ASSESSEMENT OF THE EFFICACY OF CERTAIN ANTIBIOTICS AND VOLATILE OILS FOR THE TREATMENT OF INDUCED *MYCOPLASMA GALLISEPTICUM* INFECTION IN BROILER CHICKENS

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Summary

This study was undertaken to investigate the effectiveness of different antibiotics containing active principles (pefloxacin, tylosin and spiramycin) as well as volatile oils (containing eucalyptus and peppermint) on the treatment of experimental *Mycoplasma gallisepticum* (MG) infection in broiler chickens. Two hundred day-old Hubbard broiler chicks were used. At day old, randomly 20 chicks were sacrificed and their sera were collected for serological examination using serum plate agglutination (SPA) test, and then examined bacteriologically to prove their freedom from egg borne MG infection. One hundred and eighty birds were divided into six equal separate groups; 30 chicks each (groups 1, 2, 3, 4, 5 and 6). Group (1) was kept as blank control (non infected non treated) group. Just before challenge (at 14 days old), the birds in each group were weight, and then three serum samples of each group were subjected to SPA test. Moreover, these birds were sacrificed and subjected to post-mortem and bacteriological examinations to exclude presence of natural MG infection. Each bird of groups (2, 3, 4, 5 and 6) was inoculated intranasally with 0.4 ml of MG culture containing 10^5 CFU/ml viable organisms at 14 days of age. Chickens of group (2) were left as control positive (infected non treated) birds, however, chickens of groups (3), (4), (5) and (6) gained preparations containing active principles pefloxacin, tylosin, spiramycin, and volatile oils (eucalyptus and peppermint) in the drinking water, respectively. Treatment with these compounds was given to the birds immediately after appearance of the clinical signs (5 days post challenge). The treatment was continued once daily in the drinking water for a period according to the manufacturer's recommendations. For assessment of the tested medicament efficacy, different criteria including (clinical signs, mortalities, gross lesions, body weights, re-isolation of MG and serological examination) were evaluated at different intervals (weekly post challenge) till the end of the study (35 days of age).The results indicated presence of not only significant (p<0.05) difference between non infected non treated group and the infected non treated one, but also significant (p<0.05) difference between the treated groups and the only infected control group. The different treatments 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 groups than the infected control group. Moreover, significant (p<0.05) improvement in the mean body weights was observed in the treated groups than the infected control one. All the tested drugs and volatile oils were efficacious in controlling of MG infection in broiler chickens, but pefloxacin medication was the best in controlling of such infection. Although volatile oils were the least efficacious, however it could be recommended as an alternative natural safe way to the drugs in controlling MG infection to overcome the problems of drug resistance and drug tissue residues common under our field conditions.

Introduction

Respiratory diseases cause significant economic losses to poultry industry. Mycoplasma infections in poultry pose one of the major economic problems in the poultry field. Infection by *Mycoplasma gallisepticum* (MG) is of particular concern because it's well known for its ability to produce great economic losses in poultry industry. In complication, MG causes chronic respiratory disease (CRD), poor feed conversion, low growth rate, increased mortalities, increased carcass condemnation at processing and increased vaccination and medication costs **(Kerr and Olson, 1967 and Ley and Yoder, 1997)**. In commercial layer flocks, MG is involved in severe egg production losses **(Domermuth and Gross, 1962; Mohamed *et al.,* 1987; Kleven, 1998 and Levisohn and Kleven, 2000)**; also, it leads to deterioration of egg quality **(Pruthi and Kharole, 1981)**. Indirect losses caused by MG is associated with increased the susceptibility of the infected birds to other infectious agents like infectious bronchitis, infectious laryngeotracheitis or Newcastle disease viruses and *Escherichia coli* **(Grimes and Rosenfeld, 1972; Carpenter *et al.,* 1981 and Yoder, 1991)**.

Controlling disease while improving utilization of feed, decreasing waste, and providing a wholesome consumer product are goals of the food production industry; therefore the control of MG infection in the poultry flocks is very important.

Although MG has been successfully eliminated from broiler flocks in many of the major areas of poultry production, in those areas where infection remains endemic, measures to control the infection often rely heavily on the widespread use of antimicrobials **(Lin, 1987)**. Many different antibiotics have been used at various dosages in an attempt to control the air-sacculitis lesions and egg production losses resulting from MG infection **(Yamamoto and Adler, 1956; Barnes *et al.,* 1960; Olesiuk *et al.,* 1964; Gale *et al.,* 1967; Hamdy and Blanchard, 1969 and 1970; Ose *et al.,* 1979; Gordon and Jordan, 1982; Hamdy *et al.,* 1983; Siva *et al.,* 1986; Glisson *et al.,* 1989; Hinz and Rottmann, 1990; Jordan *et al.,* 1991b; Arzey and Arzey, 1992 and Jordan *et al.,* 1998)**.

Among the most commonly used antibiotics against mycoplasma infections is the fluroquinolone compounds which is widely used in clinical veterinary practice because of their wide spectrum bactericidal activity and high degree of bioavailability **(Eliopoulos *et al.,* 1984 and Andon, 1993)**. Different studies have been produced to

evaluate the efficacy of using flouroquinolons alone or in combination with old antimycoplasma drugs like macrolides and tertacyclins against avian mycoplasmosis (**Arai** *et al.,* **1992; Jordan** *et al.,* **1993 and Sumano** *et al.,* **1998). Megaki** *et al.,* **(1993) and Tanner** *et al.,* **(1993)** reported superiority of danofloxacin compared to tylosin for control of MG infection in broiler chickens. Enrofloxacin was shown to protect chicks and poults against mortality, growth depression and air-sac lesions and to reduce the prevalence of isolation of MG and sero-conversion in different experimental models (**Kempf** *et al.,* **1988; Jordan** *et al.,* **1989; Hinz and Rottmann, 1990; Jordan** *et al.,* **1991a; Kempf** *et al.,* **1992 and 1994, Lu** *et al.,* **1995; Burch and Stipkovits, 1997; Jordan** *et al.,* **1997; Stipkovits and Burch, 1997; Stipkovits** *et al.,* **1997; Kempf** *et al.,* **1998 and Stanley** *et al.,* **2001).** Enrofloxacin was shown to be highly effective in reducing the level of egg transmission of MG (**Ortiz** *et al.,* **1995),** and the lateral and vertical transmission of *M. Iowae* (**Jordan** *et al.,* **1991b and 1993). Takahashi and Yoshida, (1989)** concluded that ofloxacin has a promising antimicrobial agent for treatment of mycoplasma infection.

Pefloxacin is a new member of fluoroquinolones that penetrates intracellularly and acts on DNA gyrase enzyme (intracellular enzyme) (**Badet** *et al.,* **1982; Kayser, 1985; Smith, 1986 and Brown, 1996)**, so it exhibits broad spectrum rapid bactericidal activity at low concentration (**Neer, 1988; Vancutsem** *et al.,* **1990 and Brown, 1996)**. Pefloxacin has *in vitro* potency against the causes of bacterial infectious diseases in poultry such as coliform infections, salmonellosis, infectious coryza, avian mycoplasmosis, and complication due to chronic respiratory disease, fowl cholera, avian tuberculosis, clostridial infections and avian chlamydiosis (**Wille** *et al.,* **1988; Gonzalez and Henwood, 1989; Wolfson and Murray, 1989 and Mohamed and Dardeer, 2001).**

