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IMMUNE RESPONSE TO MYCOPLASMA GALLISEPTICUM INFECTION IN CHICKEN

(With 3 Fig. & 7 Tables)

By

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الرد المناعى نتيجة لعدوى الميكوبلازما جاليسبتكم فى الدجاج

محمد حسانين ، يوسف شحاته ، ليلى بايزيد ، أحمد عبد الصادق
ليلى الشعبينى ، محمد مدكور ، ثناء عبد الرحمن

تم حقن خمسون كتكوت عمر أسبوعين بالميكوبلازما جاليسبكم بتقسيمها إلى ٤ مجموعات تبعاً لطريقة الحقن مع الأخذ فى الاعتبار مجموعة ضابط التجربه ، وتم فحص الأنسجه والدم أسبوعياً بعد الحقن ووجد أن نسبة العزل ١٧ ٪ والاصابات المرضيه الظاهره كانت طفيفه وباجراء الفحوص السيرولوجيه على دم الدجاج المصاب بإستعمال إختبار التلازن وجد أن عدد الإيجابى وقوته تزداد مع الأسبوع الأول من العدوى وتقل تدريجياً بعد ذلك ، وبإستعمال اختبار مانع التجمع الدموى وجد أن الأجسام المضاده تظهر متأخره عنها فى حالة إختبار التلازن وتزداد تدريجياً وكانت نسبتها أعلى فى الدجاج المحقون فى الأكياس الهوائيه.

أما بالنسبه للإختبارات البيوكيميائيه فقد وجد أن البروتينات الكليه زادت زياده معنويه خلال مدة التجربه فى الدجاج المصاب وبعمل تحليل لمصل الدجاج بواسطة الفصل الكهربائى شوهدت زيادة معنويه فى نسبة الجلوبيولين الكليه وألفا ١ و ألفا ٢ و بيتا جلوبيولين ، كما كانت هناك زيادة معنوية فى الترنسفيرين فى بداية التجربه ثم تقل عند نهايتها ، ووجد أيضاً زيادة معنوية فى الجلوبيولينات المناعيه M تقل فى درجة حدوثها مع زيادة العمر بعد العدوى ، بالنسبه للألبيومين فقد نقص معنوياً فى مصل الدجاج المصاب خاصة فى نهاية التجربه ، وقد أثبتت هذه الدراسه وجود إرتباط معنوى إيجابى بين جلوبيولين المناعه M وإختبار التلازن وكذلك بين إختبار مانع التجمع الدموى و جلوبيولينات المناعه G فى مصل الدجاج المحقون مما يؤكد الدور الهام الذى يمكن أن تلعبه الطرق الكيميائيه الحيويه فى تدعيم طرق البحث التقليديه.

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SUMMARY

Fifty day old Hubbard chicks were maintained and equally fed, at 2 weeks of age, they were divided into 4 groups each of 12 chicks according to the route of infection with M. gallisepticum. Blood and tissue samples were examined, it was found that the reisolation rate of mycoplasma was low (17%), macroscopic lesions were very mild, serological examinations were adopted, the number of positive sera and intensity of agglutination were high in the first week of infection, decreased gradually later on. Haemagglutination inhibition antibodies appeared later than the agglutinating antibodies, and increased later on and they were higher in air sac infection. Biochemical examination was performed, total serum protein increased in infected chicks and eletcrophoretic pattern using poly acrylamide gel electrophoresis showed that serum albumin decreased, total globulin $O<_1$, $O<_2$ and B globulins and transferrin increased due to M. gallisepticum infection. IgM and IgG immunoglobulins also increased. These results correlated with grading scores of serum agglutinating titres depending on IgM antibodies in the first week of infection and IgG antibodies detected by haemaglutination inhibition test and that detected by the biochemical method in the fourth week. The present study confirms the correlation between antibody response to M. gallisepticum infection by serological methods and that detected by biochemical methods proving the role of biochemistry in supporting the classical methods of examination.

INTRODUCTION

M. gallisepticum causes considerable economic loss within the poultry industry as infection commonly leads to an increase in carcass condemnations, decreased hatchability and egg production, beside the increased medication cost.

Following mycoplasma infection an antibody response is induced and can be detected in serumby serological tests including the adapted enzyme linked immunosorbent assay system *(ERHARD et al., 1992)*, also can be studied by immunoglobulin

analysis of serum antibodies *(WISE and FULLER, 1975; FERNALD, 1989a and EL-SHABINY, 1984).*

The aim of this investigation is detecting antibody response to M. gallisepticum infection by the electrophoretic pattern of various serum proteins with special reference to immunoglobulins G. and M.

MATERIAL and METHODS

Samles:

1. **Birds:** Fifty, one-day old Hubbard chickens were obtained from General Poultry Company, they were negative for mycoplasma, no medication was previously given to them, they were identically fed, maintained during the period of the experiment.
2. **Blood:** Blood samples were collected from infected and control chickens after one and four weeks of infection.
3. **Tissue:** Fresh parts of lungs, tracheas and air sacs were taken from slaughtered chickens.

An experimental infection was performed by dividing the birds at 2 weeks of age into 4 groups, each of 12 according to the route of infection with 0.2 ml 24-hour broth culture of M. gallisepticum strain PG31, 8^{10} C.F.U. colony forming unit without thallium acetate after several passages in chicken egg embryo. Inoculation was made according to *KUBA* et al. *(1974)*, group one intranasally, group 2 intratracheally, group 3 via air sac and group 4 was a control group. The period of the experiment was one month during which the samples were examined in the first, and fourth week of infection.

