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SEROLOGICAL STUDIES ON MYCOPLASMA GALLISEPTICUM INFECTION IN CHICKENS

(With 12 Tables)

By

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دراسات سيرولوجيه على العدوى بالميكوبلازما جاليسيبتكم فى الدجاج

أبوالخير محمد ابراهيم عيسوي ، حامد عبدالمجيد الامام شلبي ، خالد عبدالرحمن الغريب ديب ، محمود حمدى محمد حامد شلبي

30 كتكوت عمر يوم خاليه من الميكوبلازما قسمت الى 6 مجاميع (كل منها 5 كتاكيت). المجموعه الأولى تركت كضابط بدون اى معاملات، والثانيه أخذت العدوى وتركت كضابط أيضا، والثالثه أخذت العدوى عند عمر 16 يوم وتم علاجها بالأجسام المناعيه المستخلصه من مح الدواجن المحصنه ضد الميكو بلازما جاليسيبتكم (المناعه السلبيه)، والمجموعه الرابعه أخذت أجسام مناعيه مختصه بالميكوبلازما جاليسيبتكم ثم أخذت العدوى عند عمر 16 يوم ثم عولجت بالأجسام المناعيه، والخامسه تم تحصينها بالقاح الحى المضعف (العتره- اف) ثم أخذت العدوى عند عمر 16 يوم، والمجموعه السادسه أعطيت مضاد حيوى (انروفلوكساسين) فى عمر 1-5 يوم ثم أخذت العدوى عند عمر 16 يوم ثم أخذت جرعه اخرى من المضاد الحيوى مرة اخرى عند عمر 20-25 يوم. تم تجميع عينات دم فى نهاية الأسبوع الأول والثانى والثالث والخامس من المجاميع الأولى والرابعه والخامسه والسادسه وفى نهاية الأسبوع الثالث والخامس من المجموعتين الثانيه والثالثه. وذلك لعمل الأختبارات السيرولجيه والبيوكيميائه والعد النوعى لكرات الدم. العدوى بالميكوبلازما جاليسبتيكم (المجموعه الثانيه) أظهرت زيادة معنويه فى العدد الكلى والنوعى لكرات الدم البيضاء، زياده معنويه فى انزيمات الأسبرتيت والألنين أمينوترانسفيريز والفوسفتيز القلوى والبروتين الكلى والجلوبيولين وحامض اليوريك ونقص فى الألبيومين ولكن الكرياتينين لم يتأثر. وظهرت استجابه مناعيه منذ اليوم الخامس من العدوى وحتى نهاية التجربه وذلك باستخدام الاليزا كما أعطى اختبار التلازن 5/3، 5/5 فى الاسبوع الثالث والخا مس. وبفحص الأجزاء الداخليه أظهر وجود احتقانات بالأوعيه الدمويه للرئتين مع وجود بؤر من الألتهابات والتهاب فيبرينى أو مصلى بالأكياس الهوائيه. وفى المجموعة الرابعة (استخدام الأجسام المناعيه المستخلصة من بيض الدجاج المحصن ضد الميكو بلازما جاليسبتيكم "المناعة السلبية" قبل العدوى) لم يحدث تأثيرا معنويا على العد الكلى والنوعى لكرات الدم البيضاء ووظائف الكبد والكلى، ماعدا وجود زيادة معنوية فى البروتين الكلى والجلوبيولين فى الأسبوع الثانى من العمر.

حدثت استجابة مناعية نتيجة لحقن الأجسام المناعية باستخدام الآليزا. كما أعطى اختبار التلآزن صفر/5 في الأسبوع الأول والثانى. وباستخدام الأجسام المناعية فى الدجاج المعدى بالميكوبلآزما كانت فى الأسبوع الثالث مماثله للمجموعه الثانية وفى نهاية الأسبوع الخامس عادت كل الأختبارات الى الحد الطبيعى ماعدا وجود زيادة فى البروتين الكلى والجلوبيولين. وكانت الآليزا ايجابية فى الأسبوع الثالث والخامس. وكان اختبار التلازن 5/3 ،5/2 في الثالثة والرابعه فى الأسبوع الثالث على التوالى بينما كانت 5/1 فى الأسبوع الخامس بالنسبة للمجموعتين. بالنسبة للمجموعة الخامسة المحصنه باللقاح الحى (العترة-F) وجد بها زيادة فى عدد كرات الدم البيضاء طوال فترة التجربة ولم يحدث تأثير معنوى على وظائف الكبد أو الكلي ما عدا زيادة البروتين الكلى والجلوبيولين. وأوضحت الآليزا وجود مستوى عالى ومتزايد من الأجسام المناعية ابتداء من الأسبوع الثانى وحتى الأسبوع الخامس. وأوضح اختبار التلآزن صفر / 5- 5/1 – 5/4 -5/5 في الأسبوع الأول والثانى والثالث والخامس على التوالى. وفى المجموعة السادسة فكان استخدام الانروفلوكساسين فى الاسبوع الأول أدى الى نقص معنوى فى كرات الدم البيضاء فى الآسبوع الأول بينما وجد زيادة معنوية فى انزيمات الكبد وحمض البوليك والكرياتنين ولم يحدث أى تأثير على البروتين الكلى والألبيومين والجلوبيولين. وكانت الآليزا سلبية واختبارالتلازن صفر / 5 فى الأسبوع الأول والثانى على التوالى. أما بالنسبة لاستخدام الانروفلوكساسين فى الدجاج المعدى بالميكوبلازما جاليسيبتكم ففى الاسبوع الثالث من العمر كانت التغيرات مثل المجموعة الثانية. ولكن فى الاسبوع الخامس رجعت كل الاختبارات الى معدلاتها الطبيعية ما عدا زيادة فى البروتين الكلى والجلوبيولين. الاليزا كانت ايجابية واختبار التلازن أعطى 5/3 -5/1 فى الاسبوع الثالث والخامس على التوالى. وتلاحظ وجود بقع نزيفية فى الرئتين مع وجود سائل مصلى فيبرينى. مما سبق نستنتج أن العدوى بالميكو بلازما تؤدى الى تغيرات مرضية فى كل من الدم والكبد والرئتين .وان استخدام كل من المناعة السلبية أو التحصين أو المضاد الحيوى له القدرة على العلاج. وكان من أفضلهم اللقاح الحى حيث أنه أعطى مستوى عالى من المناعه ولم يؤثر على الجسم وتأتى بعد ذلك المناعه السلبية ثم العلاج بالمضاد الحيوى.

