In vitro and *In Vivo* Evaluation Of Some Antimicrobials Activity Against *Mycoplasma Gallisepticum* Isolates From Day Old Chicks In Egypt

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ABSTRACT

The present study was conducted to compare the *in vivo* and *in vitro* activities of some antimicrobial agents against *Mycoplasma gallisepticum* strains (MG) isolated from day old chicks .For this aim, fifty broiler chickens, 6-weeks old serologically and culturally negative for MG and MS, were divided into 5 equal groups reared separately. The first, third, fourth and fifth groups were experimentally infected via intra tracheal route with MG strain (Eis-C3-091, 0.2ml (10^9) for each bird. While, the second group was kept as negative control. After the infection has been detected, the birds were treated with tilmicosin, tiamulin and tylosin for three successive days. The birds were weighted and tracheal swabs were collected for re-isolation and PCR. Tilmicosin gave the lowest Minimum inhibitory concentrations (MICs) (0.009, 0.009 and 0.018 µg/m) while tiamulin gave 0.037, 0.075 and 0.150 µg/m and tylan gave higher MICs than the previous two antimicrobials 0.075, 0.150 and 0.310 µg/ml for the isolate 1,2 and 3 respectively. Treatment with Tilmicosin resulted in the highest mean body weight (2.410g) during three weeks post-treatment which was significantly at $P< 0.05$ higher than the mean body weight of positive control group. Tiamulin and Tylosin had equal body weight (2.370 g) which was higher insignificantly than the positive control mean at $P< 0.05$. Mycoplasma couldn't be recovered from the group treated with tilmicosin starting from the first week post-treatment until the end of experiment.

INTRODUCTION

Mycoplasma gallisepticum (MG) infects approximately 85% of the commercial table egg layer chickens in US, once infected, chickens remain infected for life and no antibiotic is effective. It s presence is estimated to cost the industry in excess $140 million annually *(1).* MG infection continues to be an important cause of loss in poultry production due to the loss due to MG; these were a reduction in egg production of 10-20%, an increase in embryo and chick mortality of 5-10% and a reduction in weight gain and feed conversion efficiency 10-20% *(2).* *MG* infection commonly induces chronic respiratory disease in chickens *(3).* The clinical signs include nasal discharge, sneezing coughing, tracheal rales and mild conjunctivitis *(4).* *MG* infection is transmitted both horizontally and vertically and it remains in the flock constantly as sub clinical form *(5).*

Control of avian mycoplasmosis by vaccination is limited as only few vaccines are available. Total eradication through test and slaughter is the most effective control method; the emergence of multiage complexes in the commercial layer industry makes this approach impractical *(6,7).* Therefore the control of MG infection in broiler breeder by chemotherapy is the most practical way to minimize economical losses.

Many antimicrobial agents, such as macrolides an lincosamides (e.g., tylosin, spiramycin, linomycin and clindamycin), tetracyclines, tiamulin and fluoroquinolones (e.g., enrofloxacine and danofloxacine) have been shown to possess *in vitro* activity against various veterinary mycoplasmas. *(8).*

The *in vitro* activates of eight antimicrobial agents were tested against the three MG strains(previously isolated from day old chicks) *(9).*Three of them having the lowest MIC (tilmicosin,tiamulin and tylosin 0.009,0.037 and 0.150 respectively) were used for the in vivo study.

So the aim of this work is to compare the *in vitro* and in vivo efficiency of these antimicrobial agents against MG infection.

MATERIAL AND METHODS

I. Samples

Three MG field isolates were subjected to antibiotic sensitivity test and the more effective three antimicrobials (tilmicosin, tiamulin and tylosin) were used for *in vitro* evolution.

II. Determination of Minimal inhibitory concentration (MIC)

Micro-broth method: tests were performed in duplicate exactly as previously described *(10)*. The antimicrobials were tested in serial two-fold dilutions at concentrations ranging from 16 to 0.008µg/ml and tests were repeated if the back-titration of CCU was carried out alongside and did not fall in the required range of 10^3 to 10^4/0.2ml.

III. Isolation and identification of *MG*

1. Liquid and solid media were prepared and used for isolation and propagation of Mycoplasma *(11)*.
2. Genus determination and biochemical characterization were carried out *(12)*.