Serological tests performed on the serum samples Were:

1- Slide agglutination test *(Alexander* et al., *1958)* using M. gallisepticum strained antigen supplied by *Salsbury Laboratories, U.S.A.*

2- Haemagglutination inhibition test *(MESZAROS, 1964).*

3- Isolation method: tissue samples were cultivated on heart infusion medium *(HAYFLICK, 1965)* as described by *EL-EBEEDY (1973).*

The reisolated strains were examined by digitonin test for genus determination as described by *FREUNDT (1973).* Biochemical characterization was performed according to *SABRY (1968).* Identification was carried out using growth inhibition test *(CLYDE, 1964).* M. gallisepticum cultures and antisera were obtained from National Institute of Allergy Bethesda, Maryland,

U.S.A.

Biochemical analysis:

The serum was used for determination of:

1- Total serum proteins according to *STAVENS (1965)* using the auto-analyzer, it is a modification of biuret reaction *(WEICHSELBAUM, 1946).*

2- Electrophoretic pattern of serum proteins using polyacrylamide gel columns, the technique used was that of *RAINER MAURER (1971)* using Gelman vertical polyacrylamide gel electrophoresis apparatus. Reading of the gels was carried out by scanning through filter 1 Beckman and model R-112 to identify the zone, MR or R value of each zone was determined following the method of *GLICK (1968).*

$$\text{MR value} = \frac{\text{distance migrated by each fraction}}{\text{distance migrated by transferring}}$$

Statistical analysis was applied according to *TURNER (1970), CROSSLAND (1971) and GOLDSTEIN (1965).*

RESULTS

Gross lesions showed slight congestion of lungs, slite turbidity in air sacs, exudate in the trachea and slight liver congestion and perihepatitis in infected birds.

Serological examination using slide agglutination (RPT) showed increased intensity of agglutination in the 1st week of infection (+++) in intra air sac and intratracheally infected chickens and (++) in intranasally infected ones, decreased gradually whatever was the route of infection.

Haemagglutination inhibition test (HI) gave low titres (1/20) in the first week of infection then increased to 1/40-1/80-1/160 in the 4th week. The hihest HI titre was in intra air sac infected chickens followed by intratracheally and intranasally infected ones.

The reisolation rate of M. gallisepticum from infected birds was very low (17-33%) in intratracheally inoculated chickens and 17% in intra air sac and intranasally, while there was significant increase ($P<0.001$) in chickens intratracheally infected (after one week).

Regarding to the biochemical results;

Total serum protein and its fractional pattern: The estimates of total proteins, prealbumin, albumins and total globulins in the 1st week have been statistically presented in

Tables (1a and 1b), illustrated in (Fig. 1 and 2). It was clear that, total serum protein increase, serum albumin decreased in infected groups. Statistical analysis of Alphal and Alpha 2 and B-globulins were presented in Tables 3a and 3b) in the 1st week and in Tables (4a and 4b) in the 4th week and illustrated in Fig. (2). It was found that, these parameters increased in infected groups.

The results of total gamma-globulins, transferrin, IgG and IgM in the 1st week were shown in Tables (5a ad 5b) and in Tables (6a and 6b) in the 4th week, illustrated in (Fig. 3). It was clear that these parameters increased in infected groups as compared with the control ones.

DISCUSSION

Mycoplasmas are primarily pathogens of mucosal surfaces and therefore resistance at this site provides the first line of defense which is composed of phagocytic cells and complement, these together with the rapidity with which the host can mount an immune response and the ability of mycoplasmas to overcome these defense mechanisms, determine the extent of subsequent disease *(RAZIN and BARILE, 1985).*

Humoral immunity can be detected by serological tests, slide agglutination test (RPT) is particularly useful as a flock screening test detecting maily IgM antibody to mycoplasma infection *(CHABRA and GOEL, 1980 and 1982).* Haemagglutination inhibition test (HI) is used to detect IgM, IgA and IgG antibodies but the activity is usually in the IgG fraction.

In the present study, macropathological lesions were slightly congested lungs, slightly thickened air sacs and mild perihepatitis agreed with *DOMERMUTH et al. (1967) and YODER (1985).* RPT showed high grades at the first week of infection and decreased gradually as the disease progressed, while HI showed low titres 1/20-1/40 at the beginning of the experiment, increased gradually from 1/40-1/80 to 1/160 after that, this may be due to increased IgM antibodies as early defense mechanism against mycoplasma infection then IgG antibodies that appeared later on and increased with advancement of infection as was previously proved by *CHABRA (1980 and 1982), WHITBY et al. (1985), GOISE et al. (1972) and KLEVEN and POMEROY (1971).*

The results were confirmed by the significant positive correlation between grading scores of RPT antibody titre and IgM at the first week of infection and HI titres and IgG at the fourth week of infection shown in Table (7).

Asfor the biochemical parameters, total serum protein

levels were increased due to M. gallisepticum infection as presented in Tables (1 and 2) and Fig. (1) which could be attributed to increased level of globulin fractions which were generally considered to contain most of the antibody response activity according to *BRATTIN and GRABAR (1967)* and also due to affection of the liver as was reported by *KRACZKOWSKI (1964); KUMAR and CHANDIERMANI (1979)* in chickens infected with M. gallisepticum. Serum albumin levels of infected chickens significantly decreased as the disease progressed (Tables 1 and 2; Fig. 1) and this may be due to the disturbed synthesis of albumin because of affection of the liver *(BERTIL LAURELL, 1985 and SELLS, 1976)* who attributed the reduction of albumin in sera of chickens infected with myoplasma to the fact that in hypergamma globulinemic states, albumin synthesis falls as a result of colloidal control mechanism, for albumin synthesis located within the hepatocytes. The loss of appetite observedin chickens may be a factor responsible for albumin decrease *(SHELTON and OLSON, 1960 and KONDO* et al., *1984)*, also may be due to the shift of albumin to orosomucoid biosynthesis to increased synthesis of protective proteins particularly acute phase proteins *(BERTIL LAURELL, 1985)*, also, *CLYDE and TOMAS (1973 a,b)* referred to increased permeability of blood vessels and swelling of endothelium of the infected host as a cause for decreased albumin level.

