nares to the lungs. The beauty of temperature sensitivity is that the colonization is limited and all a bird has to do to limit the colonization is maintain its core temperature. It does not need to be immunocompetant.

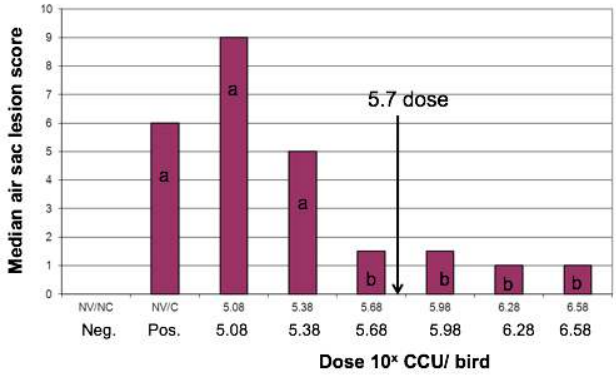
The need to eye drop Ms-H vaccine

- The minimum infective dose for *ts*+ strains is inherently high
- This provides benefits in terms of possible horizontal spread
- Eye drop administration is therefore necessary

MS-H no reversion-to-virulence

- Stability of attenuation assessed by
  - 5 *in vivo*, and
  - 10 *in vivo* passages
- The final passages after both methods retained the ts+ phenotype
- The final passages were apathogenic when inoculated directly into the air sac of 2-week old SPF chickens

Dose Response: Air sac lesion 2 week post challenge



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

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Benefits of Using MS-H Vaccine

- Several studies have also shown benefits in terms of egg numbers and reductions in substandard eggs

MS-H, EAA protection study: Deventer

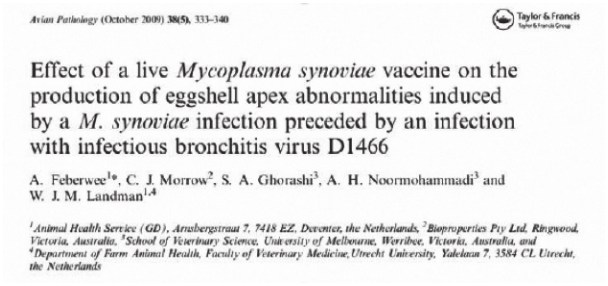
	IBV & Ms EAA	IBV-Ms vac & Ms EAA
Mean egg/hen/day	0.48	0.54↑
% EAA	22.9 (148/646)	11.9 (88/741)↓
Strength non-EAA*	33.7	37.0↑
Strength EAA*	22.8	22.8=

**Newton*

Mechanisms of Immunity

- Not fully understood
  - Not humoral antibody
    - Birds with no humoral antibody can be immune
  - Not competitive exclusion
- Predicted that Ms-H vaccine needs to colonize the upper respiratory tract for life to maintain immunity
  - NB Antibiotic treatment of flocks could interfere with immunity maintenance.

Serological response is variable but typically low and slow to develop (some unchallenged flocks to not reach peak serological response till after 40 weeks). Monitoring vaccine administration can not be done reliably with serology. It is best to monitor vaccine cold chain and administration (Like with Mareks vaccination or coccidiosis vaccination). If you need to find the vaccine you should use PCR with sequencing or HRM (high resolution melting point analysis). Mucosal immunity is short lived and requires repeated antigenic stimulation for persistance.


Avian Pathology (October 2009) **38(5)**, 333–340

Taylor & Francis

Effect of a live *Mycoplasma synoviae* vaccine on the production of eggshell apex abnormalities induced by a *M. synoviae* infection preceded by an infection with infectious bronchitis virus D1466

A. Feberwee[1]*, C. J. Morrow[2], S. A. Ghorashi[3], A. H. Noormohammadi[3] and W. J. M. Landman[1,4]

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Field Based Experience from 10 years use of live MS-H Vaccine

- Prevents respiratory, egg shell problems and infectious synovitis due to wild Ms.
- Has been used in the USA to displace wild strains and decrease local challenge.
- Occasionally spreads to adjacent sheds but not to other farms.
- Massive decrease in antibiotic usage in areas adopting vaccination.
- Sensitive to all anti-Ms antibiotics (note Ms is innately resistant to erythromycin).

These are a summary of our experience around the world and not claims.
Experiments where you see increased egg production (supposedly non specific) to routine antibiotic administration are probably due to the effect on MS.