Tylosin is a member of macrolides group which was discovered in 1955, approved for improving feed efficiency in the 1960s, and approved as an aid in the control of chronic respiratory disease in 1970s. There were different studies concerning the efficacy of using tylosin compounds in the treatment of chronic respiratory disease in different avian species. **Yoder and Hofstad, (1965)** detected that tylosin had a strong inhibitory effect on MG and greatly reduced the egg transmission of this organism. Tylosin tartrate when administered in the drinking water at a concentration of 0.55 g/liter for the first three days after hatching, was

highly effective in controlling the adverse consequences of MG infection **(Wise and Fuller, 1975). Ose** *et al.,* **(1979)** concluded that higher doses of tylosin reduced markedly the spread of MG infection in chicken flocks. The efficacy of tylosin in the feed for prevention of MG infection was also studied by **Stoianova–Zaiakova** *et al.,* **(1980) and Cummings** *et al.,* **(1986)** and found complete protection of the infected chickens from mycoplasmosis. The antimicrobial activity of acyl derivatives of tylosin were evaluated *in vitro* and *in vivo* by **Okamoto** *et al.,* **(1980a,b)**, who reported that 3-acetyl-4-isovaleryl tylosin had good and superior antimicrobial activity compared with tylosin. Moreover, **Skelly** *et al.,* **(1986)** demonstrated that when 3-acetyl-4"-isovaleryl tylosin tartrate formulation administered in drinking water to chickens infected with a macrolide-sensitive or macrolide-resistant strain of MG resulted in no detection of mycoplasma in the air sacs and in MG-negative sera. **Bywater, (1991)** corroborated that tylosin was very active against mycoplasmas in chickens particularly MG, easily administered, and of wide margin of safety as well as of low level of toxicity. In addition, **Baba** *et al.,* **(1998a,b)** proved that tylosin tartrate not only enhanced the humoral immune functions by significantly increasing the amount of antibody production and by increasing the amount of antibody producing cells, but also enhanced the cellular immune functions in tylosin treated chickens than untreated group.

Spiramycin is another member of macrolides group that produced by the growth of certain strain of *streptomyces ambofaciens* **(Brander** *et al.,* **1991).** Spiramycin exerts its effect by interfering with protein synthesis of the cells **(Alexander, 1994).** Within two hours from administration of spiamycin, the drug in its active form is accumulated intracellular in macrophages to a concentration 10 to 20 times that found in the extracellular medium **(Zenebergh and Trouet, 1982).** Its antibacterium spectrum includes mainly Gram positive organisms and mycoplasma species. The effect of using spiramycin in the treatment of MG infection in turkeys **(Sanger and Gale, 1961 and Inglis and Cook, 1964)**, broiler **(Kempf** *et al.,* **1989)** and layer chickens **(Arzey and Arzey 1992)** were tested and the drug proved its efficacy.

Due to the wide spread and haphazard using of antimicrobials to control MG infection in the field resulted in development of drug resistance and presence of drug

tissue residues, so it is very important to search another alternative natural and safe way to control such infection. Volatile oils proved their therapeutic action in human and veterinary medicines as local stimulants, diuretics, carminatives, antiseptics and anthelmintics **(Brander and Pugh, 1977 and Balbaa *et al.,* 1981).** The previous work by **Lu *et al.,* (2004)** proved that the essential oil of *Eucalyptus globulus* reduced the inflammatory cell infiltration into the epithelium of trachea and bronchioles caused by lipopolysaccharide (LPS) in rats. Also, using of volatile oils was tested recently and gave good results in prevention and control of birds' MG infection. The previous experimental and field trials demonstrated that the volatile oils containing eucalyptus and peppermint has been proven safe for broilers and layers and it is effective in preventing respiratory problems, improving performance, and stimulating the immune system when it was used in drinking water **(Bragg *et al.,* 1999; Awaad *et al.,* 2002; Bouzoubaa, 2004; Bragg, 2004; Barbour and Dankar 2005; Barbour *et al.,* 2006 and Tayfun *et al.,* 2008).**

Accordingly, the goal of this study was testing and comparing the effectiveness of some antibiotics (pefloxacin, tylosin and spiramycin) as well as other natural alternatives like volatile oils (eucalyptus and peppermint essential) in the control of induced MG infection in broiler chickens.

Material and Methods

1. Experimental chickens:

A total of two hundred, one day-old Hubbard broiler chicks of mixed sex were used in the present study. The chicks were obtained from a breeder flock known to have no history of mycoplasmosis problems. The birds were kept under complete observation in separate thoroughly cleaned and disinfected pens and provided with adilbitum feed and water. All the birds were vaccinated against Newcastle disease using HB1 vaccine and against infectious bronchitis at 5 days of age, also against infectious bursal disease using 228E vaccine at 13 days of age. Lasota vaccine against Newcastle disease was given at 14 days of age (before challenge). All the vaccines were given via eye drop instillation.

2. The ration used:

Commercial starter and finisher broiler chicken ration was used. The starter ration contained crude protein-not less than 21%, crude fat-not less than 2.94%, crude fibers-not less than 2.35%, metabolizing energy-not less than 3054 Kcal/kg ration and

used for the first 4 weeks. The finisher ration contained crude protein-not less than 17.15%, crude fat-not less than 2.5%, metabolizing energy-not less than 3020 Kcal/kg ration and used for the remaining of the experimental period. No antibiotics, coccidiostats and other growth promoters were added to the ration.

3. The challenge organism:

Morphologically, biochemically, serologically and molecullary characterized strain of MG which was obtained from the project "Epidemiological, diagnostic and preventive studies on mycoplasma infections in breeder chickens" was used in this experimental study. After thawing of the strain, an aliquot of the strain was filtrated once before being used and was tested for identity and purity (**Tully, 1983**). The stock culture was diluted in fresh mycoplasma broth medium to give an inoculum of 10^5 colony forming unit (CFU) per milliliter (**Jordan et al., 1989**). The titer of the inoculum was estimated retrospectively, by making appropriate 10-fold dilutions of culture in Frey's broth (**Frey et al., 1968**) and then plating six discrete 25 µl drops of each dilution onto surface dried mycoplasma agar plates (**Rodwell and Whitcomb, 1983 and Jordan et al., 1991a**). Plates were incubated at 37ºC under reduced oxygen tension for up to seven days, and visible colonies were counted. At 14 days old, each bird in the challenged groups was inoculated with 0.4 ml of MG culture containing 10^5 CFU/ml viable organisms via intranasal route (**Talkington and Kleven, 1985 and Kempf et al., 1998**).

4. The different treatments used:

Different commercial antibiotics and volatile oils preparation were tested. These compounds were containing the followings active principls:

- Pefloxacin (10%) solution is an active principle was obtained from United Co. for Chem. and MED preparation. Egypt. The Reg. No. of the drug was 1706/200. The drug was given for five days.

- A water soluble powder containing acetyle isovaleryl tylosin tartarate as an active principle (20 mg/kg body weight) obtained from Arab Company for Medical products (ARABCOMED). The drug was given for three days.

- A water soluble powder containing spiramycin as an active principle in a dose of 1 gm/liter of the drinking water for one day. The drug was obtained from the Arab Company for Medical Products (ARABCOMED), with a Reg. No. 2/52/2003 Vet.

- A natural product containing eucalyptus and peppermint essential volatile oils as an active principle. This product was obtained from EWABO Chemikalien GmbH & Co. KG, Kolpingstrabe 4 D-49835 Wietmarschen. Batch No. 08/2005/10330 and was given for five days.

All the tested compounds were used in the drinking water just after appearance of the clinical signs (5 days post challenge). The medicated drinking water was prepared such that the desired concentration in the drinking water matched the designed dose levels of the company's recommendations.