Regarding total serum globulin and its fractions, Tables (1-6) and Fig. (2) showed significant increase in serum globulins as well as their fractions alphal, alpha2, B- and gamma-globulins in chickens after one week of M. gallisepticum infection and as the disease progressed except B-globulin that showed lower level at the end of the experiment.

Our results are in accordance with *YOUSSEF (1972) EL-SHABINY (1984).* They demonstrated significant elevation in alphal, alpha2, B- and gamma-globulins in chickens experimentally infected with M. gallisepticum. Sells gamma-globulins in sera of chicks infected with M. synoviae. Evidence of icnreased levels of total serum globulins with M. gallisepticum infection in chickens may be attributed to increased synthesis of acute phase proteins which act as buffer or protective proteins against rapid intracellular spread of active proteolytic enzymes and reactive molecules released from injured tisse cells, and from activated phagocytes. These acute phase proteins include alphal, alpha2, acid glycoproteins, ceruloplasmin and C-reactive protein. The significant rise in serum beta globulin after one week of infection in Tables (3 and 4) is in agreement with *GEWURZ (1982) and KUSHNER* et al.

(1982). It was demonstrated that alpha globulin is linked with mucoproteins and glucoproteins of mycoplasma *(BERTTIN and GRABER, 1967)*, changes in this fraction have been attributed to hypo-albuminemia injury regardless the type or cause. The significant rise in total alpha globulins (Tables 3 and 4) may be due to destruction and disintegration of tissue, damage of liver and kidney as was reported by *BERTTIN and GRABER (1967)*.

Beta globulin is the carrier of lipids and particularly cholesterol. *BERTIL and GRABER (1967); BERTILL and LAURELL (1985)* observed that immunoglobulins chiefly of IgM class, have an electrophoretic mobility dispersed in the B zone.

The rise in qualitative contents of B-globulins as shown in Tables (3 and 4) and Fig. (2) may be attributed to violation of lipid metabolism due to hepatic disorder and or to antibody response by the infected chickens *(KUSHNER et al., 1982 GEWURZ, 1982 AND EL-SHABINY, 1984)*.

Gamma-globulin is generally considered to contain most of antibody activity. *BERTTIN and GRABER (1967)* observed that immunoglobulin of IgG class have an electrophoretic mobility disperse in gamm[a] zone.

The increase in gamma-globulin observed in the present study and reprsented in Tables (5 and 6) may be attributed to the production of IgG following M. gallisepticum infection. Serum transferrin levels significantly increased in chickens after one week of infection, disappeared after the 4th week of infection via air sac and intranasal but slightly increased in case of infection intratracheally as shown in Table (6 a,b) and Fig. (3). This increase in transferrin level may be due to its action as gamma-globulin pattern in response to M. gallisepticum infection *(MEDINA et al., 1971). LATNER (1978)* also demonstrated significant increase in transferrin in sera of chickens due to anemia as was the case in mycoplasmosis.

CHERRY and TAYLOR-ROBINSON (1973) reportedthat M. gallisepticum infection causes formation of hydrogen peroxide, a factor leading to haemolysis to RBCs and haemolytic anemia, this confirms the finding of *EL-SHABINY (1984)* who reported significant decreas in iron binding capacity in serum of M. gallisepticum infected chickens.

Immunoglobulins of IgG class increased significantly in sera of chicks after one week of infection, particularly those infected via air sac and intratracheally, increased after 4 weeks of infection whatever was the route of infection (Tables 5 and 6; Fig. 3). Immunoglobulins of IgM class showed significant increase throughout the experiment period whatever was the route of infection especially earlyin the infection

(Tables 5 and 6). These results are in accordance with those of *FERNALD (1969), KLEVEN and POMEROY (1971)* in turkey poults, *CHABRA (1980 and 1982)* in chickens infected with M. gallisepticum.

A correlation between IgM and agglutination titre at the 1st week and IgG and HI titre were presented in Table (7) and showed positive correlation.

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Table (1 (a,b) : Statistical analysis of total proteins, pre-albumin , albumin, and total globulins in sera groups of control and infected chickens at the 1 st week of infection.

a- control group

Statistical parameters		Total g/dl proteins	pre-albumin mg/dl	albumin g/dl	Total globulin g/dl
Arith. mean ($\bar{x}$) ± S.E.		2.733±0.084	28.417 ± 2.249	0.529 ± 0.02	2.176 ±0.067
Measures of individual variability	Range	(2.5-3.0)	(0-54.636)	(0.449-0.577)	(2.002-2.429)
	±S.D	0.207	22.037	0.049	0.163
	C.V %	7.56%	77.55%	9.25%	7.50%
95% confidence limite of universal mean.		(2.516-2.950)	(23.952-32.88)	(0.478-0.580)	(2.005-2.347)
Expected limites of the normal range of individual estimates.		(2.433-3.033)	undected (0-79.046)	(0.458-0.600)	(1.939-2.413)