SUMMARY

30 One day old Mycoplasma free broiler were divided into 6 groups (grs) (each of 5 chicken). The 1st gr. used as control without any treatment, 2nd gr. was infected with Mycoplasma gallisepticum (M.G.) S6 via the thoracic air sac on the 16th day of age and left till the end of the experiment (as infected control gr.), 3rd gr. was infected on the 16th day then passively immunized by I/P injection of egg yolk antibodies (IgY), 4th gr. was passively immunized I/P by (IgY) and infected with M.G., then passively immunized, 5th gr. was vaccinated with the living vaccine (F-strain) of M.G. then infected with M.G.S6 and the 6th gr. was orally given the therapeutic dose of enrofloxacin in the drinking water for the 1st 5 days of age, then infected with M.G.S6 and the chickens were given another dose of enrofloxacin from 20th -25th day of age. Blood samples were collected from each bird in grs. (1, 4, 5 and 6) at the

end of the 1st, 2nd, 3rd and 5th weeks of age and from grs. (2 and 3) at the end of the 3rd and 5th weeks for serological, hematological and biochemical tests. The lungs, trachea and air sacs were collected for the isolation of M.G. The infected birds gr. (2) revealed Leukocytosis, heterophilia and monocytosis in the 3rd and 5th weeks of age. There was a highly signififent increase in the AST, ALT, AP, Total proteins, globulin and uric acid. The serum albumin was decreased, while the creatinine not significant changed. The ELISA showed positive values as early as the 5th day post infection (PI) till the end of the experiment. The result of serum plate agglutination test (SPA) illustrated 3/5 and 5/5 at the end of the 3rd and 5th weeks of the age respectively. The Pulmonary tissue was congested and showed focal pneumonic areas. The passively immunized birds of gr. (4), before infection, showed non-significant change in leukogram but there are a significant increase in the total protein and globulin at the end of the 2nd week. There was a positive result of the EILSA due to the injected (Ig). The SPA was 0/5 and 0/5 in the 1st and 2nd weeks of age respectively. The passively immunized and infected chickens grs. (3&4) showed similar results to gr. (2) at the end of the 3rd week. All the parameters returned to nearly the normal value in the 5th week of age except for a significant increase in the serum total protein and globulin. The ELISA showed a positive result in the 3rd and 5th weeks of age. The SPA showed 3/5 and 2/5in grs. (3&4) respectively in the 3rd week and 1/5 in the 5th week of age in both grs. The actively immunized birds of gr. (5) showed leukocytosis, heterophilia, lymphocytosis and monocytosis at the end of the 1st, 2nd, 3rd and 5th weeks of age. All the parameters were not significantly changed except the total proteins and globulin which showed highly significant increase. The ELISA was gradually increased from the 2nd till the 5th week of age. The SPA showed 0/5, 1/5, 4/5 and 5/5in the 1st, 2nd, 3rd and 5th weeks of age respectively. The enrofloxacin treated birds gr. (6) before infection especially at the end of the 1st week of age, showed leukopenia, heterophilia, lymphopenia and a significant increase in AST, ALT, AP, uric acid and creatinine. Meanwhile, the serum total proteins, albumin and globulin were non-significantly changed. The ELISA showed non-significant change. The SPA is 0/5in the 1st & 2nd weeks of age. The enrofloxacin treated birds gr. (6) after infection and at the end of the 3rd week of age showed similar hematological finding to gr. (2) at the end of the 5th week of age. All parameters nearly regained to their normal values except for a significant increase in the total proteins and globulin. The ELISA showed a significant increase. The SPA were 3/5 and 1/5 at

the end of the 3rd & 5th weeks respectively. The chickens which were sacrificed at the end of the 3rd week, revealed focal hemorrhage in the lung and serofibrinous exudates. It is recommended to vaccinate the chickens with F-strain living vaccine of M.G.

Key words: *Mycoplasma gallisepticum, chickens, immunology*

INTRODUCTION

M.G. infections can cause significant economic losses on poultry farms from chronic respiratory disease, reduce feed efficiency, decreased growth and decreased egg production. The carcasses of birds send to slaughter may also be down graded. M.G. infections are notifable to the World Organization for Animal Health (2007). M.G. infection vary from asymptomic to sever, depending on the infecting strains and other factors. More sever infections are seen when the birds are infected concurrently with New-Castle disease virus, infectious bronchitis virus *E. coli* or other pathogens World Organization for Animal Health (2007). M.G. is the most economically significant mycoplasma pathogen of poultry, and has a world wide distribution. Phenotypic variation of major surface antigens occurs at high frequency, which is probable explanation for chronic infection by M.G. despite of strong immune response (Levisohn and Kleven, 2000). The presence of M.G. predisposes birds to other infections which together causes mortality, poor growth and condemnation of the carcasses (Kempf *et al.,* 1992).