5. Experimental design:

Two hundred, day-old Hubbard broiler chicks of mixed sex were used. At day old, randomly 20 chicks were sacrificed and the serum collected from them were then subjected to serological examination serum plate agglutination (SPA) test, then examined bacteriologically to prove their freedom from egg borne MG infection. One hundred and eighty birds were divided into six equal separate groups; 30 chicks each (groups 1, 2, 3, 4, 5 and 6). Group (1) was kept as blank control negative (non infected non treated) group that was inoculated with sterile mycoplasma broth. Just before challenge (at 14 days old), the birds in each group were weight, then three blood samples were collected randomly from the wing veins birds in each group and the sera of them were subjected to SPA test. In addition, these birds were sacrificed and subjected to post-mortem and bacteriological examinations to exclude presence of MG infection. Each bird of groups (2, 3, 4, 5 and 6) was inoculated intranasal with 0.4 ml of MG culture containing 10^5 CFU /ml viable organisms at 14 days of age. Chickens of group (2) was left as control positive (infected non treated) birds, however, chickens of groups (3), (4), (5) and (6) gained preparations containing active principles (pefloxacin, tylosin, spiramycin) and volatile oils (eucalyptus and peppermint) in the drinking water, respectively. The different treatments were given to the birds immediately after appearance of the clinical signs (5 days post challenge). The treatment was continued once daily in the drinking water for a period according to the manufacturer's recommendations. During this period the water consumption of these groups was monitored. The birds were kept under observation till 35 days of age (experimental period).

6. Assessment of the treatment efficacy:

The efficacy of the used antibiotics and volatile oils was assessed in the experimental groups based on evaluation of the following parameters:

A. Clinical signs:

The infected and treated groups were daily observed for clinical signs just after infection till the end of the observation period (5 weeks old). The respiratory symptoms were scored individually as described by **Kempf *et al.,* (1998)** as the followings:

1= No respiratory signs.

2= Slight symptoms (sneezing and few tracheal rale).

3= Moderate symptoms (sneezing or tracheal rale).

4= Severe symptoms (sneezing or frequent tracheal rale, despnea).

B. Mortality rate:

The number of dead birds/group was recorded daily till 35 days of age (end of the experiment).

C. Gross lesions:

Five birds from each group were sacrificed weekly after challenge (at 21, 28 and 35 days of age). The post-mortem lesions of the sacrificed as well as the dead birds were recorded. The typical lesions of mycoplasma infection (air-sac lesions) were monitored and scored according to the criteria described by **Kleven *et al.,* (1972)** as follows:

- No air-sac lesion observed (lesion score= 0), the air sac membranes of the birds were completely clear without gross alterations.
- Air-sac lesion score= 1, the membranes were slightly cloudy without marked alterations.
- Air-sac lesion score= 2, the membranes were slightly thickened and usually with small accumulations of cheesy-like substances exudates.
- 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.
- 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.

D. Body weights:

The body weights of the birds in each group were detected just before infection at 14 days old and then weekly (at 21 and 28 days old) till the end of the treatment at the day 35 of age.

E. Re-isolation of MG:

Air-sac swabs were collected from day-old birds, 14 days old chickens (just before infection) and from the dead as well as the sacrificed birds each week with air-sac lesions for re-isolation of MG. The isolation and purification were performed as described by **Adler *et al.,* (1958).** The swabs were inoculated in liquid and solid mycoplasma media either immediately or after storage at 0-4ºC for up to 48 hours. Subcultures were made on solid media and the plates were incubated at 37ºC under reduced oxygen tension for 7 days as they were examined frequently for mycoplasmas growth, and they were considered negative if no growth appeared after 14 days of incubation. The proportion of the recovered mycoplasmas was identified microscopically.

F. Serological examination:

The rapid serum plate agglutination (SPA) test was used to test the collected sera for antibodies to MG. Blood samples were randomly collected from sacrificed 20 chicks at day-old and also from the wing veins of three birds in each treatment just before infection and at the end of the experiment at 35 days of age. The blood samples were centrifuged at 3000 rpm for 10 minutes and the sera were separated. The antigen used was a commercial stained product (*Mycoplasma gallisepticum* antigen Nobilis) (Intervet International B. V. Boxmeer, Holland). The technique was adopted according to the previously published method of **Kempf *et al.,* (1994).**

7. Statistical analysis of the data:

The collected data were tested using the method of **Sendecor and Cochran (1980).** Differences of $p < 0.05$ were considered significant.

Results and Discussion

This work was designed to evaluate the efficacy of using different antibiotics (pefloxacin, tylosin and spiramycin) as well as volatile oils containing eucalyptus and peppermint on the experimental MG infection of broiler chickens. Evaluation of the treatment efficacy was based on determination of certain parameters including, clinical signs, mortality rate, gross lesions, body weights, re-isolation of MG as well

as detection of MG antibodies (**Megaki** *et al.,* **1993; Tanner** *et al.,* **1993; Hanafy** *et al.,* **1995; Jordan and Horrocks, 1996; Charleston** *et al.,* **1998; Kempf** *et al.,* **1998 and Manal and Mona, 2002).**

Considering the results of the effect of using different treatments on the mean clinical score of non infected-non treated group as well as MG infected and treated groups were given in table (1). There were no clinical signs recorded in non infected non treated chickens along 5 weeks experimental period (mean clinical score=1). The first clinical signs of MG infection were sneezing and/or rals that observed in the infected groups 5 days post experimental infection. The severity of the clinical signs was increased by the following few days to reach the greatest value (2.86) in the infected non treated control group at the last week of the observation period. Statistically, there were significant (p<0.05) differences in the mean clinical score between the infected control positive group and the treated ones. Among the treated groups, there were no significant differences (p<0.05) between them in the clinical score at the last period of the observation (29-35 days old) and the drugs succeeded in reliving of the respiratory signs. At the pervious interval, chickens treated with pefloxacin showed the least mean clinical score (1.01), followed by those treated with tylosin (1.21) and spiramycin (1.19), while those treated with volatile oils showed the highest score (1.23). These results are partially in accord with those reported by **Kempf** *et al.,* **1998** who found that using of either fluoroquinolones (difloxacin 10 mg/kg) or (enrofloxacin 10 mg/kg) was equally effective in treating respiratory symptoms in MG infected broiler chickens. The effect of volatile oils in clearing of respiratory signs was investigated by **Bragg** *et al.,* **(1999)** who concluded that using of these oils in the drinking water of layer chickens could greatly assisted in reducing the clinical signs after experimental infection with C-3 serovar of *H. paragallinarum* organism even in birds vaccinated against this infection. The mechanism by which the volatile oils can relief the respiratory sings was explained by **Wurges, (2001); Gawronski, (2002); Page, (2004); Zakay-Rones** *et al.,* **(2004) and Salari** *et al.,* **(2006).** Those authors suggested that the active ingredients of *Eucalyptus* spp. can protect the first line of defense in the poultry host through the thinning of the mucus in the respiratory tract which could help in its outward flow, pushing with it the microorganisms, preventing their colonization, and thus protecting the cilia from consequent damage. **Barbour** *et al.,* **(2006)** evaluated the histopathological changes of eucalyptus and peppermint oils treated versus deprived broilers subjected to three

different natures of challenges (MG, H9N2, and a combination of MG/H9N2) and found that this treatment resulted in significant decrease in tracheal deciliation in MG- and MG/H9N2-challenged birds, significant decrease in tracheal goblet cells degeneration in MG-and MG/H9N2-challenged birds, significant decrease in tracheal mucus accumulation in MG-challenged birds, and significant decrease in heterophil infiltration in MG/H9N2-challenged birds. So, the previously mentioned authors detected that there is synergism among the active ingredients of *Eucalyptus* spp. and peppermint for providing protection of the goblet cells in the upper respiratory system. The maintenance of the goblet cell structure and function is important in respiratory diseases to keep the mucus flow in the air passages, thus pushing the germs and other particles, with the help of the maintained cilia, anteriorly and outwards through the mouth or nostrils.