Table (1) b. Infected groups

Serum proteins estimates in infected chickens		% of incidence frequences among 6 infected chickens infected via : $\bar{x}$± S.E. , (Range in brackets) for estimates.		
		intra air sac route	intra nasal route	intra tracheal route
Total serum proteins g/dl	i	in 83% (5/6) 2.760 ± 0.1031 (2.50 - 3.00)	in 67% (4/6) 2.95 ± 0.029 (2.90 - 3.00)	in 33% (2/6) average 2.6 (2.50 - 3.00)
	ii	in 17% (1/6) 3.5. 15%	in 33% (2/6) average 3.15 + 7%*	in 50% (3/6) 3.4 ± 0.1 (3.1 - 3.5) +15%*
Pre-albumin mg/dl	i	in 100% (6/6) 30.224 ±1.947 (0 - 60.944)	in 100% (6/6) 45.070 ± 2.506 (0 - 60.944)	in 83% (5/6) 31.460+ 2.189 (15.99 -68.89)
	ii	—	—	in 17% (1/6) 96.57 . + 22%*
Albumin g/dl	i	in 100% (6/6) 0.528± 0.018 (0.468 - 0.595)	in 50% (3/6) 0.536 ± 0.017 (0.504 - 0.561)	in 67% (4/6) 0.565±0.047 (0.545 - 0.584)
	ii	—	in 50% (3/6) 0.430 ± 0.01 (0.410-0.440) -10%*	in 33% (2/6) average 0.417 (0.389-0.444) -9%*
Total globulins g/dl	i	in 33% (2/6) average 2.099 (2.175 - 2.023)	in 33% (2/6) average 2.391 (2.372 - 2.409)	in 33% (2/6) average 2.133 (2.119 - 2.146)
	ii	in 50% (3/6) 2.623 ± 0.147 (2.474 -2.918) +12%* in 17% (1/6) 1.880 -3%*	in 67% (4/6) 2.517 ±0.052 (2.454 - 2.671) + 7%*	in 67% (4/6) 2.552 ±1.171 (2.532 - 2.913) + 9%*

i = within the normal rang e
ii = significantly deviated from normal

Table (2 a, b): Statistical analysis of total serum proteins albumin pre albumin, and total globulins in sera groups of control and infected chickens at the 4th weeks of infection.

a) Control group

Statistical parameters		Total proteins g/dl	Pre-albumin mg/dl	Albumin g/dl	Total glob. g/dl
Arith. mean ($\bar{x}$) ± S.E.		(2.84±0.060)	(35.358±2.830)	(0.677±0.071)	(1.772±0.072)
Measurs of individual variability	Range	(2..3-2.7)	(0-75.790)	(0.470-0.854)	(1.589-2.004)
	S.D.	0.147	27.729	0.175	0.175
	C.V. %	5.93%	78.42%	25.84%	9.89%
95% confidence limite of universal mean		(2.329-2.637)	(29.740-40.976)	(0.498-0.856)	(1.588-1.956)
Expected limits of the normal range of individual estimates		(2.269-2.697)	undetected (0-99.063)	(0.419-0.935)	(1.518-2.026)

Table (2) b.) Infected groups

Serum proteins estimates in infected chickens		% of incidence frequences among 6 infected chickens infected via : $\bar{x}$± S.E. , (Range in brackets) for estimates.		
		intra air sac route	intra nasal route	intra tracheal route
Total serum proteins g/dl	i	in 83% (5/6) 2.48 ± 0.082 (2.40 - 2.60)	in 33% (2/6) average 2.55 (2.5 - 2.6)	in 33% (2/6) average 2.55 (2.50 - 2.60)
	ii	in 17% (1/6) 2.8. + 4%*	in 67% (4/6) 2.78±0.048 (2.70-2.90) +5%*	in 67% (4/6) 2.925 ± 0.075 (2.80 - 3.10) +11%*
Pre-albumin mg/dl	i	in 100% (6/6) 10.62 ± 0.363 (0 - 31.400)	in 100% (6/6) 29.37± 0.721 (0 - 71.968)	in 100% (6/6) 26.32± 0.636 (0 -57.930)
	ii	—	—	—
Albumin g/dl	i	in 67% (4/6) 0.598±0.025 (0.516 - 0.675	in 50% (3/6) 0.552 ± 0.026 (0.504 - 0.595)	in 50% (3/6) 0.671 ± 0.047 (0.521 - 0.754)
	ii	in 33% (2/6) average 0.412 (0.408 - 0.415) - 2%*	in 50% (3/6) 0.413 ± 0.002 (0.410 - 0.415) -17%*	in 50% (3/6) 0.403 ± 0.012 (0.379 - 0.416) -19%*
Total globulins g/dl	ii	in 67% (4/6) 1.876 ± 0.057 (1.777 - 1.986)	in 17% (1/6) 1.971 .	in 17% (1/6) 2.006.
	i	in 33% (2/6) average 2.183 (2.143 - 2.223) +12%*	in 83% (5/6) 2.218 ± 0.075 (2.028 - 2.386) 13%*	in 83% (5/6) 2.283 ± 0.0576 (2.120 - 2.421) + 18%*

i = within the normal range
ii = significantly deviated from normal

Table (3 a,b): Statistical analysis of alpha$_1$-glob.,alpha$_2$-glob.and B-glob. Estimates in sera groups of control and infected chickens at the 1st week of infection.

a) Control group

Statistical parameters		alpha$_1$-glob. mg/dl.	alpha$_2$-glob. mg/dl.	B-glob. mg/dl.
Arith. mean ($\bar{x}$) ± S.E.		(339.420±30.113)	(343.707±8.196)	(292.692±23.165)
Measurs of individual variability	Range	(2.002-2.429)	(324.960-368.508)	(250.628-404.880)
	S.D.	73.762	20.077	56.743
	C.V. %	21.73%	5.84%	19.39%
95% confidence limite of universal mean.		(262.011-416.829)	(322.637-364.777)	(233.144-352.240)
Expected limits of the normal range of individual estimates		(232.424-446.416)	(314.584-372.830)	(210.308-375.001)

