ASSESSMENT 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 intranasal with 0.4 ml of MG culture containing 10⁵ 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 fluoroquinolons alone or in combination with old
antimycoplasma drugs like macrolides and tetracyclins 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 spiramycin, 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 adlibitum 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 molecularly 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 37°C 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 principles:

- 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 acetyyle 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 0-4°C for up to 48 hours. Subcultures were made on solid media and the plates were incubated at 37°C 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 50S 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.

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<th>Group number</th>
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<th>Mean clinical score ± SD</th>
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<tr>
<td>1</td>
<td>Blank control</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Infected control</td>
<td>1.78±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.30±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Pefloxacin</td>
<td>1.86±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.45±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Tylosin</td>
<td>1.70±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.55±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Spiramycin</td>
<td>1.75±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.58±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>Volatile oils</td>
<td>1.81±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.54±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-d</sup> 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.

<table>
<thead>
<tr>
<th>Group number</th>
<th>Group treatment</th>
<th>Mortalities at intervals/days</th>
<th>The total mortalities (15-35)days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>15-21</td>
</tr>
<tr>
<td>1</td>
<td>Blank control</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Infected control</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Pefloxacin</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Tylosin</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Spiramycin</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Volatile oils</td>
<td>30</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>*</sup>No.= Number of birds/total number of birds in the group.
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.

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

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.

<table>
<thead>
<tr>
<th>Group number</th>
<th>Group treatment</th>
<th>Re-isolation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intervals of age/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>1</td>
<td>Blank control</td>
<td>0/5</td>
</tr>
<tr>
<td>2</td>
<td>Infected control</td>
<td>6/6</td>
</tr>
<tr>
<td>3</td>
<td>Pefloxacin</td>
<td>3/6</td>
</tr>
<tr>
<td>4</td>
<td>Tylosin</td>
<td>3/5</td>
</tr>
<tr>
<td>5</td>
<td>Spiramycin</td>
<td>4/6</td>
</tr>
<tr>
<td>6</td>
<td>Volatile oils</td>
<td>4/6</td>
</tr>
</tbody>
</table>

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