Logistics of MS-H Vaccine

- MS-H must be stored below -70˚C.
- The vaccine must be shipped in insulated boxes in dry ice
- MS-H can be stored for up to to 4 weeks @ -20 ˚C
  - Use ‘chest’ rather than ‘vertical’ freezers
  - AVOID ‘frost-free’ and ‘bar-fridge’ freezers
- Thaw in a large volume (5L) of water

But most important from a logistics point of view, is any event or procedure causing the vaccine to thaw and then re-freeze will have an immediate and fatal effect on the living vaccine organism. This should remain uppermost in one’s thinking, from the manufacturer through to the end users of the vaccine.

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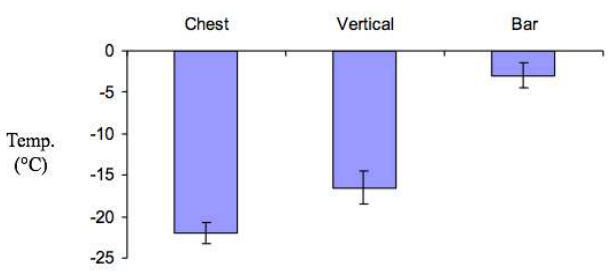
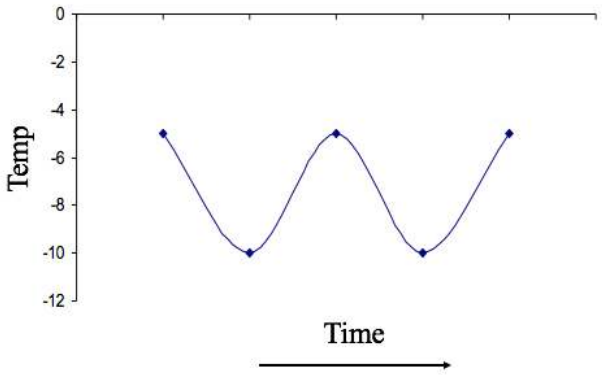
Beware Performance of some domestic freezers



Temperature profile of frost free freezer



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Practical use of MS-H Vaccine

- Thaw quickly (~350˚C) in a water bath
  - Use a large volume of water (5 – 10L)
  - Remember temperature sensitive !
  - Remove once ice melted
- Thaw should take 3-5 minutes
- Keep cool after thawing
- Use within 2 hrs.
- Do not shake the vials as the MS-H is fragile
  - Roll not shake!
- Do **NOT** re-freeze once thawed

- 1000 dose vials
- Eye drop (30 µL per bird)
  - 1 drop per bird
- MUST be well organised for storage, transport, thawing and administration.
- Ensure each bird receives a full dose
- Allow a full drop to form
- Place drop in the middle of an open eye
  - Ensure drop does not roll off the eye due to surface tension
- Pause until bird blinks before releasing

Blue dye added to vaccine for training. This makes it easier to see the drop in low light intensity.

Blue mareks dye added at 4-5 drops from a needle per MS-H vial

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- Must be sero-negative before vaccination
- ? PCR to confirm status.
- Use possible between 5 weeks of age and 5 weeks prior to onset of lay. Vaccinate as early as possible if challenge likely in rear.
- Possibly medicate prior to vaccination: 2 week gap required.

Monitoring Vaccination

- Audit administration
- Serology can give an indication but is not definitive.
  - New biochek ELISA being evaluated in vaccinated flocks
  - Small slowly developing antibody response is seen and absence is not necessarily correlated with vaccination failure.

Serological results after vaccination with MS-H

Antibody Response after vaccination :@ 6 weeks		Alternative explanation	Follow up
High	Can happen up to 40 weeks	Field challenge	PCR
Med	Usual	Early field challenge	PCR re-bleed
Low to Zero	Can happen especially before lay	Poor vaccination	PCR re-bleed

T = +5 sec

T = +30 sec

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Summary

- Mycoplasma synoviae remains a persistent threat to the poultry industry
- MS-H vaccine provides effective & persistent immunity against disease challenge

Do's & Don'ts

- Store & Transport at the correct temperature
- Do not thaw and re-freeze
- Do not cut dose
- Do not spray
- MS does cause a significant economic impact in poultry
- Believe in MS-H because it is one of the most effective vaccines you will ever sell.

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