

The *In-vitro* and *In-vivo* Evaluation of Tiamulin and Tilmicosin for the Treatment of *Mycoplasma gallisepticum* Infected Broiler Chickens

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Abstract: This study was undertaken to evaluate the efficacy of some antimicrobials containing tiamulin and tilmicosin (as active principles) against *Mycoplasma gallisepticum* (MG) infection both *in-vitro* and *in-vivo*. For *in-vitro* investigation, the Minimum Inhibitory Concentration (MIC) of tiamulin and tilmicosin against MG organism was done. However, the *in-vivo* evaluation of tiamulin and tilmicosin against field MG infection was carried out on a commercial broiler chicken farm taken from MG infected farm and proved to have such infection through bacteriological and serological examination at day old. Once the birds suffered from respiratory signs at 22 days of age, this flock was divided into three separate houses. Chickens in house (1) were kept as MG-infected without treatment; chickens in the house (2) were treated with tiamulin in the drinking water (1 gram/1.5 liter) for 3 successive days and the birds in house (3) were also treated with tilmicosin in the drinking water (0.3 ml/liter) for 3 successive days. Just after appearance of the first clinical signs and mortalities, the clinical signs score was recorded, the birds were weighed and the serum samples were collected for serological examination. The clinical signs and the mortalities in each house were recorded daily during and after the treatment course till the end of the study (42 days of age). The body weight of the birds in each house was determined weekly till 6 weeks of age. Twenty birds from each house were sacrificed weekly for recording the air-sac lesion score and for re-isolation of MG. The air-sac lesion score and the re-isolation of MG were also detected from the dead birds. Serum samples were collected from sacrificed 20 birds just after appearance of clinical signs and from each house at the end of the work (42 days of age) for detecting the presence of antibodies for MG infection using serum plate agglutination test. The results of the *in-vitro* assessment revealed that the MIC of tiamulin and tilmicosin ($\mu\text{g/ml}$) was 0.1 and 0.05; respectively. *In-vivo* evaluation of tiamulin and tilmicosin denoted that there were significant ($p < 0.05$) differences between MG-infected non-treated house and the treated houses, Tiamulin and tilmicosin succeeded in inducing significant reduction ($p < 0.05$) in the mean clinical score, mortality rate, mean gross air-sac lesion score, re-isolation rate of MG and absence of MG antibodies in the treated houses than the infected control group. Moreover, significant ($p < 0.05$) improvement in the mean body weights was observed in the treated chickens than the infected ones. Both tiamulin and tilmicosin were efficacious in the treatment of MG infection in broiler chickens; nevertheless tilmicosin medication was superior in controlling of such infection. It is recommended that testing the efficacy of the drugs *in-vitro* before application *in-vivo* to overcome the problem of drug resistance, also tiamulin and tilmicosin are effective in eradication programmes of field MG infection in the broiler chickens.

Key words: *Mycoplasma*, tiamulin, tilmicosin, treatment, chickens

INTRODUCTION

Mycoplasma gallisepticum (MG) continues to be of primary concern to the poultry industry world wide as it commonly affects intensively reared chickens and turkeys causes large economic losses (Evans *et al.*, 2002).

Uncomplicated cases of MG infection don't always cause overt clinical signs or mortality, but can result in sub-optimum production, increase feed conversion ratio and down grading of the carcasses (Charleston *et al.*, 1998). However, MG infection can cause severe respiratory manifestations, mortalities and decrease in egg production (Domermuth and Gross, 1962; Pruthi and Kharole, 1981; Kleven, 1990; Ley and Yoder, 1997; Kleven, 1998; Levisohn and Kleven, 2000). A further complication of MG infection is that birds are more susceptible to other bacterial or viral pathogens as well

as other *mycoplasma* species (Charleston *et al.*, 1998). Attempts to control MG must be balanced against the need for greater efficiency in production costs (Evans *et al.*, 2002). The most effective control methodology for MG is total eradication through test and slaughter (Yoder, 1991). Not only is this expensive but the emergence of multi-antigen complexes in commercial layer industry makes this approach impractical (Evans and Hafez, 1992; Whithear, 1996; Kleven *et al.*, 1997; Levisohn and Kleven, 2000). In spite of the availability of vaccines, antimicrobial use continues to be the most economic method (Arzey and Arzey, 1992; Jordan *et al.*, 1993; 1997; 1998; 1999).