IV. Experimental design

Fifity broiler chickens 6-weeks old serologically and culturally negative for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* were divided into 5 groups 10 of each in separated units. The birds were provided with chick starter mash from which antibacterial additives has been omitted but containing maduramycin as anticoccedial.

After 1 week, 4 groups were challenged intra-tracheal with 0.2ml (10^9CFU) of *Mycoplasma gallisepticum* isolate (Eis-C3-091, Gene bank accession number Gu134346).

Group 1: positive control.

Group 2: negative control.

Group 3: treated with tilmicosin of concentration 250mg/ml in drinking water, (Mycosin-sigma pharmaceuticals industries lot No 080808) with dose 1ml/3 liter for 3 days.

Group 4: treated with tiamulin 45%W.S.P of concentration 55.55gm/100gm tiamulin hydrogen fumarate equivalent to 45gm tiamulin base (Arabcomid company, B. No 0101/08) with dose 1gm/1.5 liter for 3 days.

Group 5: treated with tylosin 100% (tylofort W.S.P contain tylosin tartarate 100gm, unifarm company, lot No 320309) with dose 0.5gm/liter for 3 days.

Tracheal swabs were collected and placed onto Frey's Mycoplasma broth medium for incubation at 37°C. All culture tubes showing evidence of growth (color change from red to yellow) were then inoculated onto agar and incubated at 37°c for 3-5 days. The remaining culture tubes were inoculated into agar after 5 days of incubation. All broth media were subjected to PCR after 7, 15, 21 days from the last treatment. Mortality and body weight gain were recorded with each sampling.

V. Polymerase chain reaction (PCR)

1. **Extraction of chromosomal DNA *(13)*:** A five ml of overnight culture from each Mycoplasma isolate was centrifuged in a microcentrifuge at 13000 r.p.m. for 3 minutes. The cell pellets were washed twice in 100 µl of 150 mM phosphate-buffered saline (PBS, pH 7.2) and suspended in 25 µl PBS. The cell suspension was heated directly at 100 °C for 10 minutes in a heat block and collected on ice for 10 minutes. Finally, the cell suspension was centrifuged for 3 minuets, and chromosomal DNA was collected and stored at 4°C.
2. **Primer selection *(14)*.** Two specific oligonucleotide primers were selected for the detection of MG. The sequence of primer (F) was: 5\- GCT TTG TGT TCT CGG GTG CTA-3\. The sequence of primer (R) was: 5\- CGG TGG AAA ACC AGC TCT TG-3\. .
3. **Procedure for DNA amplification:** The reaction mixture (total volume 50 µl) was 5 µl of 10 X reaction buffer (Applied Biosystem), 1.5 µl 25 mM MgCl2, 1 µl of nucleotides mix(10 mM),(sigma),DNA 5 µl (containing 400 ng of each forward and reverse primers),0.5 µl (2 units of taq DNA polymerase (Applied Biosystem)was added and the mixture was completed by ultra pure

distalled water to 50µl . PCR was performed on progene (programmable thermal controller (UK). Amplification was performed by heating the sample for 3 minutes at 94°C .After this step 40 cycles were performed as follows: denaturation for 20 sec.at 94°C, annealing for 40sec. at 55°C and extenuation for 1 minute at 72°C and a final incubation at 72°C for 5 minutes. The analysis of PCR amplified products was done by using 8 µl of amplified PCR products, mixed with 2 µl loading dye and electrophoresed through 1% agarose gel and DNA was visualized by UV fluorescence after ethedium bromide staining and then photographed.

4. **Primer, *(13).***

The oligonucleotide primer used in this study was M16SPCR5. The M16sPCR5`, primer was based on the sequence of 16SrRNA of MG (Gene Bank Acc. No. M22441). Lists the sequence, size, guanine plus cytosine content, and melting temperature of this primer. Base sequence and size of the arbitrary primer used:

Primer	base sequence	No. of bases	G+C %	Meltin point
M16sPCR5`	5'AGGCAGCAGTAGGGAAT3'	17	52.9	43.7 °C

G + C =Guanine plus cytosine.