Our results illustrated in table (2) revealed the effect of different treatments on the mortality rate of non infected-non treated group as well as MG infected and treated groups. It has been demonstrated that there was no mortalities along the period (15-35 days of age) in the non infected-non treated group; whereas, the number of dead birds in the infected non treated group during this period was (10/30) with a percentage of 33.33. Out of 30 birds in each of the treated group, the total number of dead birds from the day post infection (15) till the end day of the study (35) in pefloxacin, tylosin, spiramycin and volatile oils treated groups were 3, 4, 6 and 6; respectively that representing percentage of (10, 13.3, 20 and 20); respectively. The above mention results are in concur with these recorded by **Manal and Mona (2002)** who revealed that using of pefloxacin (10mg/kg body weight) in the drinking water for five successive days induced only 1% mortality rate in comparison with 88% in MG infected control broiler chickens. Furthermore, **Khan *et al.,* (2006)** proved that tylosin is the drug of choice followed by oxytetracyline against MG infection in broilers on the basis of measuring mortality, morbidity and case-fatality profiles, whereas, **Arzey and Arzey (1992)** found that single oral treatment of MG infected layer chickens with 100 or 200 mg spiramycin induced 100% curacy in comparison with 85% in tylosin treated birds.

The air-sac lesions of the sacrificed birds prior to experimental infection were absent. The results of the effect of different treatments on the mean air-sac lesion

score of non infected-non treated group as well as MG infected and treated groups were summarized in table (3). During 35 days experimental period, dead as well as sacrificed birds from infected-non treated control group showed the highest mean air-sac lesion score (3.16), however sacrificed chickens of non infected-non treated control group showed no air-sac lesions (lesion score=0). Such results agree with **Yoder (1984 and 1991) and Ley (2003)** who concluded that MG incriminated as a pathogen responsible for pathological changes in the respiratory tracts and air-sacs of the infected birds. Along the whole observation period, a significant ($p<0.05$) reduction in mean air-sac lesion score was seen in the medicated groups when compared with the only infected control group. By the end of the study (29-35 days of age), the lowest mean air-sac lesion score of dead and sacrificed birds was achieved in group treated with pefloxacin (0.91), followed by those treated with tylosin (1.05), spiramycin (1.35) and volatile oils (1.78). Previously mentioned results refer to the capacious role of the treatments in deterring the development of air-sac gross lesions in the treated birds. Our results are partially in agreement with **Kempf *et al.,* (1989)** who recorded that both of spiramycin and tylosin have the potential and significant effect in reducing symptoms, mortalities and lesions of MG infected day old chicks than untreated birds. **Tayfun *et al.,* (2008)** determined that treatment of broilers with compound containing eucalyptus and peppermint oils could reduce *E. coli* related lesions and mortalities in birds with acute infectious bursal diseases and Newcastle disease vaccination reactions, could have a positive effect on Newcastle disease-haemagglutination inhibition antibody response produced just after its vaccination and also could improve the food conversion ratio of the treated broilers.

From table (4), the effect of different treatments on the mean body weights of surviving birds in non infected-non treated group as well as MG infected and treated groups could be recognized. Efficacy assessed in the mean body weights of birds that survived at various ages to the end of the experiment showed that there were no significant differences in the mean body weights among all groups prior to infection at 14 days of age. At the different intervals (21, 28 and 35 days of age), there were significant ($p<0.05$) differences between non infected-non treated blank control group and the only infected control one; moreover, significant ($p<0.05$) improvement in the mean body weights was observed in the treated groups (pefloxacin, tylosin, spiramycin and volatile oils) than the infected non-treated control group. Further, no

significant (p<0.05) differences were seen between pefloxacin as well as tylosin treated groups with the blank control group at all intervals. Similarly, **Manal and Mona (2002) and Adayel and Abdalla (2007)** reported on the effectiveness of using pefloxacin in improving the performance after infections with MG and *Salmonella* spp.; respectively. The improvement of body weights in the infected-pefloxacin treated group might be attributed to the bactericidal effect of the drug on MG and consequently improving the general health conditions **(Alexander, 1985).** In spite of birds treated with volatile oils showed the least mean body weights (1187.4) among the treated groups at the end of the study, but statistically it was significantly (p<0.05) better than non treated infected birds. The last result consent with **Awaad *et al.,* (2002)** who recorded that treatment of broiler chickens with volatile oils containing eucalyptus and peppermint significantly improved the zootechnical performance of the treated birds after challenge with *ornithobacterium rhinotracheale* (*ORT*) and velogenic viscrotropic strain of Newcastle disease (VVND) virus. The above mentioned investigators presumed this improvement due to the action of volatile oils in liquefaction and loosening of the respiratory thick sticky exudates which could be expelled by the birds and consequently reduced the hypoxia, improved breathing and finally increased the feed intake and body weights.

Concerning the results of bacteriological examination of day-old chicks and 14 days old birds for the presence of MG, the air-sac swabs collected from those birds revealed negative results or absence of any MG natural infection. From table (5) which showed the effect of different treatments on the re-isolation rate of non infected-non treated group as well as MG infected and treated groups, it could be detected that air-sac swabs collected from sacrificed birds in non infected non treated group were negative to MG (re-isolation rate= 0%) along the whole experimental period. In contrast to this, the recovery rate was 100% from the air-sacs of dead and sacrificed birds in infected-non treated group at all intervals of the study (15-35 days of age). Attempts to re-isolation of MG was failed (re-isolation rate of 0%) in all treated groups (pefloxacin, tylosin, spiramycin and volatile oils) at the last week of observation (29-35 days of age). These results are in agree with that cited by **Abdel-Aziz *et al.,* (1996) and Ewing *et al.,* (1998)** who concluded that newer flouroquinolones (pefloxacin is one of them) have bactericidal activity at lower concentrations over naldixic acid and other quinolones. Also, **Manal and Mona (2002)** found that treatment of MG infection in broilers using pefloxacin reduced the

re-isolation rate of the organism to (20%) in comparison with (100%) of the control non treated group. Tylosin acts by inhibition of the bacterial protein synthesis by inhibiting of 5 0S ribosome, which is the essential structure for growth and multiplication of bacteria particularly MG, so it was selected to control MG in an infected flocks **(Bywater, 1991 and Razin *et al.*, 1998).** Absence of MG in volatile oils treated group is supposed by the manufacturer to the possible bactericidal action of these oils or perhaps attributed to their effect on the cell mediated immunity which help in clearing the bacteria from the cells.

The rapid serum plate agglutination test of the tested birds at day-old and at 14 days old (just before infection) proved negative agglutination results. These indicated absence of antibodies to the egg borne or natural infection with MG. At the end of the experiment (35 days of age), the serum samples collected from non infected non treated group revealed negative results, On contrary, the serum samples of the infected non treated group showed (100%) positive agglutination. All the infected-treated groups were serologically negative to MG at the end of the study. The previously mentioned results are in coincide with these obtained by **Kempf *et al.*, (1998). Barbour and Danker (2005)** found that the administration of eucalyptus and peppermint oils following vaccination with Newcastle disease, infectious bronchitis and infectious bursal disease viruses in MG/Avian influenza-infected broilers boosted the bird's immune response and ameliorated their performance. Consequently, the administration of these oils could have similar alleviating effect on signs and lesions of MG- and/or Avian influenza-infected broilers.

Eventually, the present investigation clearly showed that the tested antibiotics (pefloxacin, tylosin and spiramycin) and volatile oils (eucalyptus and peppermint) were efficacious in the treatment of induced MG infection in broiler chickens as indicated by reduced clinical signs, mortality rate, gross lesions, re-isolation rate and serological reaction as well as improved body weights of the treated groups when compared with non treated infected chickens. With reference to aforementioned parameters, pefloxacin was the best to the other treatments in controlling of such MG infection. Although volatile oils containing eucalyptus and peppermint gave the lowest efficacy in comparison with the other tested drugs in controlling MG infection, but it advised to be used as a safe natural alternative to antibiotics to avoid the common problems of drug resistance and drug tissue residues occurring in the field.