Decrease in efficacy of antibiotics against avian mycoplasmosis is a phenomenon frequently observed in the field, particularly in broilers as it was attributed to the acquisition of antibiotic resistance (Zanella *et al.*,

1998). *Mycoplasma* strains showed considerable variations in sensitivity to the given antibiotics, probably due to extended or improperly used administered therapy (Osborn and Pomeroy, 1958; Osborn *et al.*, 1960; Kiser *et al.*, 1961; Newnham and Chu, 1965). An important factor in the successful control of *mycoplasma* infections is the correct selection and use of the antimicrobial to achieve an effective concentration in the blood of a bird so that the organism can be destroyed. Periodic *in-vitro* antibiotic sensitivity testing of field isolates of *mycoplasma* is indicated as means of monitoring the impact of masses medication programmes and as a guide to therapy (Levisohn, 1981). The *in-vitro* sensitivity tests of different avian *mycoplasma* species to assess the sensitivity to a wide spectrum of chemotherapeutic agents were carried out intensively by Newnham and Chu (1965); Zanella (1966); Kleven and Anderson (1971); Hinz (1980); Ziv (1980); Levisohn (1981); Whithear *et al.* (1983); Jordan and Knight (1984); Lin (1987); Hannan *et al.* (1989); Jordan *et al.* (1989); Kempf *et al.* (1989); Tanner and Wu (1992); Bradbury *et al.* (1994); Lin *et al.* (1994); Jordan and Horrocks (1996); Hannan and Windsor (1997); Jordan *et al.* (1998); Lin (2006); Pakpinyo and Sasipreeyajan (2007).

There are many standardized procedures for testing the *in-vitro* sensitivity of *mycoplasmas* to antibiotics. The micro-broth dilution procedure [Minimum Inhibitory Concentration (MIC)] method was chosen as the most convenient and reliable standardized one because it is relatively simple, quick to perform and requires only small volumes of the media (Taylor-Robinson, 1967).

Tiamulin, 14-deoxy-14 [(2-dihylaminoethyl)-mercapoaceoxy] mutilin hydrogen fumarate, is a semi-synthetic derivative of the diterpen antibiotic pleuromutilin (Egger and Reinshagen, 1974). Tiamulin is highly active against gram-positive bacteria (Drews *et al.*, 1975), a wide range of *mycoplasma* (Drews *et al.*, 1975) and certain spirochetes (Baughn *et al.*, 1976).

The *in-vitro* activity of tiamulin against field *mycoplasma* isolates using MIC was studied by Hinz (1980); Ziv (1980); Forster *et al.* (1982); Lin (1987); Koh *et al.* (1993); Lin *et al.* (1994); Stipkovits and Burch (1997); Jordan *et al.* (1998); Burch and Valks (2002); Cerda *et al.* (2002); Gautier-Bouchardon *et al.* (2002); Lin (2006); Pakpinyo and Sasipreeyajan (2007).

Tiamulin has been used for over 20 years in pigs to combat the main target pathogens *Brachyspira hyodysenteriae* and *Mycoplasma hyopneumoniae*. Various studies showed the antibiotic activity of tiamulin against MG, *M. synoviae* (MS), *pasteurella* and *Ornithobacterium rhinotracheale* (ORT) infections (Laber and Schutze, 1975; Baughn *et al.*, 1978; Devriese *et al.*, 2001). The antimycoplasmal activity of tiamulin in birds (*in-vivo*) was first observed and reported by Baughn *et al.* (1974) and was confirmed and extended by Laber and Schutze (1975); Stipkovits *et al.* (1977); Baughn *et al.* (1978); Jordan *et al.* (1991); Arzey and Arzey (1992);

Stipkovits *et al.* (1992); Burch and Stipkovits (1993), Stipkovits *et al.* (1993); Jordan *et al.* (1998) where the drug was highly effective and was more superior when compared to other drugs against avian *mycoplasma* infections.