5.Amplification condition

The reaction mixture (total volume 50 µl) was 10ml of 10 x reaction buffer (promega), 8 micro-liter, 25mM $MgCL_2$, 16 µl of 10 mM of each nucleotide (dATP, dCTP, dGPT and dTTP, sigma, 2 µl primer (containing 400 ng of each left and right primer), 5 µl DNA template (containing 40 ng DNA), 0.5 µl (2units) of tag DNA polymerase (promega) and complete the mixture with distilled water. PCR was performed on a PTC-100 programmable thermal cycler controller (M.J. Research Inc.) The amplification conditions was three cycles of 94°C for 15 seconds, 28°C for 2 minutes and 74°C for 3 minutes and for 35 cycles of 94°C for 15 sec, 45°C for 2 minutes and 74°C for 3 minutes.

RAPD patterns analysis was performed by Image analysis by Image Quant TL-2003 software of Amersham Bioscience. Each RAPD analysis gel was standardized by comparison of *Mycoplasma gallisepticum* unknown isolates to reference strains. Isolates were considered identical when major differences could not be visualized.

RESULTS

Table 1. Minimum inhibitory concentrations (MICs µg/ml) for the eight antimicrobials against three different MG field isolates used.

Antibiotics	Isolate 1	Isolate 2	Isolate 3	C-max
Doxycyclin	0.627 *	2.500	10.000	4.47
Enrofloxacin	1.250	0.310	1.250	2.44
Erythromycin	0.009	20.000	2.500	2.44
flurophenicol	1.250	5.000	2.500	3.50
Spiramycin	0.009	1.250	0.310	1.3
Tilmicosin	0.009	0.009	0.018	2.09
Tiamutin	0.037	0.075	0.150	1.70
Tylosin	0.150	0.075	0.310	2.09

*= µg/ml

Table 1 revealed that the Doxycyclin, Erythromycin and Flurophenicol were not effective. They had higher minimum inhibitory concentrations (10 µg/ml "for isolate 3", 20 µg/ml "for isolate 2" and 5 µg/ml "for isolate 2" respectively than their C-max. The other five antibiotics were effective and had lower MIC than C-max.

In the present study, we chosen the best three antibiotics (for *In-vivo* treatment) Tilmicosin, Tiamutin and Tylan which their MICs didn't exceed 0.310 µg/ml for Tylan against "isolate 3" and we excluded Enrofloxacin and Spiramycin which had higher MICs (1.250 µg/m) against isolate 1 and 3 (in case of Enrofloxacin) and isolate 2 (in case of Spiramycin).

Tilmicosin gave the lowest MICs (.009, .009 and .018 µg/ml) for the isolate 1,2 and 3 respectively while tiamulin gave 0.037, 0.075 and 0.150 µg/ml and tylan gave higher MICs than the previous two antimicrobials (0.075, 0.150 and 0.310 µg/ml).

Table 2. Body weight gain for positive and negative control groups and different groups treated with Tilmicosin, Tiamulin and Tylosin.

Group	1st week	2nd week**	3rd Week***	4th week	5th week
Positive control	1.195*	1.420	1.850	2.140	2.310
Negative control	1.210	1.490	1.950	2.350	2.520
Tilmicosin	1.280	1.480	2.120	2.450	2.650
Tiamulin	1.150	1.390	2.100	2.410	2.610
Tylan	1.240	1.420	2.150	2.380	2.570

*= Average body weight for the group **= First week post-challenge
***= First week post-treatment

There was an obvious increment in body weight gain in all of the groups treated with antimicrobials than positive control and there were significant differences between the groups at P< 0.05 and P< 0.01(by ANOVA test) Table (2).

Treatment with Tilmicosin resulted in the highest mean body weight (2.410g) during three weeks post-treatment which was significantly at P< 0.05 higher than the mean body weight of positive control group. Tiamulin and Tylosin had equal body weight (2.370 g) which was higher insignificantly than the positive control mean at P< 0.05.

Table 3. Culture re-isolation and PCR results for different groups

Group	No. of birds	1stWeek Preinfection		2nd week**		3rd Week***		4th week		5th week	
		Cult.	PCR	Cult.	PCR	Cult.	PCR	Cult.	PCR	Cult.	PCR
Positive control	10	0	-*	9	+	8	+	7	+	7	+
Negative control	10	0	-	0	-	0	-	0	-	0	-
Tilmicosin	10	0	-	9	+	0	-	0	-	0	+
Tiamulin	10	0	-	8	+	0	-	5	+	5	+
Tylosin	10	0	-	9	+	0	-	6	+	5	+

*= Every group (10 samples) were pooled and tested as one sample
= First week post-challenge *= First week post-treatment

The recovery of *Mycoplasma gallisepticum* from chickens as in table (3) Mycoplasma couldn't be isolated from any of the groups before infection as well as from uninfected group throughout the experiment.

