Etiology, pathogenesis, clinical signs, diagnostics in different species, sampling and laboratory methods for the detection of Mycoplasmosis

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Mycoplasmosis

Mycoplasma species

Mycoplasma – the organism

Mycoplasma – the main diseases

Mycoplasma – sampling and diagnosis

Control
### Taxonomy

<table>
<thead>
<tr>
<th>Division</th>
<th>Tenericutes</th>
<th>cell-wall less bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>Mollicutes</td>
<td>soft skin</td>
</tr>
<tr>
<td>Genera:</td>
<td>Ureaplasma, Spiroplasma, Acholeplasma, Anaeroplasma, Astereoleplasma, Entomoplasma, Phytoplasma, Mesoplasma and <strong>Mycoplasma</strong>.</td>
<td></td>
</tr>
<tr>
<td>Family:</td>
<td>Mycoplasmataceae – contains Ureaplasma and Mycoplasma</td>
<td></td>
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</tbody>
</table>

**More than 125 named**

**Mycoplasma** species
Mycoplasma (class Mollicutes)
The Organism

- Smallest organism that is capable of self-replication.
- Generally host-specific, few opportunist zoonoses.
- Small: cell = 300-800nm; genomes = 0.58-1.38Mbp
- Nutritionally fastidious, dependent on host.
- TCA cycle is absent, cannot synthesise own amino acids, nucleotides or fatty acids.
- **Lack a cell wall.**
Significant Mycoplasma Diseases

OIE Listed (Office International Epizooties)

Contagious Bovine Pleuropneumonia (CBPP) *M. mycoides* subsp *mycoides* (SC)

Contagious Caprine Pleuropneumonia (CCPP) *M. capricolum* subsp *capripneumoniae*

Contagious Agalactia (CA) *M. agalactiae*

* M. capricolum* subsp *capricolum*
* M. mycoides* subsp. *capri*
* M. putrefaciens
Significant Mycoplasma Diseases

OIE Listed (Office International Epizooties)

Avian Mycoplasmosis

*M. gallisepticum*  Chronic respiratory disease (CRD)
*M. synoviae*  CRD and sinusitis

(M. *meleagridis* affects Turkeys—Not OIE listed but in EU Directive 2009/158).
Significant Mycoplasma Diseases

Cattle, Sheep and Goats, Pigs, Man

Cattle:  *Mycoplasma bovis*
        *Mycoplasma dispar*
        *Mycoplasma bovigenitalium*
        *Mycoplasma (Eperythrozoon) wenyonii*
        *Ureaplasma diversum*

Sheep and Goats:
        *Mycoplasma ovipneumoniae*

Pigs:  *Mycoplasma hyorhinis*
        *Mycoplasma hyopneumoniae* - Enzootic pneumonia

Man:  *Mycoplasma pneumoniae* - Community acquired pneumonia
        *Mycoplasma genitalium* – genital infections
        *Mycoplasma hominis* - genital infections
        *Ureaplasma urealyticum* - genital infections
Mycoplasma species occurring in Avian species

23 species. In addition, new species and unusual isolations such as 
* M. capricolum* and *M. verecundum* like isolates and *M. bovis* from chickens
Mycoplasma gallisepticum - Chronic Respiratory Disease (CRD).

Clinical Signs

Coughing, nasal and ocular discharge, poor productivity, slow growth, leg problems, stunting, inappetance, reduced hatchability and chick viability, occasional encephalopathy and abnormal feathers.

Post-mortem lesions

Airsacculitis.
Pericarditis.
Perihepatitis (especially with secondary E. coli infection).
Catarrhal inflammation of nasal passages, sinuses, trachea and bronchi.
Occasionally arthritis, tenosynovitis and salpingitis in chickens.

Cost worldwide estimated to be $780M per annum
Mycoplasma gallisepticum

In other birds -
Parrots, duck, geese and finches (USA)
Mycoplasma synoviae

**Mycoplasma synoviae** - Synovitis, Airsacculitis,

**Clinical signs**

There may be no signs, depression, inappetance, ruffled feathers, lameness, swelling of hocks, shanks and feet, faeces may be green in acute infections. Effects on egg production.

**Post-mortem lesions**

Joints and tendon sheaths have viscid grey to yellow exudate, may lead to amyloidosis (aggregated protein lumps), green liver, exudate becomes caseous later, sternal bursitis, airsacculitis, may see swollen liver, spleen and kidney
Mycoplasma synoviae

Eggshell apex abnormalities (EAA)
**Mycoplasma meleagridis**

*Mycoplasma meleagridis* - Turkeys (and some birds of prey)

Venereal transmission in breeders

**Signs**

Reduced hatchability, slow growth, leg problems, stunting, mild respiratory problems, crooked necks, infected parents may be asymptomatic.