Tilmicosin is a relatively new semisynthetic macrolide antimicrobial [20-deoxy-20 (3, 5-dimethylpiperidin-1-yl) desmycosin], that was prepared by chemical modification of desmycosin which have licensed to treat a number of diseases in cattle and pigs. The drug showed good *in vitro* activity against MG with MIC of 0.048 µg/ml (Os, 1987). Tilmicosin also showed good activity in the treatment of MG infection *in-vivo* when incorporated either in the drinking water or in feed (Shryock *et al.*, 1994; Jordan and Horrocks, 1996; Kempf *et al.*, 1997; Charleston *et al.*, 1998; Jordan *et al.*, 1999). The pharmacokinetics properties of tilmicosin include rapid absorption from oral administration, long half life and good tissue penetration (Debono *et al.*, 1989).

Accordingly, the aim of this work was testing the efficacy of antimicrobials containing tiamulin and tilmicosin as active principle against MG infection *in-vitro* as well as evaluating these drugs in the treatment of field MG infection in broiler chicken farm.

MATERIALS AND METHODS

Mycoplasma strain: Morphologically, biochemically, serologically and molecularly characterized strain of *Mycoplasma gallisepticum* (MG) used for this study was obtained a commercial broiler chicken farm proved to be positive for MG infection. The *in-vitro* sensitivity of this strain to the tested antimycoplasmal drugs using Minimum Inhibitory Concentration (MIC) method was carried out.

The media: The growth medium used for sensitivity test was Frey medium (Frey *et al.*, 1968) enriched with 12% inactivated swine serum. The broth used for the propagation of the culture was sterilized by filtration through a Millipore filter equipped with 0.2 µm cellulose membrane. Agar medium was prepared by autoclaving (121°C for 20 min) the above medium with 1.5% agar. Cysteine, penicillin, thallium acetate and swine serum were then added aseptically after autoclaving and was stored at 4°C.

The tested drugs: The tested antimycoplasmal drugs were commercially available. These drugs were containing the followings active principals:

- C Tiamulin (water soluble granules) produced by Novartis Animal Health Austria GmbH Biochemiestrasse 10, A-6250 Kundl, Austria. The registration No. was 1045/2006 and the batch No. was P 183. The drug was given in a dose of 1 gram for each 1.5 liter of the drinking water for 3 days. This results in a daily dose of approximately 25 mg/kg body weight

C Tilmicosin (aqueous concentrate) as the phosphate form containing 250 mg/ml was obtained from Elanco Animal Health Company. The lot No. of the drug was A350799 and the registration No. was 1498/2000. The drug was given in a dose of 0.3 ml/liter of the drinking water for 3 days.

The drugs were given in the drinking water of the treated birds just after the appearance of the clinical respiratory signs (at 22 days of age) and continued for 3 successive days.

The *in-vitro* sensitivity of the tested antimycoplasmal drugs:

The Minimum Inhibitory Concentration (MIC) method that applied was the broth micro-dilution technique described by Whithear *et al.* (1983). A 24 hours broth culture in Frey broth medium of MG strain was used as inoculum. A 1 ml aliquot was added into 4 ml of Frey broth and incubated at 37°C until *mycoplasma* growth turned the medium orange (pH 6.8-7.0). A 2 ml amount of this broth was added to 18 ml of Frey broth and homogenized. This gave sufficient inoculum to set up two replicate plates. Each drug stock solution was passed through a 450 nm membrane filter (Millipore) and then dispensed aseptically. Stock solution was stored at -70°C for up to 1 week. Solutions were diluted in Frey broth to the required concentration. Replicate doubling dilutions of the tested drugs were made in 50 µl volumes of Frey broth in sterile tissue culture quality 96-well U-bottomed microtitration plates. When the dilution sequence of each drug was complete, 150 µl of the broth, pH 7.8, containing the desired density of the organisms was inoculated into each well with a multichannel dispenser. Culture controls containing Frey broth plus organisms without the drugs were included in all tests. Plates were covered with plastic lunch wrap and incubated in a stationary position at 37°C for 14 days under microaerobic conditions. The lowest concentration of the drug that completely prevented colour change in the medium was regarded as the MIC. The MIC was read when the phenol red indicator in the culture control had just changed from red to orange-yellow. This was determined visually by comparison with the colour of sterile Frey broth adjusted to pH 7.8. The MIC values were expressed in µg/ml of active compound.

