Review of the activity of antibiotics against *Mycoplasma* spp and their use in the prevention of vertical transmission in breeder layers

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Introduction:

Control of pathogenic avian mycoplasmas can consist of one of three general approaches, according to Kleven (2008): - Maintenance of flocks, which are free of infection, medication or vaccination. To keep a flock free of infection is difficult, especially in areas where large populations of chickens have grown up, as the industry has expanded. To maintain freedom from mycoplasma requires a mycoplasma-free source, on a single age, 'all-in all-out' site, with good biosecurity and an effective monitoring system. The use of mycoplasma vaccines in breeding hens has grown over recent years to reduce the impact of infections, but these can confuse the usual serological monitoring systems. They may control an infection in the hen clinically but there is still a potential risk of vertical transmission to the egg and chick. Medication of a flock only rarely eliminates a mycoplasma infection but has been successfully described for *Mycoplasma synoviae* (MS) (Fiorentin et al, 2003). Overuse of antibiotics can lead to resistance development, which can have serious consequences on future disease control. However, targeted use for limited periods can be helpful to overcome production difficulties following an infection in a breeder flock or preventing an infection of both MS and *M. gallisepticum* (MG).

Production losses associated with Mycoplasma infections:

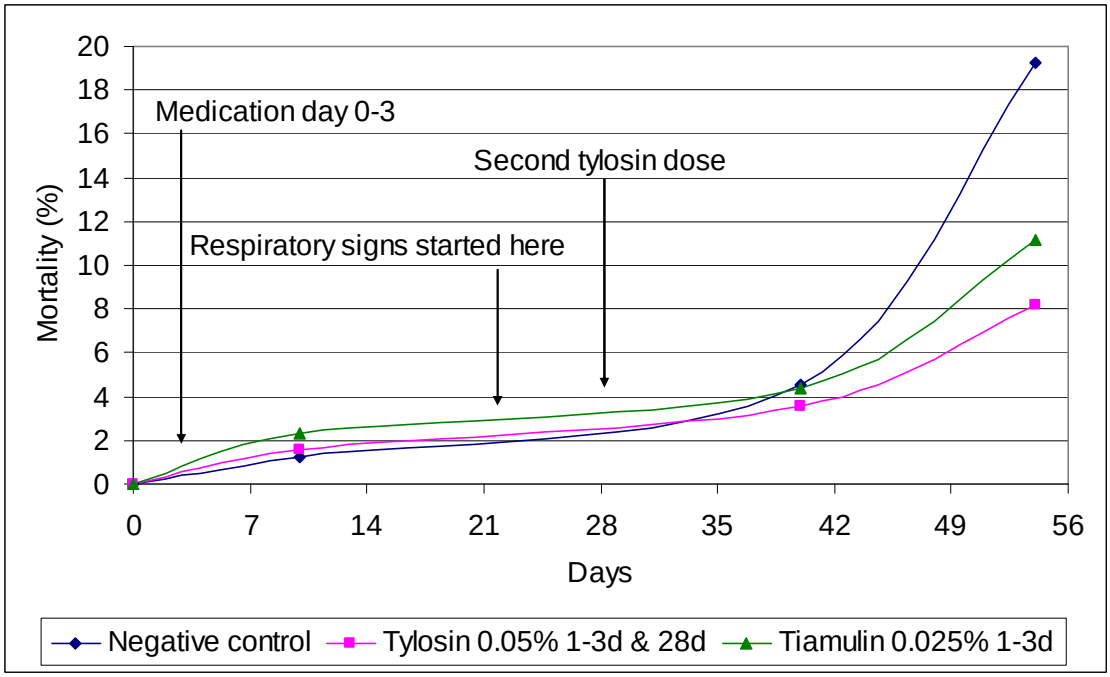
The effects of MG on poultry production associated with chronic respiratory disease (CRD) and complicated chronic respiratory disease (CCRD) plus secondary *Escherichia coli* infections, were summarised by Kleven (1990) (see Table 1).

Table 1. Production losses associated with *M. gallisepticum*

Breeder/layer	Production effect
Egg drop (acute)	10-20%
Egg drop (chronic effect)	5-10%
Embryo mortality increase / hatchability drop	5-10%
Broiler	
Poor chick quality/mortality	5-10%
Depressed weight gain	10-20%
Depressed feed conversion efficiency	10-20%
Increased mortality (due to CCRD)	20%

Mycoplasma synoviae has a much greater variability in pathogenicity and effects on production. In the past, some strains were as pathogenic as MG and could induce severe respiratory signs and mortality in broilers, associated with complicated chronic respiratory disease (CCRD) with mortalities approaching 20% (Burch and Pickles, 1982) (see Figure 1).