Efforts to reduce the effect of the disease on infected layers have included the use of antibiotics or vaccination (Robert *et al.,* 2001). Vaccination is an option for controlling M.G. when the security measures fail to prevent the infection of poultry with M.G. (Jordan, 1979). There are two types of vaccination, killed vaccines (bacterins) and living vaccines which include F-strain, TS-11 and 6/85 strain (Whithear, 1996). The most attractive application of IgY in the passive immunization therapy. At present several researchers reported that administration of IgY is quite effective in prevention of Rota viral diarrhea and enterotoxaemia E. coli infection (Yamamoto *et al.,* 1997). The antibodies have been used for over a century in the prevention and treatment of infectious diseases. Antibody treatment of the lower respiratory tract infection has a long history of success and is receiving renewed interest (Margaret and Richard, 2000). The yolk of eggs laid by immunized chickens has been widely recognized as an excellent source of polyclonal antibodies for over a decade (Jensenius and Koch, 1997).

The antibiotic therapy is another option for controlling losses associated with mycoplasmosis. Several therapeutic antibiotics with activity against mycoplasma were approved for use in poultry. Such antibiotics include Fluoroquinolone group which include enrofloxacin, amifloxacin, ciprofloxacin, danofloxacin, difloxacin, marboflxacin, ofloxacin and norfloxcin (Calnek *et al.*, 1997). Antimicrobial drugs are used in feed-producing animals to treat, prevent and control disease and to improve growth and feed efficiency. Fluoroquinolones are a modern group of antibiotics which are active against wide range of gram-negative, gram-positive and cytoplasma spp. (Abo El-Nile, 1997).

The present work was planned for evaluation of 3 methods, passive immunization (using egg yolk antibodies [IgY]) active immunization (using F-strain living vaccine) and treatment with antibiotics (enrofloxacin) to control M.G. infection. Such evaluation was based on the hematological, biochemical and immunological changes.

MATERIALS and METHODS

Antigen preparation of M.G.: It was prepared for vaccination of laying hens for production of antibodies specific for M.G. in egg yolk, for ELISA and Western-blotting techniques. It was prepared according to (Patten *et al.*, 1984).

Determination of protein content of crude antigen: It was determined to calculate the optimum dose of antigen for inoculation of laying hens for active immunization and concentration of antigen used for ELISA and Western-blotting techniques. It was estimated colorimetrically according to Lowry *et al.* (1951).

$$\text{Concentration} = \frac{\text{O.D. of sample}}{\text{O.D. of standard}} \times \text{concentration of standard}$$

Hens for production of egg yolk antibodies (IgY): 10 hens (5 month old) mycoplasma free used for the production of specific IgY against M.G. used for passive immunization, Separation and purification of IgY from chicken egg yolk. This procedure was done according to (Jensenius and koch, 1997). Sodium dodecyl sulphate – polyacrylamide Gel-electrophoresis (SDS-PAGE): It was done according to Towbin, *et al.* (1979) and Charmbach (1985) for the determination of the specificity of the produced IgY against M.G.

Experimental chickens: 32 one day old, mycoplasma free broiler chickens were obtained from Cairo Comp. for poultry. The chickens were housed in disinfected cages under controlled hygienic measures. They were feed on (starter, grower and finisher) free of mycotoxins and antimicrobial agents ration. All chickens were vaccinated with Hitchiner B 1 and Lasota for New-Castle disease (Main biological laboratories "mbl" USA) at the age of 7, 21 and 28 day and Bursal disease vaccine for Gumboro "mbl" at the age of 14 day via the drinking water. The mycoplasma free status of chicken was confirmed by serological examination of 2 chickens using serum slid agglutination test (Stipkovits, 1997).

Mycoplasma gallisepticum strain: The experimental infection was done by M.G.S6 (culture diagnostic lab. Corenell University Ithaco New York, USA, Each ml contain $3x10^7$ cfu).

Living vaccine (F-strain): M.G. live vaccine, avian isolate F-vax M.G. (Sherring Plough Animal Health Com.). It was used for active immunization of chickens in this study.

Drugs: Enrofloxacin was obtained from BAYER Com. the recommended therapeutic dose was 10 mg/kg.b. weight of drinking water for 3-5 day (Ahmed, 1996).

Experimental design: 32 one day old, Mycoplasma free broiler chickens were used. 2 chickens were used for confirmation of the mycoplasma free status and 30 chickens were divided into 6 groups (grs.) each of 5 chickens. The 1^{st} gr. kept as control (co.) without any treatment, the 2^{nd} gr. Kept as infected co. (infected with 0.1 ml M.G. broth culture containing $3x10^7$ cfu /ml (Stipkovits, 1997) at the age of 16 days via the posterior thoracic airsac), the 3^{rd} gr. were infected with M.G.as in gr. 2, then passively immunized intraperitonly (I/P) with IgY (0.7gm of egg yolk antibodies powder reconstituted in 0.5 ml PBS) at the age of 20, 24, 28 and 32 days, the 4^{th} gr. Were passively immunized with 0.5 ml of M.G. IgY on the 1^{st}, 5^{th}, 9^{th} and 13^{th} days of age, these chickens were infected on the 16^{th} day of age with M.G. then passively immunized on the 20^{th}, 24^{th}, 28^{th} and 32^{nd} days of age. The 5^{th} gr. were vaccinated with 0.02 ml of F-strain living vaccine of M.G. on the 1^{st} day of age ($1x10^5$ cfu /ml) via intranasal (I/N) installation (Rodriguez and Kleven, 1980; Kleven, *et al.*, 1984), those chickens were infected on the 16^{th} day of age with M.G.. The 6^{th} gr. were orally administered the therapeutic dose of enrofloxacin in drinking water for the 1^{st} 5 days of age, then infected on the 16^{th} day of age with M.G. and then given another dose of enrofloxacin (1 ml / 2 liter) from 20^{th} -25^{th} day of age.