Inoculum density: Five serial ten fold dilutions of MG culture were prepared in Frey broth giving a range of densities from 10³ to 10⁵ Colour Changing Units (CCU)/ml. Each dilution was then tested against the tested drug. All plates were observed regularly during incubation so that the MIC of each density was read at the exact time that the pH indicator in the medium of the appropriate culture control had changed to orange-yellow (pH 7.0).

Time of reading results: Reading was made when *mycoplasma* growth was first detected in the culture control. This was determined by visual comparison with sterile Frey broth adjusted to pH 7.8. A change in the colour of the culture control medium from deep-red (pH 7.8) to pinkish-red was taken to indicate growth. Additional readings were also made when the pH indicator in the culture control turned orange-yellow and after fixed periods of 24 h, 48 h and 14 days incubation.

The tested flock: This clinical trial was carried out on a commercial broiler chicken flock consists of 6000 Hubbard breed with mixed sex. Day-old chicks of this flock was taken from mycoplasma positive breeder flocks and the presence of MG infection of these chicks was confirmed by serological detection of antibodies using Serum Plate Agglutination (SPA) test and also by bacteriological examination using air-sacs swabs. These birds were vaccinated against Newcastle disease using HB1 vaccine at 4 days of age, infectious bronchitis at 5 days old and infectious bursal disease using 228E vaccine at 14 days of age. Inactivated avian influenza vaccine (H5N2) was given at 7 days of age while Lasota vaccine against Newcastle disease was given at 15 days of age. All the vaccines were given via eye drop instillation. This flock was fed on a commercial balanced ration containing no antimycoplasmal drugs. The feed and water was given ad-libitum. When the flock reached 22 of age, signs of sneezing, nasal and lacrimal discharge and conjunctivitis as well as first mortalities were appeared in the flock. This flock was divided into three separate houses. The birds in house (1) were kept infected without treatment; chickens in the house (2) were treated with tiamulin in the drinking water for 3 successive days and birds in house (3) were also treated with tilmicosin in the drinking water for 3 successive days. Just after appearance of the first clinical signs and mortalities, the clinical signs score was recorded, the birds were weighed and the serum samples were collected for serological examination. The clinical signs and the mortalities in each house were recorded daily during and after the treatment course till the end of the study (42 days of age). The body weight of the birds in each house was determined weekly till 6 weeks of age. Twenty birds from each house were sacrificed weekly for recording the air-sac lesion score and for re-isolation of MG. The air-sac lesion score and the re-isolation of MG were also detected from the dead birds. Serum samples were collected from sacrificed 20 birds just after appearance of clinical signs and from each house at the end of the work (42 days of age) for detecting the presence of antibodies for MG infection using (SPA) test.

The *in-vivo* evaluation of the efficacy of tested antimycoplasmal drugs:

The efficacy of the tested antimycoplasmal drugs was assessed in the treated groups by evaluating the following parameters:

The clinical signs: The treated and the untreated control birds were daily observed just after appearance of the clinical respiratory signs, during the treatment course, after the treatment till the end of this study (6 weeks old). The respiratory signs were individually observed on the chickens and were taken scores according to Kempf *et al.* (1998) as the following:

- 1 = No respiratory signs.
- 2 = Slight symptoms (sneezing and few tracheal rals)
- 3 = Moderate symptoms (sneezing or tracheal rals)
- 4 = Severe symptoms (sneezing or frequent tracheal rals, dyspnea)

The mortalities: The number of dead birds in non-treated and in the treated houses was recorded daily during and after the treatment course till the end of the work (42 days of age).