Figure 1. Broiler mortality following a pathogenic MS and secondary *E. coli* infection in comparison with prophylactically treated controls



Generally, MS is thought to be less pathogenic and this has allowed it to spread throughout many national flocks, as highlighted in a recent survey in the Netherlands (Feberwee et al, 2008) (see Table 2).

Table 2. *M. synoviae* prevalence, flock survey in the Netherlands

Type of flock	Broilers	Commercial layers
Grandparent	9%	0%
Parent	35%	25%
Broilers / layers	6%	73%

Mohammed et al (1987) demonstrated that the presence of MS in Californian flocks had little to no effect on production (0-3%). Observations by the author in the UK, suggested that MS alone had only a minor effect on egg production, as birds sero-converted approaching peak production, unless this coincided with an infectious bronchitis (IB) virus infection and sero-conversion. Landman and Feberwee (2004) showed that there was a synergy between IB and MS with regard to the development of arthritis. Lameness can be severe and this can affect egg production in hens but also fertility if cocks become lame. Landman and Feberwee (2001) associated amyloid arthropathy with *Enterococcus faecalis* infections but also found that MS was also frequently associated. Feberwee et al (2009) have also shown that MS causes egg apex abnormalities in layers in the Netherlands and that this can reduce egg quality and increase the number of broken eggs. The condition responded to antimicrobial therapy. Egg apex

abnormalities have been found in a number of countries including the Netherlands (see Figure 2).

Figure 2. Eggs showing apex abnormalities due to MS

Photo to be inserted Mike, Feberwee photo 2nd one

Mycoplasma susceptibility to antimicrobial drugs:
Hannan et al (1997) reported on the susceptibility of a number of antimicrobials against MG (20 isolates) and MS (20 isolates) from around the world using a broth dilution method to determine the minimum inhibitory concentrations (MIC).

Table 3. MICs of various antimicrobials against MG and MS from around the world

Antibiotic	MIC 50 (µg/ml)	MIC 90 (µg/ml)	MIC range (µg/ml)
M. gallisepticum			
Enrofloxacin	0.05	0.1	0.025 - 1.0
Tylosin	0.01	2.5	0.0025 - 10
Tiamulin	0.001	0.25	0.0005 - 0.25
Oxytetracycline	0.25	0.5	0.05 - 0.5
M. synoviae			
Enrofloxacin	0.25	0.5	0.05 - 0.5
Tylosin	0.025	50	0.0025 – 50
Tiamulin	0.1	0.25	0.05 – 0.5
Oxytetracycline	0.1	100	0.025 - >100

Resistance to tylosin could be observed by MG and to tylosin and oxytetracycline by MS.

Recently, Pridmore (2008) reported on the susceptibility of 32 isolates of *M. gallisepticum* (MG) and 21 isolates of MS found in Europe (see Table 4).

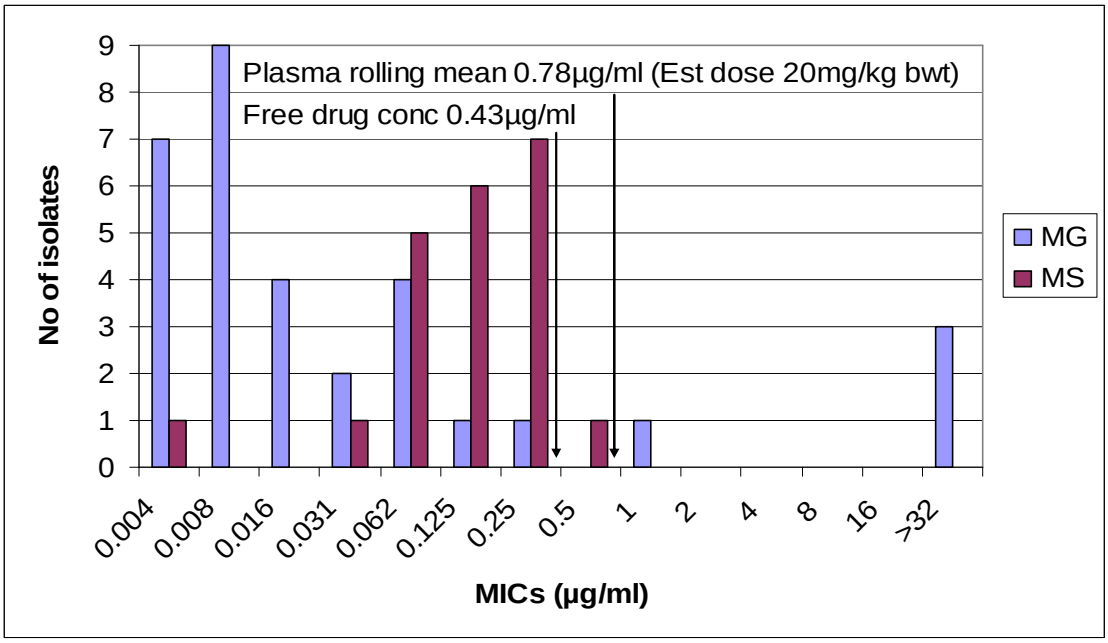
Table 4. Comparative MICs for various antibiotics against MG & MS from Europe