Blood samples were collected at the end of 1st, 2nd, 3rd and 5th weeks of age in grs. 1, 4, 5 and 6 while in grs. 2 &3 the samples were collected at the end of the 3rd & 5th weeks only. 2 blood samples were collected from each bird. The 1st samples were used for hematological (leukocytic & differential counts) studies (Nutt and Herrick, 1952, Coles, 1986). The 2nd samples for serum separation to be used for serological identification using ELISA (Engvall and perlmann, 1981) and slide agglutination test (Adler and Yamamoto, 1956 and Stipkovits, 1997) and biochemical tests using Kits of Diamond & Human. Serum Alkaline phosphates (AP) (Belfield and Goldberg, 1971), aspartate aminotransferase (AST) and alanine aminotranferase (ALT) (Reitman and Frankel, 1957), serum total protein (Henry, 1974), serum albumin (Doumas, 1971), serum globulin (Doumas and Biggs, 1972), serum uric acid (Fossati, 1980) and serum creatinine (Henry, 1979). The chickens were sacrificed at the end of the 1st, 2nd, 3rd and 5th weeks of age after blood sampling. the lungs, trachea and air sacs were collected for the reisolation of M.G. after (Stipkovits, 1997) and identification of the isolated M.G. according to (Clyde, 1983). The obtained data were statistically analysed according to (Fisher, 1953).

RESULTS

Table 1: Leukogram of chickens in grs. (1, 4, 5 & 6) at the end of the 1st week of age.

Groups	T .L. C. (thousand/ul)	Differential T .L. C . (thousand/ul)			
		Heterophils	Lymphocytes	Eosinophils	Monocytes
Control	27.47+1.25	6.25+0.50	19.75+0.95	0.95 + 0.10	0.52 + 0.02
Passive immunization	31.81+0.90	7.35+0.28	22.71 +0.75	1.05 + 0.03	0.70 + 0.12
Active Immunization	43.03+2.10	11.25+2.10	28.45 + 1.65	1.08 + 0.15	1.25 + 0.15
Enrofloxacin treatment	22.73+0.95	8.50+0.57	12.95 + 0.95	0.75 + 0.16	0.53 + 0.09

T .L .C.= total leukocytic count $P<0.05$

Table 2: Leukogram of chickens in grs. (1, 4, 5 & 6) at the end of the 2nd week of age.

Groups	T .L. C.	Differential T.L.C. (thousand/ul)

	(thousand/ul)	Heterophils	Lymphocytes	Eosinophils	Monocytes
Control	28.52+0.25	6.41.+0.25	21.35+0.55	0.75+.025	1.01+0.30
Passive immunization	30.95+0.72	6.45+0.55	21.75+0.65	1.05+0.03	0.89+0.12
Active Immunization	40.12+3.25	9.07+0.35	28.65+1.95	1.20+0.35	1.20+0.20
Enrofloxacin treatment	28.07+1.02	6.75+019	19.55+1.05	0.75+0.10	1.02+0.03

Table 3: Liver function tests of chicken in groups (1, 4, 5 and 6) at the end of 1st week of age.

Groups	AST (u/l)	ALT(u/l)	AP(u/l)	T.P. (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)
Control	85.75+2.45	70.05+1.55	234.45+4.35	3.95+0.19	1.98+0.12	1.16+0.12
Passive immunization	86.05+1.01	66.75+4.25	240.30+4.90	4.25+0.10	3.01+0.03	1.75+0.11
Active immunization	85.85+3.40	69.99+0.95	249.85+2.75	4.15+0.10	3.01+0.11	1.05+0.25
Enrofloxacin Treatment	92.00+3.00	79.85+2.35	255.09+3.95	4.25+0.18	2.98+0.02	1.37+0.14

AST =Aspartate aminotransferase ALT =alanine aminotransferas
AP =alkaline phosphatase T.P =total proteine

Table 4: Liver function tests of chicken in groups (1, 4, 5 and 6) at the end of 2nd week of age.

Groups	AST (u/l)	ALT (u/l)	AP (u/l)	T.P. (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)
Control	85.25+2.15	65.10+1.85	235.75+1.69	3.50+0.20	1.95+0.20	1.55+0.11
Passive Immunization	87.20+3.10	69.85+2.10	238.78+1.11	4.85+0.10	2.62+0.07	2.55+0.15
Active immunization	79.95+3.45	71.82+2.95	241.45+1.43	3.89+0.02	1.85+0.09	2.04+0.09
Enrofloxa-cin treatment	85.95+1.21	72.65+1.30	240.64+1.31	3.90+0.25	2.01+0.25	1.89+0.15

Table 5: Some Kidney function tests of chickens in groups (1, 4, 5 and 6) at the end of the 1st and 2nd week of age.

Group	1st week	2nd week

	Uric acid (mg /dl)	Creatinine (mg /dl)	Uric acid (mg /dl)	Creatinine (mg /dl)
Control	4.90 + 0.15	0.75 + 0.03	4.08 + 0.15	0.88 + 0.02
Passive immunization	7.95 + 0.14	0.89 + .02	4.25 + 0.35	0.90 + 0.03
Active immunization	5.01 +6 0.35	0.71 + 0.03	5.09 + 0.15	0.75 + 0.01
Enrofloxacin treatment	5.95 +0.43	0.99 + 0.01	4.85 + 0.12	0.70 + 0.02

P< 0.05

Table 6: Leukogram of the chickens in groups (1-6)at the end of the 3rd week of age.