Post-mortem gross lesions: Twenty birds from each of the treated and non-treated houses were sacrificed weekly after appearance of clinical signs till the end of the study. The air-sacs lesions of the dead and the sacrificed chickens during and after the treatment were observed. The typical air-sac lesions of *Mycoplasma* infection were recorded and scored according to those of Kleven *et al.* (1972) as the following:

- C No air-sac lesion observed (lesion score = 0), the air sac membranes of the birds were completely clear without gross alterations
- C Air-sac lesion score = 1, the membranes were slightly cloudy without marked alterations
- C Air-sac lesion score = 2, the membranes were slightly thickened and usually with small accumulations of cheesy-like substances exudates
- C Air-sac lesion score = 3, the membranes were clearly thickened and meaty in consistency with marked accumulation of clotted exudates confined to a single air-sac
- C Air-sac lesion score = 4, the membranes were with gross remarkable pathological alterations as score No. 3 but lesions were found in two or more air-sacs.

The average body weights and the cumulative feed conversion: The average body weights of chickens as well as the cumulative feed conversion in each house were detected just after appearance of the clinical signs at 22 days old and then weekly (at 29, 36 and 42 days of age).

The re-isolation of MG: Swabs from the air-sacs for re-isolation of MG were collected from the birds at day old, at 22 days of age (just after appearance of the clinical symptoms), during the treatment and also weekly from the sacrificed as well as dead birds with air-sac lesions. The swabs were immediately inoculated in liquid *Mycoplasma* media. Subcultures were done on solid

Mycoplasma media and the plates were incubated at 37°C for 7 days in Co₂ incubator, then they observed daily for the growth of *Mycoplasmas*. The plates were considered negative if no growth appeared after 14 days of incubation. The proportion of the isolated *Mycoplasmas* was identified microscopically using dissecting microscope.

Serum plate agglutination (SPA) test: The presence of antibodies to MG was detected using Serum Plate Agglutination (SPA) test. The samples of blood were collected randomly from the wing veins at day old, from sacrificed 20 birds just after detection of clinical signs and also from 20 chickens in each treatment and the control at the end of the observation period (42 days of age). The sera were separated by centrifugation of the collected blood samples at 3000 rpm for 10 min. The commercial stained *Mycoplasma gallisepticum* antigen (Nobilis) produced by Intervet International B.V. Boxmeer, Holland was used. The method of Kempf *et al.* (1994) was followed for applying SPA test.

Statistical analysis of the data: The collected data were tested using the method of Snedecor and Cochran (1980). Differences of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Mycoplasma infection continues to be an important cause of loss in poultry production (Kleven, 1990). Uses of recent anti-mycoplasmal drugs either in the prophylaxis and therapy is still recommended in the eradication programs than application of sanitary measures or usage of vaccine (Arzey and Arzey, 1992; Jordan *et al.*, 1999). Avoidance of the development of resistant MG strains to antimicrobials could be achieved through continuous application of *in-vitro* sensitivity tests (Laber and Schutze, 1975; Jordan and Knight, 1984; Tanner and Wu, 1992; Lin *et al.*, 1994; Hannan and Windsor, 1997; Schulze and Hamke, 2002; Lin, 2006; Pakpinyo and Sasipreeyajan, 2007).

Regarding the *in-vitro* sensitivity of the tested antimycoplasmal drugs, the results of the Minimum Inhibitory Concentration (MIC) of tiamulin and tilmicosin against MG field strain with a viable organism density of 10^3 to 10^5 Colour Changing Units (CCU)/ml was 0.1 and 0.05 for tiamulin and tilmicosin ($\mu\text{g/ml}$); respectively. This result confirm that of Ziv (1980) who found that MIC values for tiamulin against MG (0.05 microgram/ml) and MS (0.10 microgram/ml) were 2-4 times lower than the corresponding values for tylosin. Other study of Lin (1987) showed that the MIC₉₀ of 50 MG micrograms/ml or less of tiamulin was less than 0.4 micrograms/ml which is the best concentration when compared with other antimicrobials. The highest MIC level of tiamulin against MG in recent years is 16 times lower than that of lincomycin and 5 times lower than enrofloxacin, also tiamulin is a low inducer of resistance in mycoplasma