In the infected non treated group, MG was re-isolated from 9 out of 10 birds a week post-challenge, (8,7and 7 out of 10) at two weeks, three weeks and four weeks post-challenge respectively.

Mycoplasma couldn't be recovered from the group treated with tilmicosin starting from the first week post-treatment until the end of experiment.

In group treated with tiamulin, MG was re-isolated from 5 birds out of 10 and PCR was positive at the second and third week post treatment.

In group treated with tylosin, MG was re-isolated from 6 and 5 out of 10 at the second and third week, respectively.

Slight respiratory signs were observed in 2 birds out of 10 in infected un treated group two weeks post infection.

Table 4. The effect of antimicrobials on mortality and morbidity rates of chicks infected with Mycoplasma gallisepticum (n=10).

Group	Mortality within period (days)			Morbidity rate (days)		
	1-7	8-15	16-21	1-7	8-15	16-21
Infected untreated	1	0	0	0	2	0
UnInfected untreated	0	1	0	0	0	0
Tilmicosin treated	0	0	0	0	0	0
Tiamulin treated	0	0	1	0	0	3
Tylosin treated	0	0	0	0	0	0

The mortality in each group is shown in table (4).

One case died in control group(untreated uninfected) with severe congestion in all organs, may be due to coli septicemia in the period between 1-7 days.

One case died 8-15 day post infection in infected untreated group with liver congestion, tracheitis and airsacculitis.

At the period from 16-21 days post treatment in group treated with tiamulin with slight tracheitis and severe congestion in all internal organs in PM.

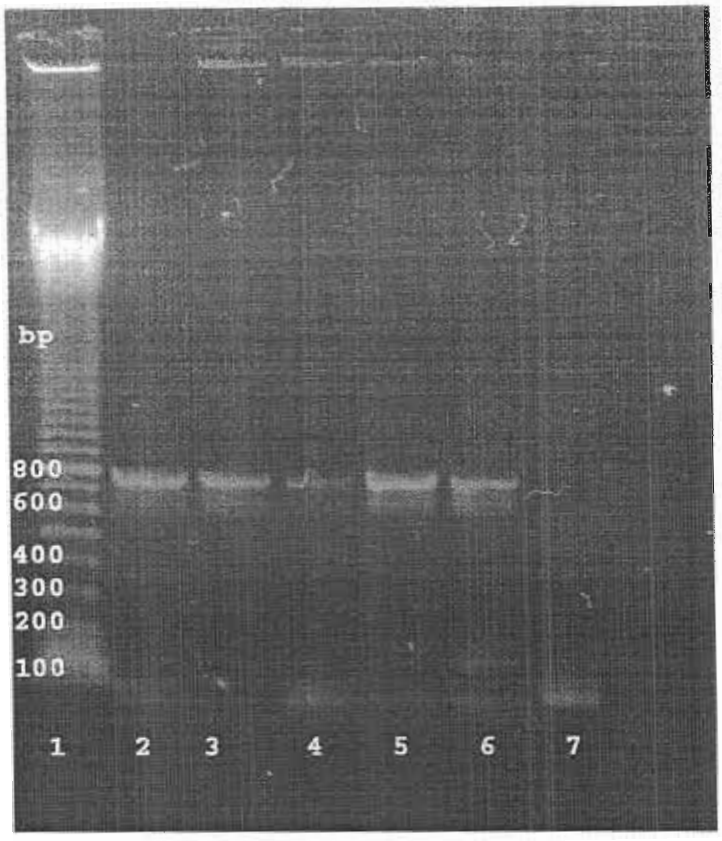

Fig. 1. PCR of *Mycoplasma gallisepticum* before and after challenge

1-100 bp DNA Ladder 2-MG field strain pre-challenge 3-MG re-isolated group1
4-MG re-isolated group4 5-MG re-isolated group5 6-MG field strain pre-challenge 7-Control negative group

Fig. 1. *Mycoplasma gallisepticum* field strain (Eis-C3-091, Gene bank accession number Gu134346) was used for challenge of the different groups and then re-isolated from group1 (positive control group), group4 (tiamulin treated group) and group5 (tylosin treated group). The figure showed that all of the strains original strain and re-isolated strains) were positive by PCR (gave band at 824 bp).