Groups	T. L. C. (thousand/ul)	Differential T.L.C. (thousand / ul)			
		Heterophils	Lymphocyts	Eosinophils	Monocytes
CO.	29.80 + 1.49	9.25 + 0.75	18.95 + 1.73	0.95 + 0.25	0.65 + 0.02
CO.+Inf.	46.26 +1.53	15.25 + 1.11	28.75 + 1.40	1.35 + 0.02	0.91 + 0.03
Inf.+ Pass.Imm..	40.74 + 1.75	12.95 +1.20	26.95 +2.39	1.09 + 0.18	1.75 +0.07
Pass.Imm.+Inf.+Pass.Imm.	35.46 + 1.59	7.03 +1.20	25.59 + 1.80	1.39 + 0.07	1.45 + 0.06
Active Imm.+ Inf.	38.95 + 0.55	8.25 + 1.04	27.85 + 1.12	1.30 + 0.19	1.55 + 0.01
Enro.+Inf. +Enro.	44.40 + 1.55	16.50 + 0.95	25.09 + 1.99	1.66 + 0.07	1.15 + 0.02

CO.=control Inf.=infection Pass.=passive Imm.=immunization. P <0.05
Enro.= Enrofloxacin

Table 7: Leukogram of the chickens in groups (1-6) at the end of the 5th week of age.

Groups	T. L. C. (thousand/ul)	Differential T.L.C. (thousand / ul)			
		Heterophils	Lymphocyts	Eosinophils	Monocytes
CO.	29.48 +2.05	9.05 + 0.75	18.45 + 1.35	0.79 + 0.25	1.09 + 0.25
CO.+Inf.	45.54 + 0.85	14.75 + 1.07	26.99 + 1.15	0.85 + 0.07	2.95 + 0.03
Inf.+ Pass.Imm..	32.75 + 1.50	9.75 + 0.75	21.04 + 1.09	0.99 + 0.06	0.97 + 0.25
Pass.Imm.+Inf.+Pass.Imm.	31.94 + 0.75	10.25 + 0.76	20.11 + .15	0.59 + 0.02	0.99 + 0.21
Active Imm.+ Inf.	36.79 + 1.15	7.59 + 1.05	22.35 + 1.55	1.15 + 0.06	0.70 + 0.02
Enro.+Inf. +Enro.	32.32 + 0.70	9.07 + 0.75	21.45 + 0.75	0.95 + 0.10	0.85 + 0.11

Table 8: Liver function tests of chickens in groups (1-6) at the end of the 3rd week.

Groups	AST (u/l)	ALT (u/l)	AP (u/l)	T.P. (gm/dl)	Albumin (gm/dl	Globulin (gm/dl)
CO.	89.45+3.75	76.05+2.95	249.50+5.09	4.15+0.29	1.99+0.15	2.16+0.25
CO.+Inf.	127.15+076	89.55+1.16	280.75+4.85	5.70+0.21	1.95+0.19	3.75+0.20
Inf.+ Pass.Imm..	103.75+2.80	85.07+1.19	260.77+5.11	4.84+0.75	1.85+0.09	2.99+0.50
Pass.Imm.+Inf. + Pass.Imm.	99.95+2.95	83.09+1.75	259.30+5.12	5.66+0.19	2.01+0.25	3.65+0.03
Active Imm.+ Inf.	90.85+2.95	81.02+1.91	250.95+1.49	4.94+0.25	1.95+0.25	2.99+0.25
Enro.+Inf. +Enro.	119.45+3.75	89.11+2.10	271.53+5.85	5.70+0.45	1.95+0.19	3.75+0.15

AST = aspartate aminotransferase ALT= alanin aminotransferase T.P.= total protein
AP= alkalin phosphates' $P < 0.05$

Table 9: Liver function tests of chickens in groups (1-6) at the end of the 5th week.

Groups	AST (u/l)	ALT (u/l)	AP (u/l)	T.P. (gm/dl)	Albumin (gm/dl	Globulin (gm/dl)
CO.	85.62+1.55	70.65+5.75	251.11+12.35	3.94+0.35	1.95+0.19	1.99+0.11
CO.+Inf.	130.75+1.45	88.45+1.60	281.16+4.35	6.30+0.25	1.95+0.12	4.35+0.15
Inf.+ Pass.Imm..	86.10+1.05	75.46+1.73	261.11+2.49	4.84+0.01	1.99+0.09	2.85+0.09
Pass.Imm.+Inf.+ Pass.Imm.	83.39+3.15	71.45+0.97	255.17+2.50	4.07+0.12	2.12+0.23	1.95+0.17
Active Imm.+ Inf.	81.25+1.95	69.45+2.35	255+951.25	4.94+0.19	1.95+0.09	2.99+0.10
Enro.+Inf. +Enro.	85.95+2.85	75.35+1.15	260.59+2.55	4.86+0.05	2.01+0.05	2.85+0.05

$P < 0.05$

Table 10: Kidney function tests of chickens in groups (1-6) at the end of the 3rd and 5th weeks.

Groups	The end of the 3rd week		The end of the 5th week	
	Uric acid (mg/dl)	Creatinine (mg /dl)	Uric acid (mg /dl)	Creatinine (mg /dl)
CO.	5.00 + 0.15	0.85 + 0.08	3.95 + 0.15	0.81 + 0.05
CO.+Inf.	5.95 + 0.09	0.90 + 0.02	9.11 + 0.19	0.81 + 0.09
Inf.+ Pass.Imm..	4.90 + 0.08	0.85 + 0.03	4.59 + 0.13	0.89 + 0.06
Pass.Imm.+Inf.+ Pass.Imm.	5.02 + 0.10	0.88 + 0.06	4.25 + 0.15	0.75 + 0.05
Active Imm.+ Inf.	4.85 + 0.15	0.79 + 0.07	4.15 + 0.11	0.73 + 0.09
Enro.+Inf. +Enro.	5.09 + 0.25	0.83 + 0.03	4.19 + 0.08	0.80 + 0.03

$P < 0.05$

Table 11: Results of ELISA in the chickens of groups (1-6).