**Post-mortem lesions**

Airsacculitis
downgrading of meat
Mycoplasma iowae

*Mycoplasma iowae* - Turkeys (wild birds)

- Vertical, venereal or horizontal transmission
- Causes embryonic mortality and reduced hatchability
- Not OIE listed, not in EU Directive or UK Poultry Health Scheme
- Few reports of cases in the EU until recently
- No serological test for *M. iowae*
Serological (Flock) Tests

Serum Plate Agglutination Test (SPA) or slide agglutination test. OIE recognised test

- Production and supply problems.
- Specificity problems.
- Requires fresh serum.

Interpretation: >15% positive
Serological (Flock) Tests

Commercial ELISA Tests

Sensitivity and specificity limitations, varies with avian species
Useful flock screening, with clinical information. However positive results can prevent export or result in PHS suspension
Immunoblot Tests

Confirmatory test

• Facilitate exports, when screening tests give “false” positive results.

• Reduce need for slaughter of birds and expensive mycoplasma culture testing for Poultry Health Scheme.

• Validated for use at AHVLA – use agreed with Defra.
Immunoblot Tests

1. Antigen growth and specific extraction
2. SDS PAGE

14 different *Mycoplasma* species, 5 Mg isolates lanes 6 -10

3. Transfer proteins to membrane

4. Antigen antibody reaction / detection on membrane

5. Interpretation – key immunogenic proteins must be present
Culture

Traditional Method

Culture (up to 28 days) dilutions to maximise recovery rate

Biochemical tests (tetrazolium broth)

Growth Inhibition Test
Culture / Identification

Fluorescent Antibody Test
Multiple Mycoplasma infections
Most culture media for *Mycoplasma* species includes:

- **Peptone** (PPLO broth – beef heart infusion, bacto peptone, sodium chloride - Vegetable peptone is also acceptable)
- Serum, DNA, Yeast Extract, glucose or arginine or urea (pyruvate)
- (Nicotinamide adenine dinucleotide – for *M. synoviae* sensitive to acid pH)

May include inhibitors to other bacteria: Thallium acetate*, Penicillin, Sulphamethazine, Polymixin B Sulphate.

Usually adjusted to pH 7.4 to pH 7.8

Commercial media available from Mycoplasma Experience: [www.mycoplasma-exp.com](http://www.mycoplasma-exp.com)

Incubation at 37°C in 5-10% CO₂ with high humidity

(Some laboratories use increased Nitrogen concentrations).

*M. meleagridis*
M. Exp Avian
Medium
5 days  Bar = 1 mm
Polymerase Chain Reaction

Mg and Ms: OIE Terrestrial Manual (2008) chapter 2.3.5.

http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.05_%20AVIAN_MYCO.pdf

Real-Time PCR

Baseline is defined as PCR cycles in which a reporter fluorescent signal is accumulating but is beneath the limits of detection of the instrument. ΔRn is an increment of fluorescent signal at each time point. The ΔRn values are plotted versus the cycle number. Threshold is an arbitrary level of fluorescence chosen on the basis of the baseline variability. A signal that is detected above the threshold is considered a real signal that can be used to define the threshold cycle (Ct) for a sample. Threshold can be adjusted for each experiment so that it is in the region of exponential amplification across all plots. Ct is defined as the fractional PCR cycle number at which the reporter fluorescence is greater than the threshold. The Ct is a basic principle of real time PCR and is an essential component in producing accurate and reproducible data.

Commercial PCR Kits and R-T PCR available,
Molecular testing

Polymerase Chain Reaction followed by Denaturing Gradient Gel Electrophoresis (PCR/DGGE) TC0672

- Allows PCR products to be separated according to sequence
- PCR based on 16S rDNA gene
- dsDNA melts in denaturing gradient of formamide and urea
- Migration stops when DNA melts
- Melting depends on seq.
- Can be used on any pathogen

Sensitive to 10⁻⁹ ng/µl DNA; Specific. Detects mixed infections in one test

1. M. gallisepticum
2. M. gallinarum
3. M. iners
4. M. gallopavonis
5. M. lipofaciens
6. M. cloacale
7. M. iowae
8. M. glycophilum
9. M. synoviae
10. M. meleagridis
The Microarray
Influence of swab material on the detection of Mycoplasma gallisepticum and Mycoplasma synoviae by real-time PCR.