over the last 25 years in comparison with tylosin and slower than oxytetracycline (Stipkovits and Burch, 1997). Moreover, Jordan *et al.* (1998) compared the MICs of different antimycoplasmal drugs with tiamulin and found that the lowest MICs were with tiamulin, followed by tylosin, enrofloxacin and a relatively high MIC for lincomycin/spectinomycin. In addition, Valks and Burch, (2001) examined the ranges of MICs of various antimicrobials against the different mycoplasma species and detected that tiamulin was superior to tylosin, oxytetracycline, lincomycin and enrofloxacin. The highest *in-vitro* efficacy of tiamulin, doxycycline and danofloxacin against almost all the isolates of both MG and MS was demonstrated by Burch and Valks (2002). Also, Cerda *et al.* (2002) detected that Aivlosin (3-acetyl-4"-isovaleryl tylosin tartrate), tylosin, and tiamulin showed the lowest MICs with MIC90s of 0.006, 0.012, and 0.05 microg/ml, respectively. Gautier-Bouchardon *et al.* (2002) observed no resistance to tiamulin in MG or MS strains after 10 *in-vitro* passages. On the other hand, Jordan and Horrocks (1996) recorded that tilmicosin showed lower MIC against the tested strains of MG than did tylosin at both the initial reading (when pH 7.0 is first seen in the dilutions under test) and the final reading at 14 days of incubation.

The above mentioned documents supported the fact that MG developed resistance over many years of use to many antimicrobials, but tiamulin and tilmicosin are unrelated to these drugs and are still highly active against MG resistant strains. Both drugs also develop resistance to mycoplasmas very slowly, therefore are becoming the recommended products for resistant-mycoplasma control.

Concerning the *in-vivo* evaluation of the efficacy of tested antimycoplasmal drugs, Table 1 showed the mean

clinical score in MG infected-non treated and the infected-treated houses. The first clinical signs of MG infection (sneezing and/or rals) were detected on the broiler chickens at 22 days of age with mean clinical score (1.98). Significant ($p < 0.05$) difference in the mean clinical score was noticed between MG infected-non treated house and the treated houses during the whole study course. Chickens in tiamulin and tilmicosin treated groups showed gradual and significant ($p < 0.05$) decrease in the mean clinical score to reach their lowest values (1.04) and (1.01) in tiamulin and tilmicosin treated chickens; respectively during 33-42 days of age. Similar results were obtained by Ziv (1980) who suggest that when tiamulin is given in the drinking water at rates of 125-250 mg/litre, better antimycoplasmal activity is to be expected *in vivo* than by giving tylosin tartrate in the drinking water at 500-700 mg/litre. Arzey and Arzey (1992) recorded that tiamulin, which was administered orally; cure was in 89% in MG infected-treated birds that was assessed by absence of nasal discharge. Similarly, Jordan and Horrocks (1996) reported that clinical signs, mainly depression and nervous manifestations were seen in two to five MG-infected and tilmicosin-treated birds while 16 of 30 birds showed clinical signs in MG-infected untreated group.

The results of mortality rate in MG infected-non treated and the infected-treated houses were represented in Table 2. From the table, it could observe that the first recording of mortalities was associated with appearance of clinical signs at 22 days of age and the mortality rate in the flock was (3.2%). Significantly, although there was no significant ($p < 0.05$) difference in the mortality rate between tiamulin and the tilmicosin

Table 1: The mean clinical score in *Mycoplasma gallisepticum* infected-non treated and the infected-treated houses

House No. treatments	Age/day			
	Mean clinical score			
	Before treatment	During treatment	After treatment	
	22	22-24	25-32	33-42
House (1) Infected-non treated	1.98±0.35 ^a	2.57±0.46 ^a	2.32±0.43 ^a	2.13±0.27 ^a
House (2) Tiamulin-treated	1.98±0.35 ^a	1.87±0.32 ^c	1.21±0.19 ^c	1.04±0.05 ^c
House (3) Tilmicosin-treated	1.98±0.35 ^a	1.79±0.27 ^c	1.15±0.14 ^c	1.01±0.02 ^c

^{a-c}Figures sharing common superscripts are not significantly different ($p < 0.05$)

Table 2: The mortality rate in *Mycoplasma gallisepticum* infected-non treated and the infected-treated houses