Age in week / Groups	1st week	2nd week	3rd week	5th week
CO.	0.05 + 0.01	0.08 + 0.03	0.10 +0.01	0.15 + 0.01
CO.+Inf.	0	0	0.15 + 0.01	1.25 + 0.15
Inf.+ Pass.Imm..	0	0	0.17 +0.02	0.75 + 0.02
Pass.Imm.+Inf. + Pass.Imm.	0.11 +0.02	0.18 + 0.02	0.22 + 0.01	0.57 + 0.01
Active Imm.+ Inf.	0.09 + 0.02	0.15 + 0.01	0.55 + 0.02	1.56 + 0.11
Enro.+Inf. +Enro.	0.07 + .09	0.08 + 0.01	0.12 +0.19	0.71 + 0.03

Table 12: Results of serum plate agglutination (SPA) of groups (1-6).

Age in week / Groups	No. of chickens Examined	1st week	2nd week	3rd week	5th week
CO.	5	0	0	0	0
CO.+Inf.	5	0	0	3 /5	5 / 5
Inf.+ Pass.Imm..	5	0	0	3 / 5	1 / 5
Pass.Imm.+ Inf.+Pass.Imm.	5	0	0	2 / 5	1 / 5
Active Imm.+ Inf.	5	0	1 / 5	4 / 5	5 / 5
Enro.+Inf. +Enro.	5	0	0	3 / 5	1 / 5

No. = number

DISCUSSION

The active vaccination using the F-strain of M.G. was used for controlling the disease. (Levisohn and Kleven, 1981) isolated M.G. from trachea of vaccinated birds up to one year, but their spread was slow. They suggested that the continued presence of non virulent M.G. in the respiratory tract would prevent the localization of the virulent strain. Moreover, it continues stimulating the local immunological response against M.G. in the chicken of flocks (Cummings and Kleven, 1986; Ahmed, 1996). The experimental infection of chickens gr. (2) with M.G. revealed leukocytosis, lymphocytosis, and heterophilia at the end of the 3rd & 5th weeks of age. Monocytosis was observed at the end of the 3rd week only. The previous result may be due to the infection in which birds respond to infection with leukocytosis, lymphocytosis heterophilia

and monocytosis. The avian species responded to wide range of pathogenic agents with leukocytosis, commonly heterophilia (Hawkey, 1983). The principle function of the lymphocytosis is the immunological response to antigens like M.G., such response leads to proliferation and differentiation of the lymphocyte to that is usually manifested by lymphocytosis. The monocyte play an essential role in the immune response after antigenic stimulation, either as phagocytic cells or antigen presenting cells to lymphocytes (Coles, 1986).

The lymphopenia usually associated with mycoplasma infection and it may be due to the use of different species of birds, different doses and strains of the microorganism or different routes of infection. The M.G. infection causes highly significant increase in AST, ALT, and AP, these elevation of serum enzymes may be due to the increased cell membrane permeability and destruction and/or necrosis of hepatic cells permitting the intracellular enzymes to escape to the blood (Eisa, 2001; El-shimy, 2002) and non-significant changes in the AST after the oral M.G.. infection (Arafa, 1993).

The M.G. infected gr. (2) showed hyperglobulinemia which lead to hyperproteinemia. The hyperglobulinemia may be due to the immunodefence of the chickens against the organism (Coles, 1986 and 1997). Hypoalbuminemia was encountered at the end of the 5^{th} week only due to inflammation destruction in the liver which is the main source of albumin synthesis in the body (Abou El-Nile, 1997). Non-significant changes in the albumin in 10 day old chickens infected with M.G. (Eisa, 2001).

The M.G. infection did not affect the renal tissues consequently, there were non significant changes in the serum creatinine. The kidneys of all infected chickens appeared apparently normal without lesions (Eisa, 2001), while Arafa (1993); Abou El-Nile (1997) and El-shabiny, *et al.* (1997) observed an increase in the creatinine level after M.G. infection in chickens on the 7^{th} and 8^{th} days of age orally and intranasal respectively. At the 3^{rd} and 5^{th} week of age the serum uric acid was increased in spite of normal kidney which may be due to disturbance in purine metabolism or increase adenosine triphosphate degeneration (ATP) (Dincer *et al.*, 2002). .

The passive immunization seems to be one of the most valuable applications of antibodies in which pathogen-specific IgG is administered to individuals for prevention of infectious diseases. Passive immunization differs from active immunization (vaccination) in that the former employs an antibody obtained from other animals. The