Table (1): The effect of different treatments on the mean clinical score of non infected-non treated group as well as *Mycoplasma gallisepticum* infected and treated groups.

Group number	Group treatment	Mean clinical score ± SD		
		Intervals of age/day		
		15-21	22-28	29-35
1	Blank control	1	1	1
2	Infected control	1.78±0.21 [a]	2.30±0.34 [a]	2.86±0.40 [a]
3	Pefloxacin	1.86±0.10 [a]	1.45±0.09 [c]	1.01±0.08 [d]
4	Tylosin	1.70±0.25 [a]	1.55±0.11 [b]	1.21±0.10 [d]
5	Spiramycin	1.75±0.30 [a]	1.58±0.17 [b]	1.19±0.14 [d]
6	Volatile oils	1.81±0.19 [a]	1.54±0.17 [b]	1.23±0.10 [d]

[a-d] Figures sharing common superscripts are not significantly different ($p<0.05$).

Table (2): The effect of different treatments on the mortality rate of non infected-non treated group as well as *Mycoplasma gallisepticum* infected and treated groups.

Group number	Group treatment	Mortalities at intervals/days				The total mortalities (15-35)days	
		No.	15-21	22-28	29-35	[*]No.	%
1	Blank control	30	0	0	0	0	0
2	Infected control	30	1	6	3	10/30	33.33
3	Pefloxacin	30	1	2	0	3/30	10
4	Tylosin	30	0	4	0	4/30	13.3
5	Spiramycin	30	1	5	0	6/30	20
6	Volatile oils	30	1	5	0	6/30	20

[*]No.= Number of birds/total number of birds in the group.

Group	Group treatment	+Mean air-sac lesion score ± SD

		Intervals of age/day		
		15-21	22-28	29-35
1	Blank control	0	0	0
2	Infected control	2.99 ± 0.31^{a}	3.10 ± 0.29^{a}	3.16 ± 0.22^{a}
3	Pefloxacin	2.91 ± 0.23^{a}	1.45 ± 0.20^{b}	0.91 ± 0.10^{d}
4	Tylosin	2.95 ± 0.25^{a}	1.57 ± 0.23^{b}	1.05 ± 0.14^{d}
5	Spiramycin	3.05 ± 0.40^{a}	1.99 ± 0.38^{c}	1.35 ± 0.18^{bd}
6	Volatile oils	2.89 ± 0.33^{a}	1.87 ± 0.22^{c}	1.78 ± 0.11^{c}

Table (3): The effect of different treatments on the mean air-sac lesion score of non infected-non treated group as well as *Mycoplasma gallisepticum* infected and treated groups.

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

[a-d] Figures sharing common superscripts are not significantly different (p<0.05).

Table (4): The effect of different treatments on the mean body weights ± SD of surviving birds in non infected-non treated group as well as *Mycoplasma gallisepticum* infected and treated groups.

Group number	Group treatment	Age/day			
		14 (just before infection)	21 (during treatment)	28 (after treatment)	35 (end of the study)
		B.wt/gm	B.wt/gm	B.wt/gm	B.wt/gm
1	Blankcontrol	301.0 ± 0.29^{a}	533.6 ± 43.1^{a}	845.8 ± 61.0^{a}	1300.5 ± 85.2^{a}
2	Infectedcontrol	309.3 ± 0.24^{a}	391.0 ± 32.4^{c}	696.8 ± 50.3^{c}	1099.0 ± 93.5^{c}
3	Pefloxacin	299.1 ± 0.31^{a}	527.4 ± 39.5^{a}	839.0 ± 41.1^{a}	1295.1 ± 87.9^{a}
4	Tylosin	305.2 ± 0.22^{a}	519.0 ± 24.7^{a}	833.5 ± 48.4^{a}	1289.7 ± 91.0^{a}
5	Spiramycin	300.6 ± 0.25^{a}	489.9 ± 50.0^{b}	799.5 ± 58.8^{b}	1194.5 ± 99.0^{b}
6	Volatile oils	302.9 ± 0.35^{a}	475.8 ± 33.1^{b}	798.7 ± 46.6^{b}	1187.4 ± 97.6^{b}

B.wt= Body weight

[a-c] Figures sharing common superscripts are not significantly different (p<0.05).

Table (5): The effect of different treatments on the re-isolation rate of non infected-non treated group as well as *Mycoplasma gallisepticum* infected and treated groups.

Group number	Group treatment	Re-isolation rate					
		Intervals of age/day					
		15-21		22-28		29-35	
		*No.	%	*No.	%	*No.	%
1	Blank control	0/5	0	0/5	0	0/5	0
2	Infected control	6/6	100	11/11	100	8/8	100
3	Pefloxacin	3/6	50	0/7	0	0/5	0
4	Tylosin	3/5	60	1/9	11.1	0/5	0
5	Spiramycin	4/6	66.7	2/10	20	0/5	0
6	Volatile oils	4/6	66.7	3/10	30	0/5	0

*No.= Number of birds from which MG was re-isolated/number of dead and/or sacrificed birds.

References

Abdel-Aziz, M. I.; El-Banna, H. A. and Eissa, S. I. (1996): Efficacy and residual pattern of some fluoroquinolones in poultry. J. Vet. Med. Associ., 56 (4): 517-562.

Adayel, S. A. and Abdalla, O. E. (2007): Efficacy of pefloxacin against salmonellosis in balady chicks. Zag. Vet. J., 35 (1): 71-78.

Adler, H. E.; Fabricant, J.; Ymamato, R. Y. and Berg, J. (1958): Symptoms of chronic respiratory disease of poultry. I. Isolation and Identification of pleuropneumonia like organisms of avian origin. Am. J. Vet. Res., 19: 440-447.

Alexander, F. (1985): An Introduction to Veterinary Pharmacology. 4th Ed. Longman (London and New York).

Alexander, F. (1994): An Introduction to Veterinary Pharmacology. 6th Ed. Longman (London and New York).

Andon, A. (1993): Les fluoroquinolones: aspects pharmacologiqes. Bull. Acad. France. 65: 207-216.

Arai, S.; Gohara, Y.; Kuwano, K. and Kawashima, T. (1992): Antimycoplasmal activities of new quinolones, tetracyclines and macrolides against *Mycoplasma gallisepticum*. Antimicrob. Agents Chemother., 36 (6): 1322-1324.

Arzey, G, G. and Arzey, K. E. (1992): Successful treatment of mycoplasmosis in layer chickens with single dose therapy. Aust. Vet. J. , 69 (6):126-128.

Awaad, M. H.; El-Gohary, A. A. and Sultan, H. A. (2002): Effect of volatile oils on the concomitant infection of *ornithobacterium rhinotracheale "ORT"* and velogenic viscerotropic Newcastle disease "VVND"" in broiler chickens. Vet. Med. J., Giza, 50 (2): 289-298.

Baba, T.; Yamasheta, N.; Kodama, H.; Mukamoto, M.; Nakamoto, K.; Nose, Y. and McGruder, E. D. (1998a): Effect of tylosin tartrate (tylosin soluble) on humoral immune responses in chickens. Zentralbl. Veterinamed., (B): 45 (5): 279-286.

Baba, T.; Yamasheta, N.; Kodama, H.; Mukamoto, M.; Nakamoto, K.; Nose, Y. and McGruder, E. D. (1998b): Effect of tylosin tartrate (tylosin soluble) on cellular immune responses in chickens. Poult. Sci., 77 (9): 1906-1911.

