

Mycoplasmas – ever present pathogens?

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By the early 1980s two of the pathogenic poultry mycoplasmas, *M. gallisepticum* and *M. synoviae* had been successfully eradicated from chicken and turkey primary breeding stock, and this success was soon to be followed by the eradication of *M. meleagridis* from primary turkey breeders.

There was then a genuine belief amongst avian mycoplasmaologists that these organisms might become the dinosaurs of the 20th century poultry pathogens.

However, another mycoplasma pathogen, *M. iowae*, was later recognised as an economic problem in turkeys and was also subjected to eradication programmes.

It seems somewhat ironic, therefore, that the first two mycoplasmas to be eradicated from primary breeders are the ones that have persisted in commercial stock and are arguably 'ever present pathogens', while *M. meleagridis* and *M. iowae* are now seldom encountered.

Economic impact

The detrimental effects of *M. gallisepticum* on chickens and turkeys are well documented but it has proved much more difficult to assess the effects of *M. synoviae*.

M. gallisepticum is recognised as a cause of respiratory disease in chickens and turkeys, decreased egg production in breeders and layers and reduced weight gain and feed conversion efficiency in broilers. *M. synoviae*, on the other hand,

M. iowae affected embryo.



Leg changes due to mycoplasma.

appears to be a less overt pathogen, causing mainly sub clinical infections.

However, it is well known that other infections, particularly those involving respiratory viruses or the pathogenic serotypes of *Escherichia coli*, can considerably exacerbate disease caused by either of these mycoplasmas.

At present *M. gallisepticum* occurs only sporadically in European chicken and turkey commercial breeder flocks and such flocks may well be slaughtered.

However, there are reports that *M. gallisepticum* is now occurring more frequently in free range layer chickens although prevalence data are not widely available.

Recently Tosi et al. (2005) in Italy used egg yolk antibodies as an indicator of infection and reported a 33.3% prevalence of *M. gallisepticum* in layer chickens on single-age farms and 77.8% on multi-age farms.

It appears that *M. synoviae* is much more widespread than *M. gallisepticum* in commercial poultry.

This seems to be true for Europe and also for North America although, as already mentioned, the economic impact of this mycoplasma remains to be quantified.

Reports from some parts of Europe have suggested that there might be some 'hotter' strains of *M. synoviae* circulating that contribute to poor weight gain and mortality in broilers and it may be significant that the OIE have now added this mycoplasma to their list of 'notifiable' diseases.

Two recent studies have reported on the prevalence of *M. synoviae* in commercial layers.

Hagan et al. (2004) investigated the presence of egg yolk antibodies in 56 randomly selected UK laying

farms and found 78.6% of farms were antibody positive.

This selection included some farms that were very small but a second study that was restricted to the large egg producing companies revealed an incidence of 69.0% *M. synoviae* antibodies.

This second study suggested an association between the presence of *M. synoviae* and age of the flock, the frequency of medication and also with a higher weekly number of second class eggs.

Dufour-Gesbert et al. (2006) cultured 53 French layer flocks over 60 weeks of age for *M. synoviae* and isolated it from 36 (68%) of the flocks.

It was significantly less frequent in single house farms than multi-house farms.



***M. synoviae* lesions in a leg joint.**

Mortality tended to be lower in uninfected flocks and numbers of eggs per hen housed was 'slightly higher'.

Genomic analyses on the isolated strains showed them to be quite homogeneous, confirming an observation made earlier on UK strains.

Emerging hosts

Vertical and horizontal transmission of all four pathogenic poultry mycoplasmas is well recognised although rates of egg transmission

are not easily measured and may be highly variable.

Horizontal transmission by direct or indirect contact is also difficult to quantify and is likely to be related to many other factors, amongst which is host density.

A pathogen that has multiple hosts but does not kill them has a greater potential to succeed than one that has a restricted host range and causes high mortality.



***M. gallisepticum* infection in a finch.**

Also a pathogen that has a prolonged period of shedding is more likely to infect other hosts. Both *M. gallisepticum* and *M. synoviae*, therefore, have several attributes of a successful pathogen.

None of the four pathogenic poultry mycoplasmas cause high mortality but their relative host ranges may offer some explanation for the difficulty in controlling *M. gallisepticum* and *M. synoviae*.

These two mycoplasmas have a number of different avian hosts and it is even possible that *M. gallisepticum* has widened its host range over recent years, which might partially contribute to recurring problems in poultry flocks.

The occurrence of *M. gallisepticum* in wild songbirds in the USA is well documented and there is evidence of the susceptibility of members of the corvid family in the UK.

Another consideration in some countries, is the increasing numbers of free range poultry flocks, which offer increased contact with wild birds. In contrast to the above, *M. meleagridis* appears to have the most restricted host range of the four pathogenic mycoplasmas as, apart from turkeys, it has only been found in birds of prey.