Antibiotic	MIC 50 (µg/ml)	MIC 90 (µg/ml)	MIC range (µg/ml)
M. gallisepticum			
Tylosin	0.016	4.0	0.008 - >256
Lincomycin	4.0	64	0.25 - >256
Tiamulin	0.008	1.0	≤0.004 - >256
Valnemulin	0.008	1.0	≤0.004 – 64
M. synoviae			
Tylosin	0.031	0.062	0.008 – 0.25
Lincomycin	0.5	2.0	0.125 – 4.0
Tiamulin	0.125	0.25	≤0.004 – 0.5
Valnemulin	0.008	0.008	≤0.004 – 0.16

There was some resistance (Pridmore, 2008) observed at the MIC 90 level for both tylosin and lincomycin against MG, but of concern, there were a small number of multi-resistant isolates from the Netherlands, which were co-resistant to all the antibiotics tested.

If the susceptibility patterns of MG and MS isolates are compared with the plasma concentration of tiamulin (Ziv, 1980), it can be seen that the majority of isolates are still inhibited by tiamulin (see Figure 3).

Figure 3. Susceptibility patterns for MG and MS compared with plasma concentrations of tiamulin



Key: Est dose = estimated dose

Antimicrobial use in breeders and eggs to prevent vertical transmission: Antibiotics have been used in a variety of ways to reduce vertical transmission either by treatment of the egg or the hen.

Egg dipping: Egg dipping in antibiotic solutions was widely used in the sixties to reduce transmission and air sac lesions and tylosin, erythromycin and spiramycin were all used with temperature differential dipping but mycoplasma could still be isolated (Bigland, 1970). This was also the finding of Stipkovits (1987), who artificially infected eggs with MG per treatment and tested a variety of antibiotics using vacuum dipping. In the second part of the study they looked at the effect of the antibiotics on hatchability in non-infected eggs and only kitasamycin had a significant adverse effect.

Table 5.Comparative efficacy of antibiotics in the prevention of MG transmission

Antibiotic ppm	Mean egg conc (µg/ml)	MG re-isolation (%)	Embryo mortality due to MG (%)	Hatchability (%)
None	0	57	27	81
Tylosin 2500	2.9	27	22	73
Tiamulin 1000	3.9	27	12	76
Kitasamycin 2000	0.6	19	36	65
Lincomycin/ Spectinomycin 2500	2.6	21	16	85
Gentamicin 2500	0.25	28	20	79

Egg injection: Do Nascimento et al (2005) described the use of tylosin and a combination of tylosin and gentamicin for the successful production of chicks free of MG and MS from a naturally infected flock (see Table 6). Injection into the air cell had a major effect on hatchability in comparison with injection into the albumen.