Badet, B.; Hugher, P.; Kohiyama, M. and Forterre, P. (1982): Inhibition of DNA replication in vitro by pefloxacin. Federation of European Biochemical

Societies (Elsevier, Holland), 145: 355-359.

Balbaa, S. I.; Hilal, S. H. and Ashgan, Y. Zaki (1981): Medicinal plant constituents. Third Edition, Central Agency for University and School Books.

Barbour, E. K. and Danker, S. (2005): Essential oils of *Eucalyptus* and peppermint improve the homogeneity of immune responses and performance in MG/H9N2-infected broilers. J. Amer. Holistic Vet. Med. Assoc., 24:23-27.

Barbour, E. K.; Rindala, G. E.; Kaadi, M. S.; Shaib, H. A.; Gerges, D. D. and Nehme, P. A. (2006): Evaluation of the histopathology of the respiratory system in essential oil-treated broilers following a challenge with *Mycoplasma gallisepticum* and/or H9N2 Influenza virus. Intern. J. Appl. Res. Vet. Med., 4 (4): 293-300.

Barnes, L. E.; Ose, E. E. and Gossett, F. O. (1960): Treatment of experimental PPLO infections in young chickens with tylosin, a new antibiotic. Poult. Sci., 39: 1376-1381.

Bouzoubaa, K. (2004): Use of Mentofin to reduce respiratory disease problems in broilers after vaccination against Newcastle Disease. Institute of Agronomy and Veterinary Medicine Hassan I, Avian Diseases Department, Rabat Institutes BP 6202 Morocco.

Brander, G. C. and Pugh, D. M. (1977): Cited in Veterinary Applied Pharmacology and Therapeutics. 3rd Ed. The English Language Book society and Bolliere Tindall, London.

Brander, G. C.; Pugh, D. M.; Bywater, R. J. and Jenkins, W. L. (1991): Cited in Veterinary Applied Pharmacology and Therapeutics. 5th Ed. The English Language Book society and Bolliere Tindall, London.

Bragg, R. R. (2004): Evaluation of the safety of the continual use of the product, Mentofin for spraying of layer chickens. Veterinary Biotechnology, Department of Microbial, Biochemical and Food Biotechnology, University of Free State, PO Box 339, Bloemfontein 9300, Republic of South Africa.

Bragg, R. R.; Albertyn, N. J. and Van Heerden, E. (1999): Effects of using Mentofin for the prevention and treatment of infectious coryza caused by *Haemophilus paragallinarum*. Veterinary Biotechnology, Department of Microbial, Biochemical and Food Biotechnology, University of Free State, PO Box 339, Bloemfontein 9300, Republic of South Africa.

Brown, S. A. (1996): Fluoroquinolones in animal health. J. Vet. Pharm. Therap., 19:

1-4.

Burch, D. G. S. and Stipkovits, L. (1997): Evaluation of the efficacy of Tetramutin-OT in the treatment of chickens experimentally infected with *Mycoplasma gallisepticum*. Proceedings of the XIth International Congress of the World Veterinary Medical Association, Budapest, Hungary. P. 79.

Bywater, R. J. (1991): The control of infectious diseases: Chemotherapy. In Veterinary Applied Pharmacology and Therapeutics. Fifth Edition. Edited by Brander, G. C.; Pugh, D. M.; Bywater, R. J. and Jenkins, W. L. Bailliere, Tindall.

Carpenter, T. E.; Mallinson, E. T.; Miller, K. F.; Gentry, R. F. and Schwartz, L. D. (1981): Vaccination with F-strain *Mycoplasma gallisepticum* to reduce production losses in layer chickens. Avian Dis., 25: 404-409.

Charleston, B.; Gate, J. J.; Aitken, I. A. and Reeve-Johnson, L. (1998): Assessment of the efficacy of tilmicosin as a treatment for *Mycoplasma gallisepticum* infections in chickens. Avian Pathol., 27: 190-195.

Cummings, T. S.; Kleve, S. H. and Brown, J. (1986): Effect of medicated feed on tracheal infection and population of *Mycolpasma gallisepticum*. Avian Dis., 30 (3): 580-584.

Domermuth, C. H. and Gross, W. B. (1962): The production of salpingitis of chickens by *Mycoplasma gallisepticum*. Avian Dis., 6: 499-505.

Eliopoulos, G. M.; Gardella, A. and Mollering, R. C. (1984): *In vitro* activity of ciprofloxacin, a new carboxy quinolone antimicrobial agents. Antimicrob. Agents. Chemother., 25: 43-56.

Ewing, M. L.; Cookson, K. C.; Philips, R. A.; Turner, K. R. and Kleven, S. H. (1998): Experimental infection and transmissibility of *Mycolpasma synoviae* with delayed serologic response in chickens. Avian Dis., 42 (2): 230-238.

Frey, M. L.; Hanson, R. B. and Andreson, D. P. (1968): A medium for the isolation of avian mycoplasmas. Am. J. Vet. Res., 29: 2163-2171.

Gale, G. O.; Layton, H. W.; Short, A. L. and Kemp, G. A. (1967): Chemotherapy of experimental avian mycoplasma infections. Ann New York Acad. Sci., 143: 239-255.

Gawronski, D.V. (2002): Medical choices. In: Gale Group.Gale Encyclopedia of Medicine.

Glisson, R. J.; Hsin, I.; Brown, J. and Stewart, R. G. (1989): The effect of

oxytetracycline on the severity of airsacculitis in chickens infected with *Mycoplasma gallisepticum*. Avian Dis., 33: 750-752.

Gonzalez, J. P. and Henwood, J. M. (1989): Pefloxacin. A review of its antibacterial Activity, pharmacokinetic properties and therapeutic use. Drugs, 37: 628-668.

Gordon, R. F. and Jordan, F. T. W. (1982): Poultry Diseases. 2nd Edn. P. 62-65. Bailliere Tindall, London.

Grimes, T. M. and Rosenfeld, L. W. (1972): Experimental respiratory disease and airsacculitis in fowls caused by *Mycoplasma gallisepticum*. Aust. Vet. J., 48: 113-116.

Hamdy, A. H. and Blanchard, C. J. (1969): Effects of lincomycin and spectinomycin water medication on chickens experimentally infected with *Mycoplasma gallisepticum* and *Escherichia coli*. Poult. Sci., 48: 1703-1708.

Hamdy, A. H. and Blanchard, C. J. (1970): Efficacy of lincomycin and spectinomycin against avian mycoplasmas. Antimicrobial Agents Chemother (Bethesda)., 10: 522-530.

Hamdy, A. H.; Thomas, R.W. and Kratzer, D.D. (1983): Lincomycin dose response for treatment of necrotic enteritis in broilers. Poult. Sci., 62: 585-588.

Hanafy, M. S. M.; Abdel-Rhaman, F. I.; Soliman, G. A.; Ahmed, A. R.; Afify, N. and Ahmed, S. E. S. (1995): Studies on the efficacy of combined immunostimulant-antibiotic therapy against experimental *Mycoplasma gallisepticum* infection in chickens. Dtsch. Tierarztl. Wschr., 102: 408-409.

Hinz, K. H. and Rottmann, S. (1990): Studies *on vivo* on the efficacy of enrofloxacin against *Mycoplasma gallisepticum*. Avian Pathol., 19: 511-522.

Inglis, J. M. and Cook, J. K. (1964): Spiramycin in the treatment of experimental mycoplasmosis in day old chicks and turkey poults. J. Comp. Pathol., 74: 470-481.

Jordan, F. T. W. and Horrocks, B. K. (1996): The minimum inhibitory concentration of tilmicosin and tylosin for *Mycoplasma gallisepticum* and *Mycolpasma synoviae* and a comparison o their efficacy in the control of *Mycoplasma gallisepticum* infection in broiler chicks. Avian Dis., 40: 326-334.