Table (3) b. Infected groups

Serum proteins estimates in infected chickens		% of incidence frequences among 6 infected chickens infected via : $\bar{x}$ ± S.E. , (Range in brackets) for estimates.		
		intra air sac route	intra nasal route	intra tracheal route
alpha-1 glob. mg/dl	i	in 67% (4/6) 337.755 ± 27.451 (291.004 - 463.435)	in 100% (6/6) 370.029 ± 15.118 (262.247 - 407.670)	in 83% (5/6) 259.128 ± 17.291 (233.103 - 327.00)
	ii	in 17% (1/6) 463.435 ± 4%* in 17%130.598 -44%*	——	in 17% (1/6) 449.267 . incre+ 8%*
alpha-2 glob. mg/dl	i	in 50% (3/6) 322.225 ± 2.410 (315.456 ± 328.644)	in 33% (2/6) average 354.561 (348.551 - 360.570)	——
	ii	in 50% (3/6) 405.771 ± 17.853 (377.524-438.809) +11%*	in 67% (4/6) 418.571 ± 9.065 (395.037-438.450) +15%*	in 100% (6/6) 430.779 ± 19.149 (390.212-499.99) +18%*
beta glob. mg/dl	i	in 830.% (5/6) 280.648 ± 26.070 (210.82-346.491)	in 100% (6/6) 268.679±5.073 (249.021 - 284.640)	in 33% (2/6) average 329.414 (300.207-358.621)
	ii	in 17% (1/6) 437.710 + 0.056 +17%*	——	in 67% (4/6) 485.880 + 30.05 (420.521-562.499) +38%*

i = within the normal range
ii = significantly deviated from normal

Table (4 a,b): Statistical analysis of alpha$_1$-glob , alpha$_2$ -glob and B-glob. Estimates in sera groups of control and infected chickens at the 4th weeks of infection.

a) Control group

Statistical parameters		alpha -glob. mg/dl.	alpha -glob. mg/dl.	B-glob. mg/dl.
Arith. mean ($\bar{x}$) ± S.E.		(260.184±23.477)	(285.175±14.542)	(242.933±15.912)
Measurs of individual variability	Range	(161.80-301.151)	(242.213-332.262)	(187.325-286.948)
	S.D.	57.506	35.621	38.978
	C.V. %	22.10%	12.49%	16.04%
95% confidence limite of universal mean		(199.835-320.533)	(247.793-322.557)	(202.029-283.837)
Expected limits of the normal range of individual estimates		(176.768-343.600)	(2.3.505-336.845)	186.395-299.471

Table (4) b. Infected groups

Serum proteins estimates in infected chickens		% of incidence frequences among 6 infected chickens infected via $\bar{x}$ ± S.E., (Range in brackets) for estimates.		
		intra air sac route	intra nasal route	intra tracheal route
alpha-1 glob. mg/dl.	i	in 33% (2/6) average 269.832 each chicks	in 100% (6/6) 217.832 ± 6.672 (195.681-239.025)	in 83% (5/6) 235.454 ± 19.190 (204.167-306.000)
	ii	in 67% (4/6) 373.674 ± 16.369 (344.456-419-216) +17%*	———	in 17% (1/6) 350.7 +9%*
alpha-2 glob. mg/dl.	i	in 83% (5/6) 276.811 ±12.478) (247.032-303.675)	in 33% (2/6) average 292.185 (273.505 - 310.864)	in 17% (1/6) 288.88
	ii	in 17% (1/6) 340.116 + 1%*	in 67% (4/6) 380.462 ± 16.616 (340.314-420.611) +18%*	in 83% (5/6) 398.809 ± 30.932 (347.848-499.00) 24%
B glob. mg/dl.	i	in 100% (6/6) 221.800 ± 11.171) (187.025-259.700)	in 67% (4/6) 250.532 ± 6.650 (233.148 - 264.005)	in 50% (3/6) 221.589 ± 17.66) (190.121-251.196)
	ii	———	in 33% (2/6) average 360.540 (300.470-420.611) +27%*	in 50% (3/6) (343.342 ± 4.324 (322.027 - 358.00) +21%*

i = within the normal range
ii = significantly deviated from normal

Table (5 a,b): Statistical analysis of total Gamma-glob. , transferrin , IgG and IgM estimates in sera groups of control and infected chickens at the 1st week of infection.

a) Control group

Statistical parameters		Total Gamma-glob. g/dl	transferrin mg/dl	IgG mg/dl	IgM (mg/dl)
Arith. mean ($\bar{x}$) ± S.E.		(1.201±0.055)	(154.261±11.67)	(809.595±38.814)	(238.422±19.547)
Measurs of individual variability	Range	(1.042-1.414)	(125.307±191.940)	(699.8-950.970)	(173.275-290.87)
	S.D.	0.134	28.592	95.074	47.879
	C.V. %	11.16%	18.53%	11.74%	20.08%
95% confidence limite of universal mean		(1.060-1.341)	(124.262-184.272)	(709.820-909.370)	(188.175-288.669)
Expected limits of the normal range of individual estimates		(1.006-1.395)	(112.793-195.741)	(671.684-947.506)	(168.97-307.874)