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Abstract

Recent reports have shown an increased recovery of cells from flocked nylon swabs which may improve the specimen quality and the real sensitivity of diagnostic tests in a clinical setting. In this study, the detection of Mycoplasma gallisepticum (MG) and M. synoviae (MS), using dry swabs of different materials (nylon flocked, cotton, and polyester), was investigated using real-time TaqMan PCR protocols. Different types of samples, including dilutions of pure broth cultures of MG and MS as well as swabs from tracheas of experimentally infected chickens and field cases of infection, were analyzed. There were no statistical differences in real-time PCR results among the different swab types (P < 0.05), indicating that this is not likely to be a significant factor in MG and MS detection by this method.
Swabs

Upper respiratory tract sampling 1-2 months post infection 10-20 samples per flock. At later stages 30-100 samples may be required (Kleven and Levisohn, 1996; Molecular and Diagnostic Procedures in Mycoplasmology Vol. II Chapter D9)

- Plastic stem swabs should be used – wood and aluminium may inhibit growth or PCR.
- The swabs used should be pre-wetted, either in PBS or Mycoplasma media.
- Transport to the laboratory should be in a suitable transport media – Mycoplasma media, Amies or Stuarts without charcoal. Dry swabs are useless.
- Samples should be transported to the laboratory without delay.
- Keep it moist, Keep it cool, Move fast
Sampling – Live Birds

- Tracheal, oropharngeal, eye, nasal, or cloacal samples

- For *M. gallisepticum* the trachea or choanal cleft is the target site for sampling.

- For *M. meleagridis* oviduct, phallus, semen and cloaca

- Sampling confidence – sampling 60 birds from an airspace gives 95% confidence of detecting infection at 5% prevalence.
Sampling – Dead Birds

- Exudates, body fluids, tissue

- To reduce the bacterial contamination dip birds up to the neck in a suitable disinfectant, taking care not to allow disinfectant in the mouth, eyes or nares.

- When airsacculitis, synovitis or other lesions are present the site can be swabbed, or the tissue tested*.

- Other sites such as oviduct, intestinal tract or brain; embyonated eggs or dead embryos; or yolk membrane of non-fertile eggs can be tested

- * lungs and air sacs may contain inhibitors to mycoplasma growth
Control

Maintain flocks free of infection, single age – all in – all out management

Maintain good biosecurity and effective monitoring systems

Short term  - antimicrobial treatment

Long term  - vaccination

For Trade, flocks should be free of disease and not vaccinated.

Bennett et al. (2013) Vet J, 198: 661 modelled *M. gallisepticum* control options in egg production and conclude that either vaccination or antimicrobial treatment can reduce potential losses.
Mycoplasma species lack a cell wall and therefore are naturally refractory to treatment with antimicrobials that act on the cell wall, i.e. the penicillins and cephalosporins.

They are also resistant to sulphonamides, which inhibit folic acid synthesis, and those aminoglycosides that inhibit microbial respiration.

Antimicrobial resistance has been reported.


Control - vaccines

Bacterin
Live – modified vaccines
Mg - Mycovac – L
strains F; R; ts-11; 6/85

Ms – MS-H temp sensitive

Feberwee reported Ms vaxsafe reduced incidence of EAA
Proficiency Testing

- Laboratory Testing – Quality Standard – ISO17025
- International trade often requires testing at a named Government Laboratory
- EU Trade may be a Government approved laboratory

Mycoplasma gallisepticum (Mg) serology PT0120
Mycoplasma meleagridis (Mm) serology PT0122
Mg and Mm culture PT0121

http://www.defra.gov.uk/ahvla-scientific/services/vetqas/
Summary

- *Mycoplasma* species are important pathogens, some in avian species.
- Mg and Ms OIE Listed
- Mg and Mm in EU Directive 2009/158
- Many *Mycoplasma* species affect avian species – often multiple infections.
- Antibody / serological testing; or antigen /culture / molecular testing has limitations
- Control options, cull, live vaccination, antimicrobial treatment (resistance) – All have trade implications.
Mycoplasma

Mycoplasma mycoides
Watercolor
by David S. Goodsell,
2011
Mycoplasma

Protein synthesis (labels in black)
1. DNA
2. DNA polymerase
3. single-stranded-DNA binding protein (protects single-stranded portions during replication)
4. RNA polymerase
5. messenger RNA
6. ribosome
7. transfer RNA (in pink) and elongation factor Tu (in blue)
8. elongation factor Tu and Ts
9. elongation factor G
10. aminoacyl-tRNA synthetases
11. topoisomerases
12. Rec system for DNA repair: a) RecA, b) RecBC
13. chaperonin GroEL (helps folding of new proteins)
14. proteasome ClpA (destroys old proteins)

Enzymes for energy production (labels in red)
15. glycolytic enzymes
16. pyruvate dehydrogenase complex

Membrane proteins (labels in blue)
17. ATP synthase
18. secretory proteins
19. sodium pump
20. zinc transporter
21. magnesium transporter
22. ABC transporter (different ABC transporters transport different types of molecules-ABC is short for "ATP-binding cassette")
23. magnesium transporter
24. lypoglycan (long carbohydrate chains connected to lipid in the membrane)

Thank you for your attention