administration of this antibody specific to certain antigens (bacteria, virus and toxin) to individuals orally or parenterally neutralizes the infectious activity or toxicity of the antigen (Yamamoto *et al.*, 1997). The hen's egg yolk contains the immunoglobulin known as IgY (Akita and Nakai, 1992). The ordinary egg yolk has been shown to contain an antibody level up to 15 mg /ml and is a good potential source of immunoglobulin (Rose *et al.*, 1974). The inoculation of hens with antigens (bacterial or viral) has been shown to generate high titers of specific antibodies in the egg yolk (Polson, 1990). Now the production of immunized egg has become an economical source of antibodies for various application (Song *et al.*, 1985). The employment of the egg yolk antibody in the normal chickens gr. (4) in 1st and 2nd weeks of age, showed non significant changes in the TLC, hrterophils, lymphocytes, eosinophils, monocytes AST, ALT, AP, albumin, uric acid and creatinine. In the 1st week of age, non significant changes were recorded in total proteins globulin. However, significant increase in both total proteins and globulin in response to the injected antibodies was observed in the 2nd week of age. Chickens in grs. (3 & 4) which infected with M.G. then passively immunized with egg yolk antibodies, revealed leukocytosis, lymphocytosis and monocytosis in addition to heterophilia, there was a significant increase in the AST, ALT, total proteins, globulin and uric acid at the end of the 3rd week of age only. This increase could be due to the infection with M.G. At the end of the 5th week of age and after the last dose of immunization, all the measured parameters returned to nearly the normal values except a significant increase in the total proteins due to increased globulin which could be due to the neutralizing effect of the injected IgY against the M.G.

The IgY obtained from hens immunized with pathogenic bacteria, inhibit the growth of pseudomonas aeruginosa (Sugita-Konishi, *et al.*, 1996), also the oral administration of IgY from chicken egg yolk, has been successfully in preventing many enteric diseases such as enterotoxigenic *E. coli* and human rotavirus (Ebina, 1996; Sarker, *et al.*, 2001). Malik, *et al.* (2oo6) succeeded in control the infectious bursal disease by intraperitoneal injection of egg yolk as a source of antibodies. The active immunized gr. (5) with F-strain of M.G., showed nearly similar pattern of infection in leukogram and proteinogram,in which leukocytosis, heterophilia, lymphocytosis and monocytosis besides hyperproteinemia due to hyperglobulinemia were observed allover the experimental periods. F-strain engulfed by macrophage and then represent the antigen into T-cell which activated and proliferated into the

cells. Activated T-cell secretes interleukin which stimulate production of inflammatory cells as netrophils, monocytes and lymphocytes. The activation lead to proliferation of the B-lymphocytes which changed to plasma cells to produce immunoglobulin. F-strain vaccine induced leukocytosis, heterophilia, lymphocytes and monocytosis. All the hepatic and renal function parameters were not significantly changed, indicated that the F-strain vaccine protected the liver and kidneys from the side effect of the microbe (Fundenberg, *et al.,* 1976; Coles, 1986; El-shimy, 2002). The F-strain vaccine can produce mild lesions in all examined tissues with no symptoms resulting in less tissue damage followed by faster recovery (Rodriguez and Kleven, 1980; Hildbrand *et al.,* 1983; Dardeer *et al.,* 2004). Live F-strain vaccine provide a reduction in the clinical signs and have been shown to replace endemic strains when used for several times and also capable for eliciting stronger antibody response and providing better protection against air sacculitis, it is able to offer protection against colonized by virulent wild type field strain (Evans *et al.,* 2000). Therefore-strain M.G. vaccine have been used, as a useful part of an eradication program as a viable tool to displace virulent wild type M.G.

The drug therapy is away of controlling the Mycoplasma infection which still plays a major role in decreasing the economic loss due to mycoplasma infection in meat and layer flocks (El-Mahi and Hofstad, 1978; Cummings and Kleven, 1986). The antibiotic medication is much more effective when used prophylatically than when used as treatment. The continuous feed medications is sometimes used, but we should remember that the antibiotic medications is expensive and resistant organisms may develop. Some antibiotics may be toxic if an over dosage is given accidentally and the residues of antibiotics are undesirable in egg or meat for human consumption. Attempts to control mycoplasmosis frequently relied on the intensive and prolonged use of antimycoplasmal drugs (Migaki *et al.,* 1993). The effect of enrofloxacin on leukogram of the healthy non infected chickens, revealed leukopenia, heterophilia and lymopenia at the end of the 1st week of age. these results may be due to drug stress condition as a result of the secretion of adrenocortical hormones that cause dissolution of these cells. After norfloxacin administration leukopenia (Gellert, 1981), leukocytosis or non significant changes in the T.L.C. (Fleischer *et al.,* 2000; Jayakumar *et al.,* 2002; El-kadeem, 2005). In our opinion, such contradiction may be attributed to the time of administration or the difference in species and individual susceptibility. At the end of the 2nd week of age, the

T.L.C. returned to nearly the normal value. This may be due to the cessation of the drug. The chickens infected with M.G. and treated with enrofloxacin, showed nearly similar picture of leukogram to those in gr. (2) at the end of the 3rd week, this may be due to the effect of microorganism. Non significant changes in leukogram of gr. (6) compared with the infected non treated birds at the end of the 5th week.

The serum AST & ALT are standard tests for hepatocellular function. Although the AST & ALT are not liver specific in birds, however the increase in their activities has been associated with hepatocellular damage in birds (Coles, 1986). ALT is more specific than the AST in early hepatocellular damage in which the AST is associated with cell necrosis of many different tissues, either muscular or hepatic. The serum alkaline phosphates activity was increased 5-6 time than the normal with diseases (Coles, 1986). In the present work, the therapeutic dose of enroflxacin induce significant increase in the activities of AST, ALT & AP in the 1st week of age due to mild toxic effect of the drug metabolites which increase the permeability of the cell membrane increasing hepatic enzymes. The enrofloxacin cause hepatic dysfunction (Halkin, 1988) and elevation of the serum AST, ALT & AP during treatment then restored its normal value after 2 weeks (Gellert, 1981; Abd El-Alim *et al.,* 2000; El-Kadeem, 2005). In the present work the enrofloxacin induce non significant changes in total proteins, albumin and globulin at the end of the 1st & 2nd weeks, these results may be due to the mild and reversible effect of the drug on the liver (Helal *et al.,* 1995; Uyanik *et al.,* 2000). The hyperproteinemia due to hyperglobulinemia in the 3rd & 5th week of age after enrofloxacin treatment, may be attributed to the effect of the infection with M.G. and may need long time to return to its normal value. Non significant changes in albumin in gr. (6) compared with gr. (2) at the end of the 5th week indicating favorable response to the treatment (Ibrahim, 1995). The uric acid is the primary catabolic product of protein, non protein nitrogen and purins in the birds (Coles, 1986). The birds are uricotelic and produce uric acid not urea as the major nitrogenous end product of metabolism. The use of enrofloxacin affect on the renal tissue leading to some renal damage (Shimada and Hori, 1992), this renal damage led to increase serum uric acid and creatinine at the end of the 1st week of age and were not significantly changed at the end of 2nd week. This means that the effect of the drug is reversible. The serum creatinine is a major non-protein nitrogen compound of avian blood (Bell and Freeman, 1971) and become elevated in the birds with renal diseases due to nephrotoxic