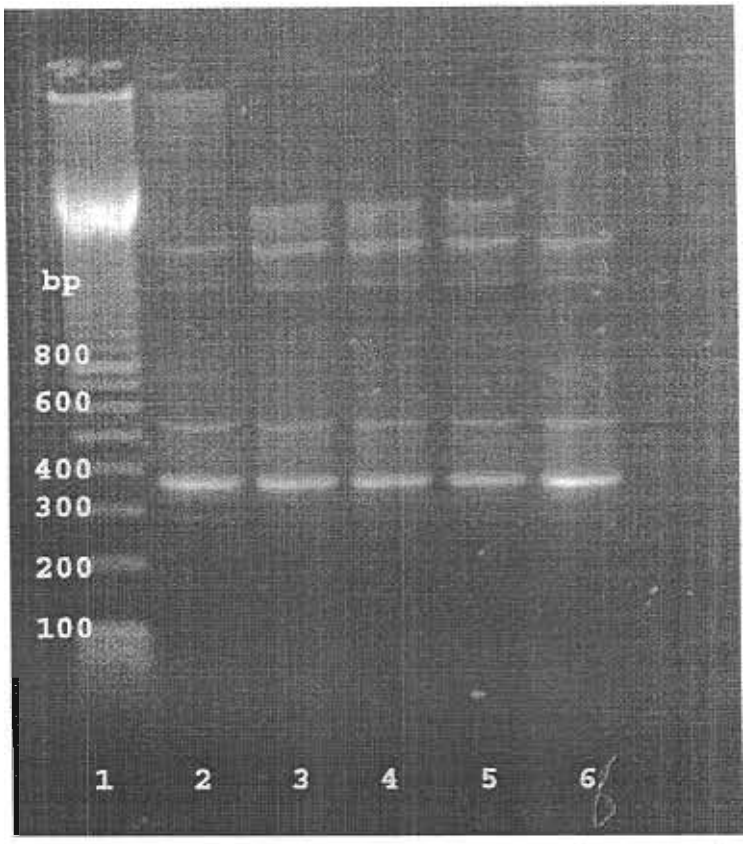

Fig. 2. Electrophoretic pattern of *Mycoplasma gallisepticum* before and after challenge 1-100 bp DNA Ladder 2-MG field strain pre-challenge 3-MG re-isolated group1 4-MG re-isolated group4 5-MG re-isolated group5 6-MG field strain pre-challenge

Table 5. Common and characteristic bands among MG field strain before infection and the reisolated strain.

	M. G field strain	Re – isolated *M. gallisepticum*		
		1	2	3
No. of bands	9	6	6	6
Common bands	5	5	5	5
Characteristic DNA bands	383, 630, 755 and 855	2700	2700	2700

Figure (2) and Table (5) in which the RAPD-PCR profile of the pre-challenge and re-isolated MG strains are present declared that *M. gallisepticum* field isolate used for experimental infection showed DNA bands ranged from 283 - 2100 bp. The re – isolated strain shared in five bands with the field isolate strain before infection, while four bands were characteristic (383, 630, 755 and 855 bp) which disappeared after infection. On the other hand, the isolated *MG* from all groups of the experiment shared characteristic band at 2700 bp which appeared after infection.

DISCUSSION

In the present study, the Minimal inhibitory concentration (MIC) of eight antimicrobials agents Doxycyclin, Enrofloxacin, Erythromycin, Flurophenicol, Spiramycin, Tilmicosin, Tiamutin and Tylosin were assessed for three field isolates in the first part of the experiment (*in-vivo* testing of the antimicrobials).

Tilmicosin, Tiamulin and Tylosin had lower MICs than other antimicrobials and were chosen for *in-vitro* treatment. Tilmicosin had the lowest MIC results followed by tiamulin and tylosin. Study the MIC of tylosin and tilmicosin for six MG strains showed that, the MIC of tilmicosin was slightly low for four MG strains than did tylosin and for the remaining two MG strains MIC for tilmicosin was equal to or less than tylosin ***(13)***. Also our results agreed with most workers who reported lower MIC for tiamulin than for tylosin ***(9,15, 16)***.