House No. treatments	Age/day			
	Mortality rate			
	Before treatment	During treatment	After treatment	
	22	22-24	25-32	33-42
House (1) Infected-non treated	65/2000 (3.2%) ^a	80/1935 (4.1%) ^a	45/1855 (2.4%) ^a	21/1810 (1.16%) ^a
House (2) Tiamulin-treated	65/2000 (3.2%) ^a	34/1935 (1.7%) ^c	11/1901 (0.5%) ^d	5/1890 (0.2%) ^d
House (3) Tilmicosin-treated	65/2000 (3.2%) ^a	21/1935 (1.0%) ^c	9/1914 (0.4%) ^d	3/1905 (0.1%) ^d

^{a-d}Figures sharing common superscripts are not significantly different ($p < 0.05$)

treated houses but there was difference ($p < 0.05$) between the infected-non treated chickens and the treated birds at all the studied intervals. Gradual decrease in mortality rate was observed in the treated groups to reach the least values (0.2%) and (0.1%) in tiamulin and tilmicosin treated chickens; respectively at the last interval of the study (33-42 days of age). In agreement with these results Stipkovits *et al.* (1977) found that tiamuline in chickens and turkeys either in a prophylactic dose of 0.0125% or in a therapeutic dose of 0.025% showed a superior efficacy than tylosin as measured by significant decrease in the clinical symptoms caused by MG infection. Besides, tilmicosin treatment at concentration of 0.125, 0.25 or 0.5 g/liter significantly reduce the mortality in the MG-treated chickens compared with the infected untreated group (Jordan and Horrocks, 1996).

Table 3 illustrated the mean air-sac lesion scores in MG infected-non treated and the infected-treated houses. Dead as well as sacrificed birds in MG-infected-non treated house showed significant ($p < 0.05$) increases in the mean air-sac lesion score in comparison with treated birds along the different intervals of the study. Moreover; there was significant ($p < 0.05$) difference between tiamulin treated and tilmicosin treated birds. The lowest mean air-sac lesion score was observed in chickens treated with tilmicosin. These results are in accord with that of Baughn *et al.* (1978) who detected that tiamulin was more effective than the other antibiotics in preventing and eradicating airsacculitis caused by MG and preventing airsacculitis and synovitis caused by MS when administered as a single subcutaneous dose. The effect of tilmicosin on the prevention of MG was studied by Shryock *et al.* (1994) who concluded that

tilmicosin at 300-500 g/ton prevented the development of airsacculitis caused by MG infection. Jordan and Horrocks (1996) noticed that the gross lesions of the airsac walls of MG-inoculated and tilmicosin-medicated birds were less than that for the inoculated-unmedicated ones. Furthermore, Kempf *et al.* (1997) and Jordan *et al.* (1999) stated that tilmicosin treatment with 100, 200 or 300 mg/liter for 5 days significantly decreased the respiratory signs and the airsac and peritonitis lesions due to MG. Charleston *et al.* (1998) investigated that tilmicosin concentrations at or less than 50 mg/1 administered for either 3 or 5 days was found to significantly reduce the severity of airsacculitis caused by MG.

Considering the results of the average body weights and the cumulative feed conversion of surviving birds in MG infected-non treated and the infected-treated houses, they were seen in Table 4. The results cleared that there was significant ($p < 0.05$) differences in the average body weights between the infected-non treated birds and the treated ones at the different weeks intervals. Although there was no significant ($p < 0.05$) differences between tiamulin treated and tilmicosin treated group, but tilmicosin treated birds showed the best average body weights. Considering the results of the cumulative feed conversion, tilmicosin treated chickens showed the best feed conversion (1.91) followed by tiamulin treated one (2.05) while the infected-non treated birds showed (2.50) feed conversion. The above mention results are in concur with these recorded by Kempf *et al.* (1997) who revealed that tilmicosin medication at dose levels of 50-300 mg/liter significantly decreased growth losses. Additionally, Jordan *et al.* (1999) proved that the mean body weight gain of poult's surviving to the end of the

Table 3: The mean air-sac lesion score in *Mycoplasma gallisepticum* infected-non treated and the infected- treated houses

House No. treatments	*Mean air-sac lesion score		
	Intervals of age/day		
	22-29	30-36	37-42
House (1) Infected-non treated	2.85±0.22 ^a	3.41±0.32 ^a	3.04±0.01 ^a
House (2) Tiamulin-treated	1.97±0.34 ^b	1.31±0.23 ^b	1.02±0.22 ^b
House (3) Tilmicosin-treated	1.46±0.23 ^c	1.12±0.20 ^b	0.83±0.10 ^c

*Mean lesion score of dead as well as sacrificed birds weekly.