drug, but less than the uric acid (Galvin, 1980). Hyperuricemia in the birds occurs with starvation, gout massive tissue destruction and renal diseases (Coles, 1986). Hyperuricemia and increase in creatinine level in the 1st week of age was due to treatment with enrofloxacin. Kobayashi, *et al.* (2004) and El-Kadeem (2005) noticed hyperuricemia and increased creatinine level after enrofloxacin treatment and Kamel and Abd El-Aziz (2002) noticed decrease in uric acid and increase in creatinine after I/M injection of norfloxacin in turkeys. The serum uric acid nearly regained its normal value in the 5th week of age after treatment, this finding may be due to antimycoplasmal activity of the drug. The enrofloxacin induce significant increase in the uric acid and creatinine, these deviations regained their normal one week post-treatment (Khodary and El sayed, 1997).

The serological tests is the preferred method of testing M.G. antibodies because of the easy of obtaining sera, the sensitivity and reproducibility of the assays (Spencer *et al.,* 2002). The antimycoplasmal antibodies were detected in the experimentally infected chickens of gr. (6) on the 5th day post-infection. The immunotherapeutic treatment using the antimycoplasmal gallisepticum egg yolk antibodies (IgY), results in non significant decrease in the antibody response on the 35th day in gr. (3) compared with the infected non treated gr. The chicken of gr. (4) which were passively immunized revealed highly significant increase of the ELISA titer in the 2nd week, this result could be due to the injected antibodies. The level of antibodies was increased in gr. (4) after the infection and was still increased specially in the 3rd week, compared with infected non treated gr. The decreased of antibody level in the 5th week was mostly due to reduced colonization of the M.G. by the passive immunization using egg yolk antibodies specific for M.G.

The vaccine stimulat the humeral response in the chickens of gr. (5) by increase in the antibody level gradually before and after infection. The live M.G. F-strain vaccine is estimated to be highly immunogenic (Abd El-Motelib and Kleven, 1993; Ferguson *et al.,* 2004). Since the level of protection by live M.G. vaccines is directly correlated with the virulence of the vaccine strain (infectivity, pathogenicity and immunogeicity) F-strain elicited stronger antibody response and provide protection against colonization by more virulent strains (Levisohn, 1984; Cummings and Kleven, 1986). The antibodies in the sera of birds vaccinated with TS-11 and challenged with M.G. began with low titer and increased gradually till the 3rd & 4th week post-challenge (Noormohammad *et al.,* 2002).

No positive results of the antibody titer were observed till the end of the 2nd week in gr. (6). After challenge and treatment with enrofloxacin the antibody titer increased in the 3rd week due to challenge. It was decreased in the 5th week in comparison with the infected non treated gr., this decrease may be due to the enrofloxacin effect on the M.G. The infection with M.G. is not completely prevented and subsequently the flock remain at risk. Also the continuous medication has the potential for reducing the M.G. tracheal population. The deleterious effect of the mycoplasma on chickens, leads to the activation of the immune system of the birds to produce more lymphocytes, leukocytes and round cells to overcome the infection (Ahmed, 2004). The serum plate agglutination test (SPA) is more rapid test detecting mycoplasma infection, therefore preferably used as screening test (Kleven *et al.*, 1984; Yoder, 1989; El-shimy, 2002; Dardeer *et al.*, 2004). The active infection started with agglutination antibodies which appeared early in the serum (one week after infection) and remained detectable for 30 days (Rodriguez and Kleven, 1980). In the present work, the SPA revealed 3/5 positive result, in the infected chickens of gr. (2) on the 5th day post-infection and reached its highest level on the 35th day of age. Concerning the immunotherapeutically treated grs. (3&4), the positive results of the SPA in the 3rd week of age, were 3/5 & 2/5 respectively, then reached 1/5 for both grs. On the 35th day of age. This decreased could be due to the passive immunization. A gradual positive result of the SPA test was found in the 2nd & 3rd weeks post active vaccination of gr. (5) and reached its maximum level in the 5th week and this may be attributed to the effect of the F-strain vaccine. Regarding to the enrofloxacin treated gr., the results of the SPA test on the 5th day post-infection was 3/5, however it dropped to 1/5 in the 5th week. this decrease could be due to the effect of enrofloxacin on the M.G. The treatment of M.G. with antimycoplasmal drugs may also prevent significant immune response and make it difficult to isolate the microorganism giving false impression that the flock is free from infection.

It could be concluded that M.G. induced hematological, biochemical and serological changes. The vaccination is the best method to control mycoplasmosis in chickens, as it stimulated the immune defense without any side effect. The passive immunization came next and the last was the treatment with antimycoplasmal drugs.

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