Table (5) b. Infected groups

Serum proteins estimates in infected chickens		% of incidence frequences among 6 infected chickens infected via : $\bar{x} \pm$ S.E., (Range in brackets) for estimates.		
		intra air sac route	intra nasal route	intra tracheal route
Total γ-globs. g/dl	i	in 67% (4/6) 1.249 ± 0.047 (1.166 - 1.377)	in 33% (2/6) Average 1.330 (1.325 - 1.335)	in 50% (3/6) 1.202 ± 0.025 (1.133 - 1.272)
	ii	in 33% (2/6) average 1.512 +13%*	67% (4/6) (1.493 ± 0.033) (1.450-1.587) +11%*	in 50% (3/6) 1.649 ± 0.015 (1.601-1.674) +23%*
Transferrin mg/dl.	i	——	in 67% (4/6) 164.359 ± 7.732 (148.29 - 183.019)	in 17% (1/6) 129.064 .
	ii	in 100% (6/6) 244.292 ± 14.068) (196.846-283.257) +33%*	in 33% (2/6) average 204.015 + 4%*	in 83% (5/6) 290.444 ± 18.584 (209.310-364.105) 58%*
IgG mg/dl.	I	in 83% (5/6) 779.615 ± 23.805 (729.450 - 868.770)	in 100% (6/6) 878.098 ± 24.005 (765.350 - 932.640)	in 50% (3/6) 715.361 ± 19.909 (681.575-778.280)
	ii	in 17% (1/6) 958.895 . +1%*	——	in 50% (3/6) 993.143 ± 8.805 (993.060-100.685) +5%*
IgM mg/dl.	i	in 50% (3/6) 229.828 ± 14.649 (197.950-274.876)	in 17% (5/6) 276.81 .	in 33% (2/6) average 291.950 (284.250-299.650)
	ii	in 50% (3/6) 335.790 ± 7.056) (313.786-349.990) +16%*	in 83% (5/6) 404.096±24.854 (415.200- 471.598) +40%*	in 67% (4/6) 311.302 ± 2.189 (3.8.03-317.698) +8%*

i = within the normal rang
ii = significantly deviated from normal

Table (6 a,b) : Statistical analysis of total γ- glob. , transferrin , IgG , and IgM in sera groups of control and infected chickens at the 4 th weeks of infection.

a- control group

Statistical parameters	Total γ-glob-g/dl	transferrin mg/dl	IgG mg/dl	IgM mg/dl
Arith. mean ($\bar{x}$) ± S.E.	0.984 ± 0.060	177.221 ± 7.197	622.625 ± 44.558	184.492 ±11.988
Measures of individual variability Range	(0.708-1.143)	(137.800-246.132)	(407.456-687.933)	(139.992-221.856)
±S.D	0.147	37.772	109.144	29.365
C.V %	14.99%	21.31%	17.52%	16.00%
95% confidence limite of universal mean.	(0.828-1.138)	(158.72-195.72)	(508.08-737.16)	(152.675.68-214.31)
Expected limites of the normal range of individual estimates.	(0.769-1.197)	(101.29-253.15)	(464.31-780.95)	(140.896-226.09)

Table (6) b. Infected groups

Serum proteins estimates in infected chickens		% of incidence frequences among 6 infected chickens infected via : $\bar{x} \pm$ S.E. , (Range in brackets) for estimates.		
		intra air sac route	intra nasal route	intra tracheal route
Total γ-globs. g/dl.	i	in 67% (4/6) 1.074 ± 0.018 (1.046 - 1.122)	in 33% (2/6) average 1.117 (1.062 - 1.171)	in 17% (1/6) 1.005 .
	ii	in 33% (2/6) average 1.242 (1.227-1.256) +5%*	in 67% (4/6) 1.439 ± 0.056 (1.282-1.543) +20%*	in 82% (5/6) 1.382 ± 0.062 (1.238-1.543) +15%*
Transferrin mg/dl.	i	in 100% (6/6) 215.134 ± 12.079 (189.048 - 251.784)	in 100% (6/6) 170.080 ± 11.604) (128.518 - 204.015)	in 67% (4/6) 1731.131 ± 15.373 (138.992-211.050)
	ii	—	—	in 33% (2/6) average 269.795 (269.111-270.478) +7%*
IgG mg/dl.	i	in 67% (4/6) 647.512 ± 28.411 (604.272 - 723.975)	in 33% (2/6) 684.504 . (675.770 - 693.238)	in 50% (3/6) 701.954 ± 23.090 (631.344-753.366)
	ii	in 33% (2/6) average 797.889 (784.925-810.852) +2%*	in 67% (4/6) 890.098 ± 30.829 (787.150-901.494) +14%*	in 50% (3/6) 879.02 ± 29.929 (804.00-932.580) +13%*
IgM mg/dl.	i	in 50% (3/6) 196.130 ± 3.857 (190.032 - 208.325)	—	—
	ii	in 50% (3/6) 238.119 ± 2.348 (233.324-245.425) +11%*	in 100% (6/6) 356.809 ± 33.435 (239.902-455.336)+ 66%*	in 100% (6/6) 323.053 ± 26.703 (234.44-455.336) +51%*

i = within the normal rang
ii = significantly deviated from normal

Table (7) : Correlation and regression analysis between serum IgM and IgG and grading scores of agglutinating titres at the 1st week in mycoplasma infected chickens and between these two immunoglobulins and grading scores of inhibition zone in serum at the 4th week old in infected chickens.

Statistical parameters	Igs and grading scores of agglut: IgM	IgG	Igs and grading scores of HI IgM	IgG
(r)	+0.953*	+0.7927	+0.9602**	+0.9593**
% degree of correlation	90.9%*	—	92.20%**	92%**
(P)	<0.02	>0.05	<0.01	<0.01
(b)%	↑+0.283%	--	↑ +0.18%**	↑ +0.22%**

r = Correlation coeffecient
P = Propability level
b = regression coeffecient.
* Significant
** highly significant.
HI = Haemagglut. inhibition.