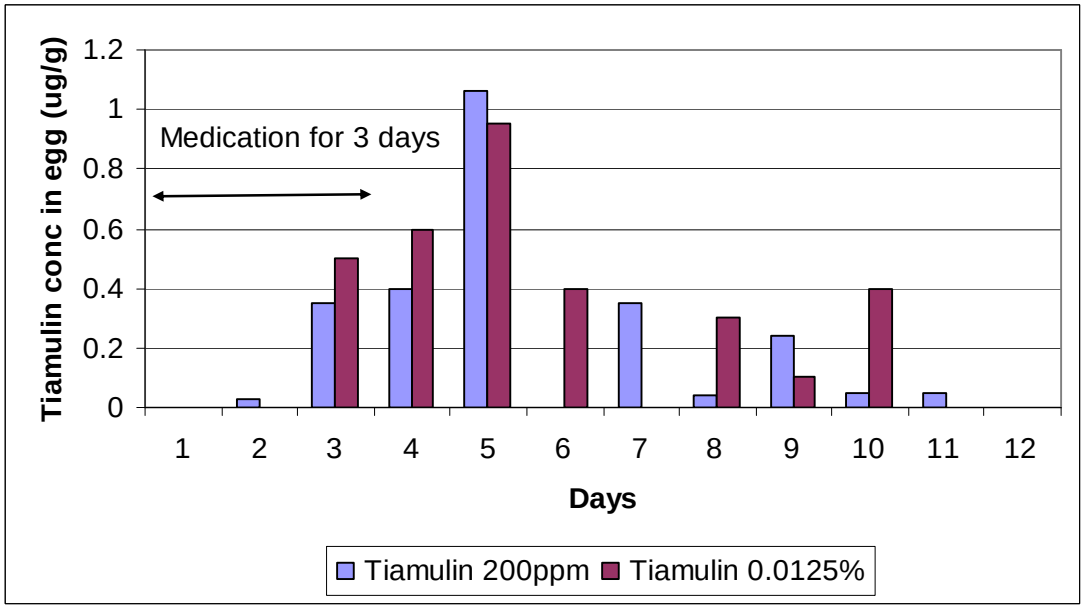
Table 6. Comparison of antibiotic dose and route injected in ovo

Antibiotic	Dose (mg/kg/route	Fertility (%)	Hatchability (%)
Tylosin	3mg / air cell	65.5	17.2
Tylosin	3mg / albumen	90.2	72.4
Tylosin	5mg / albumen	94.6	42.1
Tylosin / gentamicin	3mg + 0.6mg / albumen	88.0	72.5

Tiamulin was shown to be dose dependently embryo toxic when injected at 5, 1, and 0.2mg also into the air cell with hatchabilities of 0, 36 and 67% respectively (Romvary et al, 1985). Both tiamulin and tylosin appear to be potentially toxic when injected into the air cell.

Hen medication: Tiamulin-like activity (tiamulin and microbiologically active metabolites) was shown to accumulate and persist in egg tissues following medication both in feed at 200ppm and in water at 0.0125% (both of which give an approximate dose of 12.5mg/kg bodyweight) (see Figure 4) for several days after treatment (Laber, 1987), using a microbiological assay.

Figure 4. Tiamulin-like activity in eggs after 3 days medication with tiamulin at 200ppm in feed or 0.125ppm in the drinking water (approximate dose 12.5mg/kg bwt)



By medicating the hen, the level of mycoplasma is reduced in the hen and therefore the overall challenge. There is also a sufficient concentration of tiamulin-like activity in the egg to inhibit the growth of most mycoplasmas as well.

As a result, Stipkovits and Burch (1996) used this information to devise a programme in grandparent breeder layers to eliminate MS from the subsequent generations of the flock. The hens were treated for 3 days at 0.0125% followed by 4 days at 0.005% (approximately 12.5mg/kg bwt and 5mg/kg bwt respectively) to prolong the activity of the drug, each month. Eggs were selected from day 3 to day 14 and hatched separately. The progeny (parent flocks) and the subsequent commercial generation flocks produced remained free of MS.

This programme has been adapted in broiler breeder flocks, which are susceptible to challenge and breakdown due to the proximity of neighbouring flocks, which may be infected. The medication can be repeated on a monthly, three weekly or two weekly basis depending on the mycoplasma status of the flock or the '**risk'** of breakdown from the proximity of infected neighbours (see Figures 5 and 6).

Figure 5. Low risk programme in uninfected flocks using 200ppm tiamulin (approximate dose 12.5mg/kg bwt)