^{a-c}Figures sharing common superscripts are not significantly different ($p < 0.05$)

Table 4: The average body weights and the cumulative feed conversion of surviving birds in *Mycoplasma gallisepticum* infected-non treated and the infected-treated houses

House No. treatments	Age/day				CFC
	Before treatment	After treatment			
	22	29	36	42	
House (1) Infected-non treated	420.22±17.2 ^a	640.87±28.7 ^a	743.94±31.73 ^a	1210.5±33.21 ^a	2.50
House (2) Tiamulin-treated	533.64±12.88 ^b	755.91±27.52 ^b	940.5±38.00 ^b	1531.0±42.96 ^b	2.05
House (3) Tilmicosin-treated	591.2±17.42 ^b	765.60±27.05 ^b	1065.12±40.04 ^b	1706.9±50.33 ^c	1.91

Means within the column with no superscripts are significantly different ($p < 0.05$). CFC = Cumulative Feed Conversion

experiment was significantly greater in the uninfected and MG-infected, tilmicosin treated poult than the only MG-infected ones.

Bacteriological examination of the air-sacs collected from day-old and 22 days old chickens revealed presence of MG organism. From Table 5, it could observe the re-isolation rate of MG in infected-non treated and the infected-treated houses at weekly intervals along 6 weeks study period. The air-sacs swabs of the infected-non treated chickens showed re-isolation rate of (100%) at the different intervals, while both tiamulin and tilmicosin treated houses showed significant ($p < 0.05$) decrease in this percentage till reached (0%) at the latest interval of the study (37-42 days of age). Such results agree with that cited by Jordan and Horrocks (1996) who determined that group of MG infected chicks that tilmicosin revealed no recovery of the organism either during life or after death. As well, Kempf *et al.* (1997) and Jordan *et al.* (1999) detected significant reduction in the proportion of MG-culture-positive poult after treatment with tilmicosin at a dose level of 50 mg/liter. Tiamulin and tilmicosin are highly effective antimycoplasmal antibiotics and can cause complete bacteriological cure or elimination of MG (Burch and Roberta, 2004).

Table 5: The re-isolation rate in *Mycoplasma gallisepticum* infected-non treated and the infected-treated houses

House No. treatments	Re-isolation rate		
	Intervals of age/day		
	22-29	30-36	37-42
House (1) Infected-non treated	100%*	100%*	100%*
House (2) Tiamulin-treated	25%	34%	0%
House (3) Tilmicosin-treated	17%	0%	0%

*Significant differences ($p < 0.05$) between infected-non treated and infected treated houses

The rapid serum plate agglutination test of the tested birds at day-old and at 22 days of age (just after detection of signs) revealed presence of positive clumps (100% positive agglutination) for MG infection. At 42 days of age (end of the study), the serum samples of the infected-non treated house showed positive MG agglutination, while infected-treated houses either by tiamulin or tilmicosin were serologically negative to MG. The previously mentioned results are in coincide with that obtained by Jordan and Horrocks (1996) who observed that infected chicks with a virulent strain of MG and treated with tilmicosin at doses 0.125, 0.25 or 0.5 g/liter showed negative serological results except the infected untreated group. Moreover, Kempf *et al.* (1997) reported that tilmicosin at a dose of 300 mg/liter decreased the number of MG shedding chickens to the extent that no bird was serologically positive at the end of the study (21 days of age). Fewer positive reactors for

MG-tilmicosin medicated poult than the infected unmedicated group were detected by Jordan *et al.* (1999).

Eventually, from the aforementioned results, it can achieve the following conclusions:

- C An *in-vitro* test to assess the sensitivity of *Mycoplasma gallisepticum* isolates to antimycoplasmal drugs is the must before application of them in the field
- C Antimycoplasmal drugs containing tiamulin and tilmicosin (as active principles) are very effective in controlling field infection with *Mycoplasma gallisepticum* in broiler chickens. However, tilmicosin compound is more superior in eradication of such field infection.

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