Jordan, F. T. W.; Bradury, J. M. and Kleven, S. H. (1991a): Mycoplasmosis

(*Mycoplasma gallisepticum*) manual of recommended diagnostic techniques and recommendations for biological products, vol. 3. Office International Des Epizooties, Paris.

Jordan, F. T. W.; Horrocks, B. K. and Jones, S. K. (1991b): A comparison of Bytril, tylosin and tiamulin in the control of *Mycoplasma Iowae* infection in turkey poults. Avian Pathol., 20: 283-289.

Jordan, F. T. W.; Forrester, C. A.; Ripley P. H. and Burch, D. G. (1997): An assessment on the efficacy of valnemulin in the treatment of *Mycoplasma gallisepticum* infection of broiler chickens and its comparison with some other antimicrobials. Proceedings of the XIth International Congress of the World Veterinary Medical Association, Budapest, Hungary. P. 80.

Jordan, F. T. W.; Forrester, C. A.; Ripley P. H. and Burch, D. G. (1998): *In vitro* and *in vivo* comparisons of valnemulin, tiamulin, tylosin, enrofloxacin, and lincomycin/spectinomycin against *Mycoplasma gallisepticum*. Avian Dis., 42 (4):738-45.

Jordan, F. T. W.; Gilbert, S.; Knight, D. L. and Yavari, C. A. (1989): Effects of Baytril, tylosin and tiamulin on avian mycoplasmas. Avian Pathol., 18: 659-673.

Jordan, F. T. W.; Horrocks, B. K.; Jones, S. K.; Cooper, A. C. and Giles, C. J. (1993): A comparison of the efficacy of danofloxacin and tylosin in control of *Mycoplasma gallisepticum* infection in broiler chicks. J. Vet. Pharmcol. Ther., 16 (1): 79-86.

Kayser, F. M. (1985): The quinolones: mode of action and mechanisms of resistance. Res. Clin. Forums, 7: 17-27.

Kempf, I.; Gesbert, F.; Guittet, M. and Bennejean, G. (1994): *Mycoplasma gallisepticum* infection in medicated chickens: Comparison of diagnosis methods including polymerase chain reaction. J. V. Med. B., 41: 597-602.

Kempf, I.; Van den Hoven, R.; Gesbert, F. and Guittet, M. (1998): Efficacy of difloxacin in broiler chickens for the control of infection due to pathogenic *Mycoplasma gallisepticum*. J. V. Med. B., 45: 305-310.

Kempf, I.; Gesbert, F.; Guittet, M.; Bennejean, G. and Cooper, A. C. (1992): Efficacy of danofloxacin in the therapy of experimental mycoplasmas in chickens. Res. Vet. Sci., 53: 257-259.

Kempf, I.; Ollivier, C.; Guittet, M.; Morin, Y. and Bennejean, G. (1989): Efficacy

of spiramycin and tylosin in preventing mycoplasmosis in chickens experimentally infected with *Mycoplasma gallisepticum*. Pathol Biol., 37 (5 Pt 2):560-564.

Kempf, I.; Cacou, P. M.; Guittet, M.; Ollivier, C.; Morin, M. and Bennejean, G. (1988): Evacuation de l' efficacite de la kitasamycine et de molecule Bey VP 2674 chez des poulets infectes experimentalement par *Mycoplasma gallisepticum*. Rec. Med. Vet., 164: 653-659.

Khan, M. A.; Khan, M. S.; Younus, M.; Abbas, T.; Khan, I. and Khan, N. A. (2006): Comparative therapeutic efficacy of tylosin and oxytetracycline in broilers experimentally infected with *Mycoplasma gallisepticum*. Int. J. Agri. Biol., 8 (2): 298-299.

Kleven, S. H. (1998): *Mycoplasmas* in the etiology of multifactorial respiratory disease. Poult. Sci., 77: 1146-1149.

Kleven, S. H.; King, D. D. and Andreson, D. P. (1972): Airsacculitis in broilers from *Mycoplasma gallisepticum*: Effect on air-sac lesions of vaccinating with infectious bronchitis and Newcastle virus. Avian Dis., 16: 915-924.

Kerr, K. M. and Olson, N. O. (1967): Pathology in chickens experimentally inoculated or contact infected with *Mycoplasma gallisepticum*. Avian Dis., 11: 559-578.

Ley, D. H. (2003): *Mycoplasma gallisepticum* infection. In Diseases of Poultry. Edited by Saif, Y. M. Iowa State Press, A Blackwell Publishing Company.

Ley, D. H. and Yoder, H. W. Jr. (1997): *Mycoplasma gallisepticum*. In Diseases of Poultry. 10th ed. Calnek, B. W. (pp. 194-207): Ames, IA: Iowa state University press.

Levisohn, S. and Kleven, S. H. (2000): Avian mycoplasmosis (*Mycoplasma gallisepticum*). Rev. Sci. Tech., 19: 425-442.

Lin, M. Y. (1987): *In vitro* comparison on the activity of various antibiotics and drugs against new Taiwan isolates and strains of avian mycoplasma. Avian Dis., 31: 705-712.

Lu, X. Q.; Tang, F. D. and Wang, Y. (2004): Effect of *Eucalyptus globulus* oil on lipopolysaccharide induced chronic bronchitis and mucin hypersecretion in rats. Zhongguo Zhong Yao Za Zhi., 29:168-171.

Lu, Y. S. H.; Tsai, H. J. and Sung, H. T. (1995): Efficacy of quinolones on the experimental avian mycoplasma infections and/or colibacillosis in young

chickens. Taiwan J. Vet. Med. Anim. Husb., 65: 21-26.

Manal, M. A. and Mona, M. S. (2002): Efficacy of fluoroquinolone (pefloxacin) on mycoplasma infection in chickens. Vet. Med. J., (Giza), 50 (4): 733-739.

Mohamed, E. A. and Dardeer, M. A. (2001): Influence of combination of BCG vaccine and pefloxacin in chickens experimentally infected with *Mycoplasma gallisepticum.* J. Egypt. Vet. Med. Associ., 61 (2): 281-292.

Mohamed, H. O.; Carpenter, T. E. and Yamamato, R. (1987): Economic impact of MG and MS in commercial layer flocks. Avian Dis., 31: 477-489.

Megaki, T. T.; Avakian, A. P.; Barnes, H. J.; Ley, D. H.; Tanner, A. C. and Magonigle, R. A. (1993): Efficacy of danofloxacin and tylosin in control of mycoplasmosis in chicks infected with tylosin-susceptible or tylosin-resistant field isolates of *Mycoplasma gallisepticum.* Avian Dis., 37 (2): 508-514.

Neer, T. M. (1988): Clinical and pharmacological features of fluoroquinolones antimicrobial drugs. J. Am. Vet. Med. Assoc., 193: 577-580.

Okamoto, R.; Fukumoto, T.; Nomura, H.; Kiyoshima, K.; Nakamura, K. and Takamatsu, A. (1980a): Physicochemical properties of new acyl derivatives of tylosin produced by microbial transformation. J. Antibiot., 33 (11): 1300-1308.

Okamoto, R.; Tsuchiya, M.; Nomura, H.; Iguchi, H.; Kiyoshima, K.; Hori, S.; Inui, T.; Sawas, T.; Takeuchi, T. and Umezawa, H. (1980b): Biological properties of new acyl derivatives of tylosin. J. Antibiot., 33 (11): 1309-1315.

Olesiuk, O. M.; Van Roekl, H. and Chandiramani, N. K. (1964): Antibiotic medication of chickens experimentally infected with *Mycoplasma gallisepticum* and *Escherichia coli.* Avian Dis., 8: 135-152.