In-vivo experiment was performed using five groups (positive control, negative control, tilmicosin treated, tiamulin treated, and tylosin treated groups in which the chickens subjected to challenge (after one week) and treatment (after two weeks) and were tested for body weight gain and reisolation.

Evaluation of the antimicrobials by estimating body weight gain revealed that Treatment with Tilmicosin resulted in the highest mean body weight (2.410g) during three weeks post-treatment which was significantly at $P< 0.05$ higher than the mean body weight of positive control group. Tiamulin and Tylosin treated groups had equal body weight (2.370 g) which was higher in-significantly than the positive control mean at $P< 0.05$.

A greater mean body weight was seen in the group taken 0.5gm/liter of tilmicosin compared with the tylosin treated group ***(13)***.

The efficacy of the different antimicrobials against re-isolation of MG declared also that Mycoplasma couldn't be recovered from the group treated with tilmicosin starting from the first week post-treatment until the end of experiment except in the 3rd week post treatment which gave positive results by PCRonly.This could be explained with the fact that PCR detects DNA from viable and non viable Mycoplasmas, however, only viable Mycoplasmas should be considered as a potential source of infection ***(17)***.

On the other hand in groups treated with tiamulin and tylosin, MG was re-isolated at the second and third week post- treatment with concomitant positive PCR. Nneither tylosin nor chlorotetracycline(CTC) prevented infection of the birds, but both reduced the count of MG challenge strain ***(18)***, also this results support the published result in which continuous feeding of tylosin and CTC failed to eliminate MG infection ***(19)***.

The obtained results suggests that tilmicosin was more effective in preventing re-isolation than tiamulin and tylosin and this is in co-ordination with the previously studies ***(20, 21)***.

The original field isolates and the re-isolated strains were tested by PCR, (except group3 which was treated with tilmicosin and no re-isolation could be done) and give a characteristic band at 824 bp ***(14)***.

REFERENCES

1.Vance, A M , Branton, S l , Collier, S D, Grard, P D , Peebles, E D (2008): Effects of prelay TS-11 strain *Mycoplasma gallisepticum* inoculation and time specific F-strain M.gallisepticum inoculation overlay on internal egg& egg shell characteristics of commercial laying hens. Poultry science 87:1358-1363.

2.Kleven,S H (1998): Mycoplasmas in the etiology of multifactorial respiratory disease.poultry sci.,77(8)1146-1149.

3.Ley, D H (2003): *Mycoplasma gallisepticum* Infection. In: Saif, Y.M., H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald and D.E. Swayne (Eds.). Diseases of Poultry. Eleventh ed. Ames. Iowa, Iowa State Press A Blackwell Publishing Company, USA., pp: 722-74.

4.Kleven, S H (2003): *Mycoplasma synoviae* Infection. In: Saif, Y.M., H.J. Barnes, J. R. Glisson, A.M. Fadly, L.R. McDougald and D.E. Swayne (Eds.), pp: 756-766.

5.Bencina, D, Tadina T and Dorrer, D (1988): Natural infection of geese with *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and egg transmission of the Mycoplasmas. Avian Pathol., 17: 925-928.

6.Evans, R D and Hafez, Y S (1992): Evaluation of a *Mycoplasma gallisepticum* strain exhibiting reduced virulence for prevention and control of poultry mycoplasmosis. Avian Dis., 36: 197-201.

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

8.Bradbury, J M , Yavari, C A and Giles C J (1994): *In vitro* evaluation of various antimicrobials against *Mycoplasma gallisepticum* and Mycoplasma synoviae by the micro-broth method and comparison with a commercially prepared test system Avian pathol.23:105-115.

9.Eissa, S I , El-shater, S A , Dardeer, M A , AbdElaziz, E E ,Hanaa, A Ahmed and Anne, V Gautier-Bouchardon (2009): Detection of *Mycoplasma gallisepticum* infection in day –old chicks using molecular characterization. 5th international poultry conference 10-13 March 2009.Taba-Egypt.

10.Senterfit, L P (1983): Antibiotic sensitivity testing of mycoplasmas .In: Tully, J.G.& Razin, S. (Eds) Methods in mycoplasmology, Volume II,pp.397-401 (New York, Academic Press Inc.)

11.Frey, M C , Hanason, R P and Anderson, D P (1968): A medium for the isolation of avian mycoplasmas. Ann. Vet. Res., 29: 2164-2171.