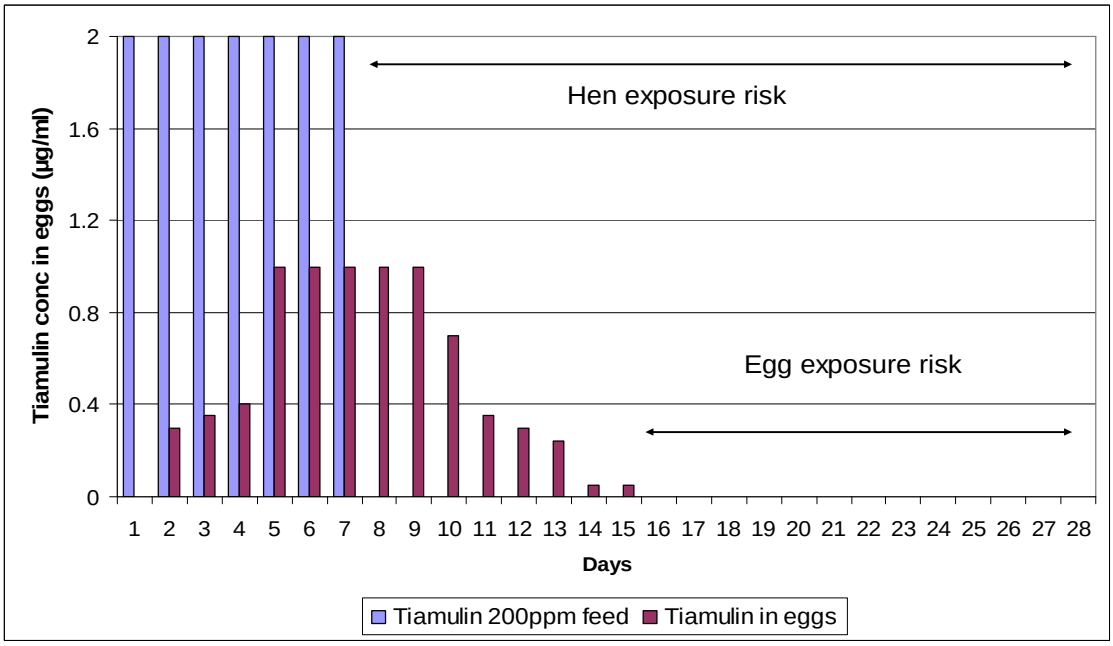
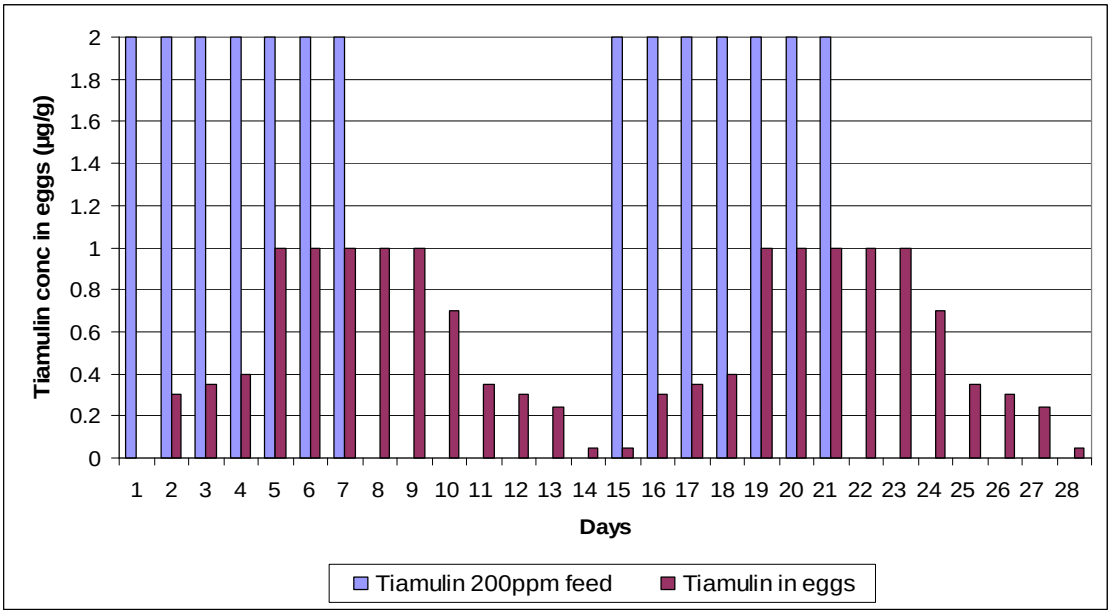


Figure 6. High risk programme or following treatment of flock breakdown with MG at 200ppm tiamulin (approximate dose 12.5mg/kg bwt)



The high risk approach has been successful in preventing MG breakdown where there is a high density of production units and also following breakdown of breeder flocks with MG and initial treatment. Infected multi-age sites might also find this of benefit until all infected flocks have been culled and there is a supply of uninfected stock.

Control of MS infections might require a higher inclusion rate of up to 500ppm tiamulin (25mg/kg bwt) depending on the susceptibility of the strain involved, as MS is generally less susceptible to tiamulin than MG.

Conclusions:
Mycoplasma infections in breeder hens are still a major problem in Europe, Asia and Latin America. Utilising the unique pharmacokinetic qualities of some antibiotics like tiamulin, which concentrate in eggs, to break the chain of infection from vertical transmission, may be one useful tool to improve poultry health and production in these regions.

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