Ortiz, A.; Froyman, R. and Kleven, S. H. (1995): Evaluation of enrofloxacin against egg transmission of *Mycoplasma gallisepticum.* Avian Dis., 39: 830-836.

Ose, E. E.; Wellenreiter, R. H. and Tonkinson, L. V. (1979): Effects of feeding tylosin to layers exposed to *Mycoplasma gallisepticum.* Poult. Sci., 58: 42-49.

Page, L. (2004): Healthy Healing: A Guide to Self-Healing for Everyone.12th ed. Sarasota FL: Book world services, 42-44.

Pruthi, A. K. and Kharole, M. U. (1981): sequential pathology of genital tract in chickens experimentally infected with *Mycoplasma gallisepticum.* Avian Dis., 25: 768-778.

Razin, S.; Yogev, D. and Naot, Y. (1998): Molecular biology and pathogenicity of mycoplasmas. Microbiology and Molecular Biology Reviews. 62 (4): 1094-1156.

Rodwell, A. W. and Whitcomb, R. F. (1983): Methods of direct and indirect measurement of mycoplasma growth. Methods in mycoplasmology, vol. 1. S. Razin and J. G. Tully, ed. Academic Press, New York, pp. 185-196.

Salari, M. H.; Amine, G. and Shirazi, M. H. (2006): Antibacterial effects of *Eucalyptus globulus* leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders. Clin. Microbiol. Infect., 12:194-196.

Sanger, V. L. and Gale, C. (1961): Spiramycin in the treatment of infectious sinusitis in turkeys. Avian Dis., 5 (2): 201-204.

Sendecor, G. W. and Cochran, W. G. (1980): Statistical Methods. 7th Ed., (Iowa State College Press, Ames, IA), 39-63.

Siva, J. L. da; Rota, A. and Osorio, M. (1986): Field evaluation of control of *Mycoplasma gallisepticum* transovaric transmission in heavy breeding hens by administration of linco-spection premix during three consecutive weeks, in each three month period. Conference-Europeenee-d-Aviculture-Paris, 1: 645-646.

Skelly B. J.; Andersen, D.; Pruss, M. and Pellegrino, R. (1986): Prophylactic efficacy of 3-acetyl-4"-isovaleryl tylosin in a *Mycoplasma gallisepticum-*induced airsacculitis infection. Avian Dis., 30 (3):505-509.

Smith, J. T. (1986): The mode of action of 4 quinolone and possible mechanism of resistance. J. Antimicrob. Chemother., 18 (suppl. d). 21-29.

Stanley, W. A.; Hofacre, C. L.; Speksnijder, G.; Kleven, S. H. and Aggrey, S. E. (2001): Monitoring of *Mycoplasma gallisepticum* and *Mycoplasma Synoviae* infection in breeder chickens after treatment with enrofloxacin. Avian Dis., 45 (2): 534-539.

Stoianova –Zaiakova, L.; Donev, B. and Shishkov, N. (1980): Clinical and pharmacological studies of technical tylosin phosphate from pharmachem in

poultry diseases. Vet. Med. Nakui, 17 (1): 26-32.

Stipkovits, L. and Burch, D. G. S. (1997): Evaluation of the efficacy of valnemulin in the prevention of model infection with *Mycoplasma gallisepticum* in chickens. Proceedings of the XIth International Congress of the World Veterinary Medical Association, Budapest, Hungary. P. 81.

Stipkovits, L.; Polgar, G. and Gautris, B. (1997): Efficacy of enrofloxacin and norfloxacin for the control of experimental *Mycoplasma gallisepticum* infection in chickens. Proceedings of the XIth International Congress of the World Veterinary Medical Association, Budapest, Hungary. P. 82.

Sumano, L. H.; Ocampo, C. L.; Brumbaugh, G. W. and Lizarraga, R. E. (1998): Effectiveness of two fluoroquinolones for the treatment of chronic respiratory disease outbreak in broilers. Br. Poult. Sci., 39 (1): 42-46.

Takahashi, I. and Yoshida, T. (1989): antimycoplasmal activities of ofloxacin and commonly used antimicrobial agents on *Mycoplasma gallisepticum*. Jpn. J. Antibiot., 42 (5): 1166-1172.

Talkington, F. D. and Kleven, S. H. (1985): Evaluation of protection against colonization of the trachea following administration of *M. gallisepticum* bacteria. Avian Dis., 29: 988-1003.

Tanner, A. C.; Megaki, T. T.; Avakian, A. P.; Barnes, H. J.; Ley, D. H. and Magonigle, R. A. (1993): A comparison of danofloxacin and tylosin in the control of induced *Mycolpasma gallisepticum* infection in broiler chicks. Avian Dis., 37 (2): 515-522.

Tayfun, K.; Kaan, O. and Elçin, G. (2008): Application of Mentofin® in broilers with clinical infectious bursal disease to reduce *Escherichia coli* related problems after vaccination against Newcastle Disease. Turk. J. Vet. Anim. Sci. 32 (1): 1-6.

Tully, J. G. (1983): Cloning and filtration techniques for mycoplasmas. Methods in Mycoplasmology, vol. 1 S. Razin and J. G. Tully, eds. Academic Press, New York, pp. 173-177.

Vancutsem, P. M.; Babish, J. G. and Schwark, W. S. (1990): The fluoroquinolones antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. Cornell Veterinarian, 80: 173-86.

Wille, A. Altay, G. and Erdem, B. (1988): The susceptibility of *Salmonella* spp to

various antibiotics. Microbiol Bul., 22 (1): 17-24.

Wise, D. R. and Fuller, M. K. (1975): Efficacy trials in turkey poults with tylosin tartrate against *Mycoplasma gallisepticum* and *Mycoplasma meleagridis*. Res. Vet. Sci., 19 (3):338-339.

Wolfson, J. S. and Murray, B. E. (1989): Values of new quinolones in the treatment and prophylaxis of infectious diseases. Eur. J. Clin. Microbiol. Infect. Dis., 8 (12): 1071-1074.

Wurges, J. (2001): Eucalyptus. In: Gale Group. Gale Encyclopedia of Alternative Medicine. Available at: http://www.findarticles.com/p/arti- cles/mi_g2603.

Yamamoto, R. and Adler, H. E. (1956): The effect of certain antibiotics and chemical agents on pleuropneumonia-like organisms of avian origin. Am. J. Vet. Res., 17: 538-542.

Yoder, Jr. H. W. (1984): *Mycoplasma gallisepticum* infection. In Diseases of poultry. 8th Ed. Edited by Calnek, B. W.; Beard, C. W.; Barnes, H. J.; Reid, W. M. and Yoder Jr. H. W. Iowa State University Press, Ames, Iowa.

Yoder, Jr. H. W. (1991): *Mycoplasma gallisepticum* infection. In Diseases of poultry. 9th Ed. Edited by Calnek, B. W.; Beard, C. W.; Barnes, H. J.; Reid, W. M. and Yoder Jr. H. W. Iowa State University Press, Ames, Iowa. pp.198-212.

Yoder, Jr. H. W. and Hofstad, M. S. (1965): Evaluation of tylosin in preventing egg transmission of *Mycoplasma gallisepticum* in chicken. Avian Dis., 9: 291-301.

Zakay-Rones, Z.; Thom, E. and Wollan, T. (2004): Randomized study of the efficacy and safety of oral elderberry extract in the treatment of influenza A and B virus infections. J. Intern. Med. Res., 32:132-140.

Zenebergh, A. and Trouet, A. (1982): Cellular pharmacokinetics of spiramycin in cultured macrophages. Ann. Immunol (Paris), 133 (3): 235-244.