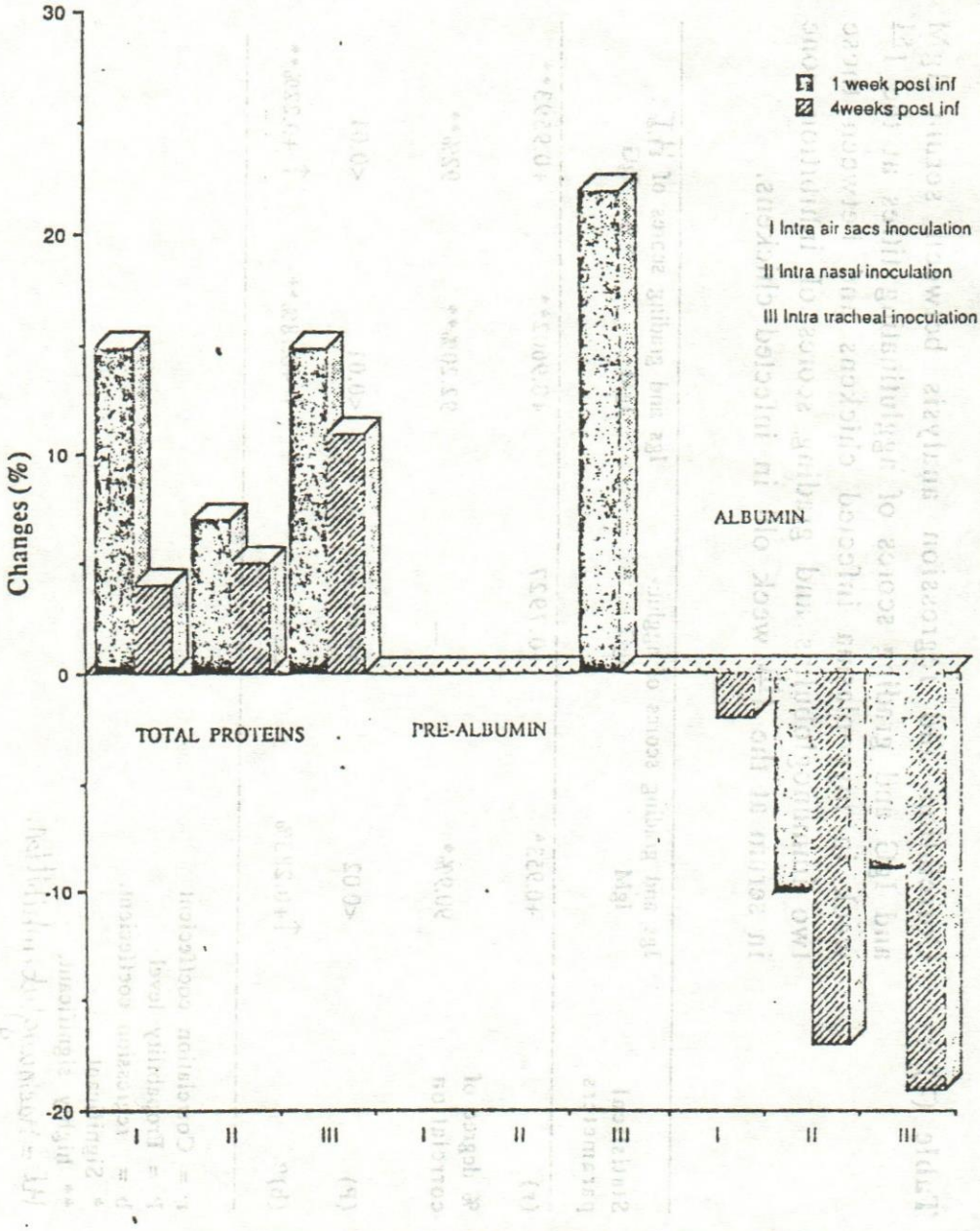

Fig. (1) Mean % deviations from the conventional limits of the normal range for total serum proteins and its various fractions of pre-albumin and albumin in *Mycoplasma gallisepticum* infected chickens.