Feberwee et al. (2005) developed an experimental model to study the

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horizontal transmission of *M. gallisepticum* from inoculated chickens to contacts in the same cage and also by airborne exposure to birds placed in separate cages at a distance of 65cm.

Different doses of inocula were used but chickens given as few as 100 colony forming units transmitted the organism to their pen-mates and also to the separated birds, although the latter appeared to have low levels of infection and excretion.

However, the authors concluded that their observations on airborne transmission could not be extrapolated to the field situation because the stocking density in their experiment was very low and the ventilation rate was high. In the field airborne transmission could well be greater.

The pathogenic mycoplasmas, despite having only a small number of genes, appear to have sophisticated genetically controlled mechanisms that enable them to persist in the host and, therefore, to reproduce and be infectious to other hosts.

Furthermore, although they are traditionally thought to have a rather short survival time in the environment recent work on some cattle mycoplasmas has shown that some may produce biofilms that could facilitate both their survival in

Free range birds are at a greater risk of infection.



the environment and their persistence in the host.

The fact that mycoplasmas survive for a long time within their host and possibly for longer than we think in the environment serves to emphasise the importance of good biosecurity on and between farms.

It has long been considered that lapses in biosecurity could account for much of the spread of *M. gallisepticum* and *M. synoviae* between flocks and this belief has been backed up by more recent epidemiological investigations.

Diagnostic methods

Routine serological testing by the rapid plate test or ELISA is still widely used to monitor flocks for mycoplasma freedom. Dubious results are investigated by repeat testing or by searching for the organism or its DNA.

Nowadays, the PCR procedure is employed more and more to detect specific mycoplasma DNA because the results are available within one to two days compared to the week or more that is required to detect the organism by culture.

However, it is important that a reliable laboratory and a reliable PCR method are used.

It has been noted that some importers of day-old chicks or

poult have had a small number of birds tested for mycoplasma antibodies by the rapid plate test or for DNA by PCR. Both these procedures are thought to be unsatisfactory for day-old birds as sera from such birds are inclined to give non-specific reactions, while PCR methods need to have been properly validated for use on such samples.

Furthermore, since consignments of day-old birds sometimes originate from a number of different source flocks, and since the rate of vertical mycoplasma transmission might well be very low, an unreasonably large number of birds would need to be tested to provide a valid result.

As a further refinement to diagnosis, some mycoplasma laboratories offer 'strain typing' for *M. gallisepticum* and *M. synoviae*.

This means that comparisons can be made between a new isolate and others of interest such as earlier isolates from the same premises, isolates from other farms within the company or isolates from birds in the same locality.

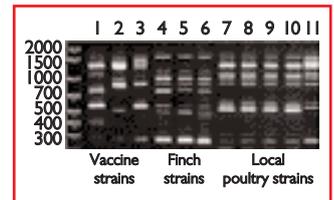
This may help to identify the source(s) of infection. *M. gallisepticum* vaccine strains are also distinguishable from field strains by such methods.

Prospects for control

Most mycoplasmologists would probably still agree that the ideal control method is to obtain mycoplasma-free breeding stock and to maintain them so with good biosecurity.

However, it is now acknowledged that there are certain situations, particularly continuous production layer sites, where *M. gallisepticum* infection is inevitable. It was for such problems that killed and live vaccines were developed.

The killed vaccines, whilst offering protection against the adverse effects of the organism, do not give protection against infection but, on the other hand, earlier live vaccines were sometimes too virulent and



RAPD analysis of finch *M. gallisepticum* strains (Ley et al. 1997).

posed a threat to turkeys, which are more susceptible to the effects of this mycoplasma.

The later live *M. gallisepticum* vaccines (TS-11 and 6/85), if given correctly to layer chickens before natural challenge, should protect against infection with field strains.

Until recently little was known about vaccine efficacy in turkeys but Ferguson et al. (2004) reported on promising vaccine trials in the USA using a strain of *M. gallisepticum* that was isolated from turkey breeders and which may have been transmitted from infected house finches. Field trials have also been conducted in Italian turkeys using TS-11 vaccine with promising results.

One of the hopes with the current live *M. gallisepticum* vaccines is that they might eventually displace existing field strains although this might take many years to achieve.

Although there are both killed and live vaccines available for *M. synoviae* in some countries, vaccination would not be considered by many producers unless it was clearly shown to have some economic benefits.

Research work continues on mycoplasma vaccine development and, as more and more knowledge accumulates about the genetic control of the infection, the disease processes and the molecular events, so we can expect more efficient and targeted vaccines to be produced.

However, in the meantime, the best prospects for control of these infections will still involve high standards of biosecurity and the judicious use of the current vaccines. ■