12.Erno, H and Stipkovits, L (1973): Bovine mycoplasmas, cultural and biochemical studies. Acta. Vet. Scand., 14: 450-463.

13.Fan, H H , Kleven, S H and Jackwood, M W (1995): Application of polymerase chain reaction with arbitrary primers to strain identification of *Mycoplasma gallisepticum*. Avian Dis.39:729-735.

14.Ferguson, N M , Hepp D , Sun S , Ikute, N , Levishon, S , Kleven, S H , and Garcia, M (2005): Use of molecular diversity of *Mycoplasma gallisepticum* by gene-targeted sequencing (GTS) and random amplified polymorphic DNA (RAPD) analysis for epidemiological studies. Microbiology, 151:1883-1893.

15.Anonymous (1981): Guide to chemotherapy. Isr.J.Med.Sci.17:661-666.

16.Hannan, P C T, O'Hanlon , P J and Rofers, N H (1989): *In vitro* evaluation of various quinolones, Antibacterial agents against veterinary mycoplasmas and porcine respiratory bacterial pathogens. Res. Vet. Sci. 46:203-210.

17.Marois,C , Fabianne, D and Kempf, I (2002): Polymerase chain reaction for detection of *Mycoplasma gallisepticum* in environmental samples.Avian pathology,31(2):163-168.

18.Cummings,T S , Kleven S H and John Brown (1986): Effect of medicated feed on tracheal infection and population of Mycoplasma in chickens. Avian Dis.30:580-584.

19.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.

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

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

الملخص العربى

دراسة معملية و على الطائر الحي لتقييم تاثير بعض المضادات الميكروبية على معزولات الميكوبلازما جاليسبتكم من كتاكيت عمر يوم.

صبرى إسماعيل عيسى، منسى أحمد عبدالحميد ،عبد الواحد محمود حسن ،عيد السعيد عبدالعزيز ، نبيل مصطفى الزفتاوى

قسم الميكوبلازما- معهد بحوث صحة الحيوان- الدقى

أجريت هذه الدراسة لمقارنة فعالية بعض المضادات الميكروبية على عترات الميكوبلازما جاليسبتكم المعزولة من كتاكيت عمر يوم.و لهذا تم استخدام عدد ٥٠ كتكوت تسمين عمر ٦ أسابيع قسمت الى خمسة مجموعات بعد التأكد من خلوها من الميكوبلازما جاليسبتكم و الميكوبلازما سينوفى و ذلك سيرولوجيا و بالعزل الأولى. أجريت العدوى الصناعية باستخدام عترة حقلية للميكوبلازما جاليسبتكم (Eis –C3-091) و ذلك بحقن ٠,٢مل (10^9) لكل طائر.بينما المجموعة الثانية أخذت كضابط سلبى للتجربة. بعد التاكد من حدوث العدوى للمجموعات المحقونة تم استخدام ثلاث مضادات ميكروبية (التلميكوسين-التيامولين و التيلوسين)و ذلك بعد التأكد من تميز هذه المضادات معمليا على العترات المعزولة من كتاكيت عمر يوم حيث تم علاج المجموعات الثالثة و الرابعة و الخامسة على التوالى لمدة ثلاثة أيام بالجرعات المستخدمة تجاريا.

أثبتت التجربة أن المجموعة الثالثة(المعالجة بالتلميكوسين) أعطت زيادة معنوية فى الوزن و ذلك بمقارنتها بالمجموعة الايجابية للتجربة.المجموعتان الرابعة و الخامسة(المعالجة بالتيميولين و التيلوسين) لم يسجل بهما زيادة معنوية فى معدل الوزن. بالنسبة لإعادة العزل لم يتم عزل ميكروب الميكوبلازما من المجموعة الثالثة طول فترة التجربة بينما كانت هذه المجموعة ايجابية باختبار انزيم البلمرة المتسلسل فى طائر واحد(١٠%) و ذلك فى الأسبوع الثالث بعد العلاج.

بالنسبة للمجموعة الرابعة كانت ايجابية لإعادة العزل بنسبة ٥٠% باختبار انزيم البلمرة المتسلسل و ذلك فى الأسبوع الثاني و الثالث بعد العلاج. أما المجموعة الخامسة فكانت ايجابية بنسبة ٥٠-٦٠% على التوالى.