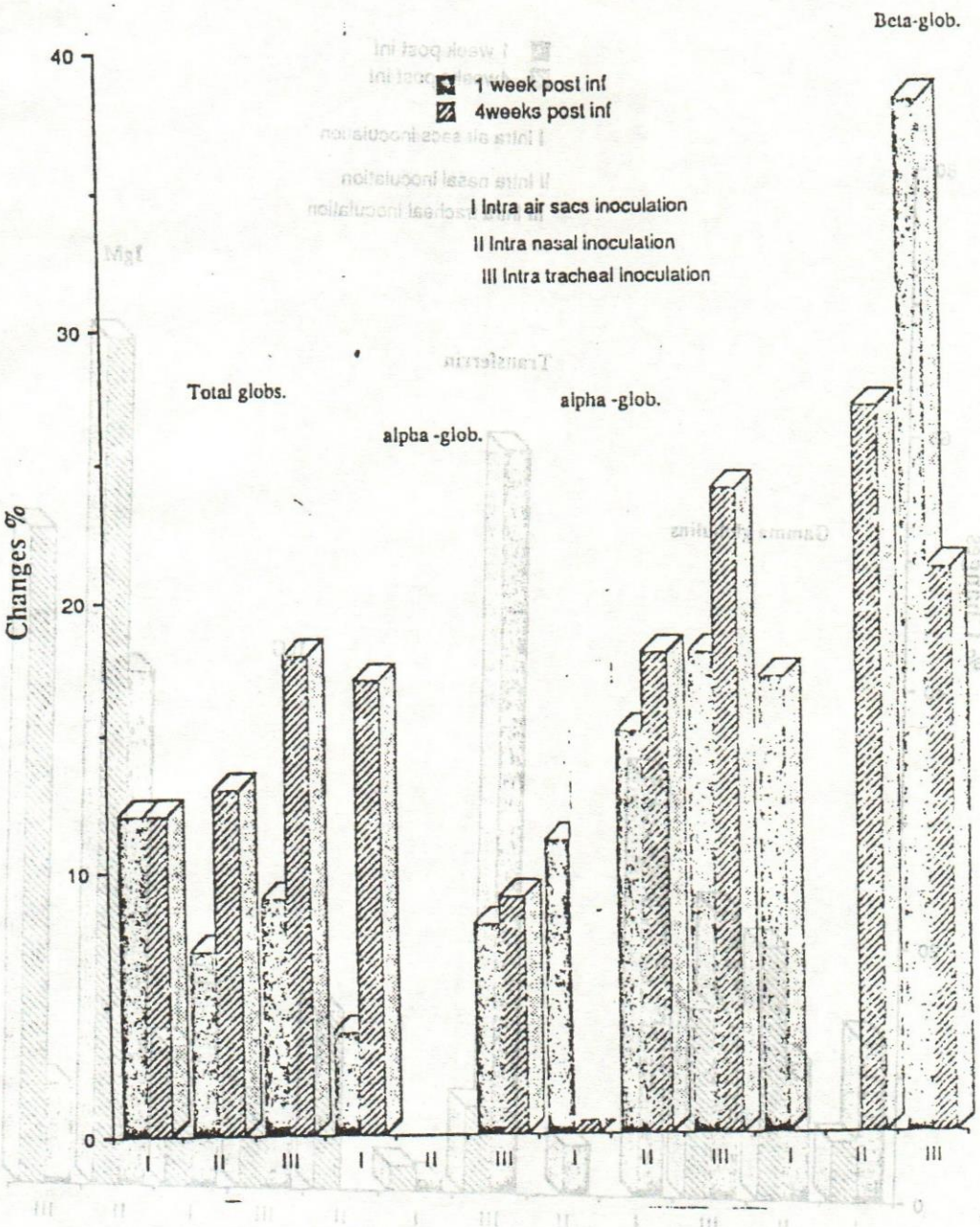

Fig. (2) Mean % deviations from the conventional limits of the normal range for α_1, α_2 and β-globulins in *M. gallisepticum* infected chickens

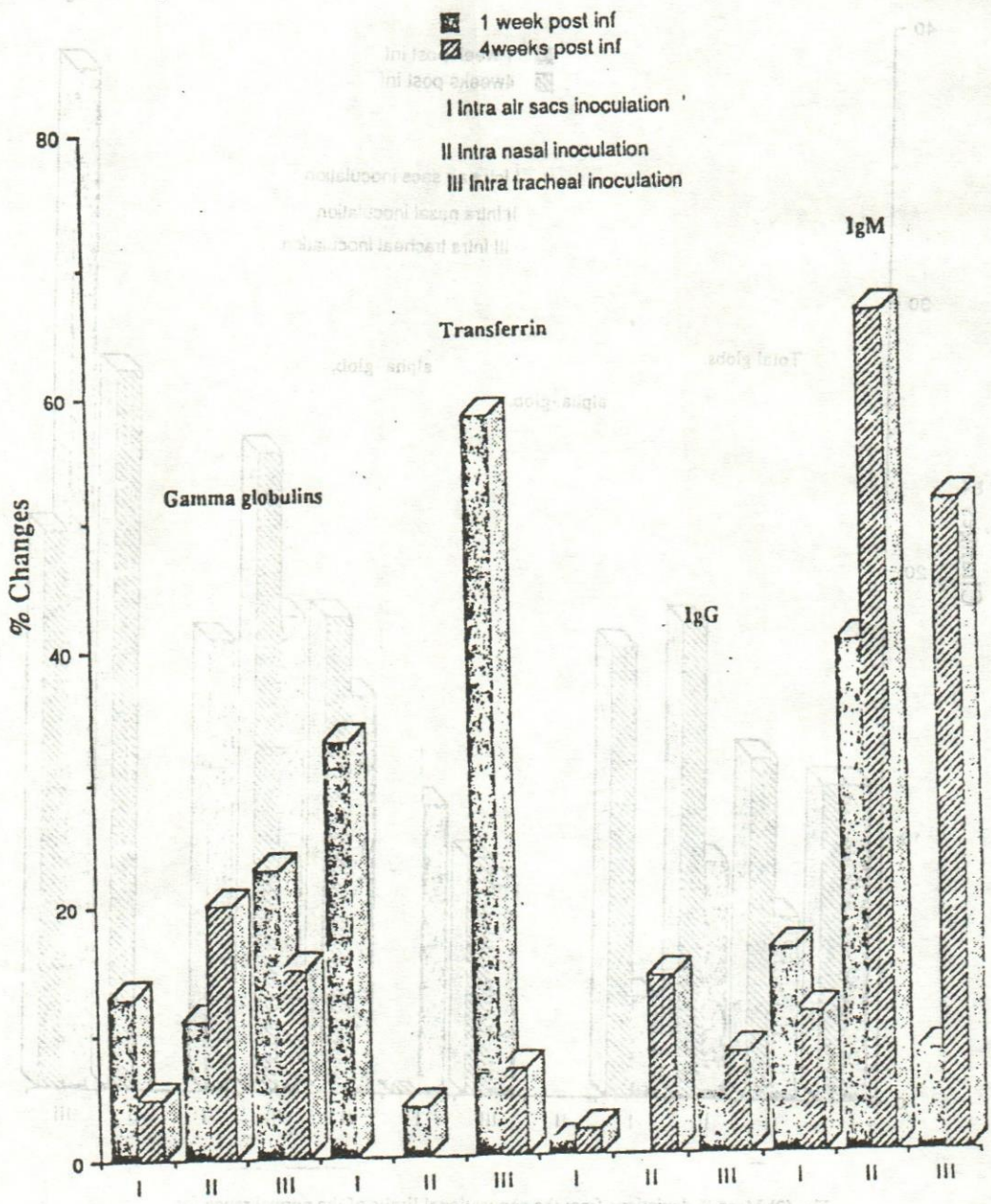

Fig. (3) :Mean % deviations from the conventional limits of the normal range for total Gamma glob. and its various fractions (transferrin ,IgG and IgM) in *M. gallisepticum* infected chickens.