INFLAMMATORY REACTION AGINST *MYCOPLASMA GALLISEPTICUM* INFECTION IN BROILER

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ABSTRACT

This study was carried out to investigate the Inflammatory reaction in broiler chicken after experimental infection with *Mycoplasma gallisepticum(MG)* , which were evaluated by using Enzyme Linked Immunosorbent Assay(ELISA) to measure pro inflammatory cytokines and IgG. one hundred day old broiler chicks were randomly divided into five groups 20 birds each group, all groups except group 5 (control) were infected at two weeks old with 0.2ml of 10^6 cfu/bird of local MG ,groups treated as follows: Group1 : infected Intravenous(I.V) with MG isolate. Group2: : infected Intraocular (I.O) ,Group3: infected Intranasal (I.N),Group4: : infected Intratracheal (I.T) infection, Group5: were not: infected with MG isolate. Blood samples were collected at 1,2,3and 4 weeks post infection to measure Interleukin 1(IL1),Tummer necrosis factor (TNF),Interferon gamma(IFG) used ELISA, also IgG was measure ELISA test at 2,3and 4 weeks after infection . The results of Serological test showed the infected groups with MG revealed significant increase in the levels of IL-1, IFG, TNF (pro inflammatory cytokines) and IgG production compared with control group ,the group 4(I.T) had higher level of these pro-inflammatory cytokines and Immunoglobulins more than other infected groups in all period post infection. From these result, it was concluded that the local isolate of *Mycoplasma gallisepticum* has the ability to in induce inflammatory reaction in infected broiler chicken.

Keywords: Mollicutes,CRD, cytokines, Immunoglobulins ,ELISA
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التفاعل الالتهابي ضد الإصابة التجريبية بجراثيم مايكوبلازما كاليسبتيكم في دجاج اللحم

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المستخلص

اجريت هذه الدراسة للتحقق في التفاعل الالتهابي للاصابة التجريبية بجراثيم المايكوبلازما كاليسبتيكم لدجاج اللحم ولهذا الغرض تم استخدم 100 فرخ بعمر يوم واحد وقسمت عشوائيا الى خمسة مجاميع وكل مجموعة عشرون فرخ وتم اصابة كل المجاميع ماعدا المجموعة الخامسة تركت كمجموعة سيطرة عند عمر اسبوعين بجراثيم المايكوبلازما كاليسبتيكم وبجرعة 0,2مل من 10^6خليةوبطرق مختلفة وكالاتي المجموعة الاولى اصيبت عن طريق الوريد والمجموعة الثانية عن طريق العين والمجموعة الثالثة عن طريق الانف والمجموعة الرابعة عن طريق الرغامي اما مجموعة السيطرة لم تعطى اصابة تم جمع عينات الدم بعد او2و3و4 اسبوع بعد الاصابة لقياس تراكيز الانتيرلوكين وعامل النخر والانتيرفيرون كاما وبعد 2و3و4اسبوع لغرض قياس الكلوبيولين المناعي G اظهرت نتائج الفحوصات المصلية ان المجاميع المصابة تجريبيا بجراثيم MG المجموعة الاولى ,الثانية,الثالثة والرابعة زيادة معنوية بتراكيزالانترلوكين-1 IL-1 وعامل نخر الورمTNFوانترفيرون غاما IFG والكلوبيولين المناعي IgG مقارنة بمجموعة السيطرة وكانت المجموعة الرابعة المصابة عن طريق الرغامي الاعلى بين المجاميع المصابة في جميع الفترات بعد الإصابة نستنتج من نتائج الدراسة الحالية ان لجراثيم المايكوبلازما كاليسبتيكم المعزولة محليا القدرة على احداث تفاعل التهابي في دجاج اللحم.

الكلمات المفتاحية: المرض التنفسي المزمن بالدجاج, رقيقة الجلد,السايتوكينات,الكلوبيولينات المناعية وفحص الاليزا
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INTRODUCTION

Avian Mycoplasmosis was primarily described in turkeys in 1926, and in chickens in 1936 (5).Mycoplasmas (class Mollicutes) are found in humans, many animal species, plants, and insects. These prokaryotes are characterized by their very small size, small genome, and complete absence of cell walls; they are bound by a plasma membrane only (22,23) *Mycoplasma gallisepticum* (*MG*) *and M. synoviae* (*MS*) *are the* most important; they are the only ones listed by the Office International des Epizooties OIE (19) *MG* is a significant poultry pathogen involved in severe economic losses of the poultry industry due to a reduction in egg production, hatchability and downgrading of carcasses(18). Both horizontal and vertical disease transmission leads to rapid spreading of this pathogen in flocks,this respiratory and reproductive tract pathogen can cause severe chronic respiratory disease (CRD) when present in concert with other poultry pathogens including Newcastle disease virus, Infectious bronchitis virus and E. coli (26,13). *M. gallisepticum* infection in chickens is characterized by coughing, nasal discharge, respiratory rales, mucous production leading to blockade of tracheal lumen, difficulty breathing and sometimes conjunctivitis (21). More recently *M.gallisepticum* was found to cause ocular infection in house finches leading to significant decline in house finch populations(30). Early studies investigating immune response to this pathogen identified that bursectomy and thymectomy of chickens significantly increased their susceptibility to infection suggesting the important role of B and T cells(1) Whereas innate immune responses are critical in early response and control of infection.(16). *Mycoplasma gallisepticum* infection in chickens is associated with severe inflammation of the trachea, air sacs and lungs (21).The aim of this study is to investigate the effect of experimental infection with *Mycoplasma gallisepticum* on induction of interleukin 1,interfern gamma tummer necrosis factor(pro-inflammatory cytokines) and immunoglobulinG (IgG).

MATERIALS AND METHODS

Preparation of poultry house: The experiment was conducted at animal house in the College of Veterinary Medicine University of Baghdad. Experiment house was prepared before the beginning of the experiment by washing, cleaning and disinfecting by formalin. The temperature was controlled by using brooders. The ground was covered by a litter containing wood mince 10 cm thick. All the chickens were related to the same breeding management. feeders and water utensils were cleaned and disinfected, commercial ration and tap water were used freely in this experiment.(3).

paration of inoculum of local strain *Mycoplasma gallisepticum*

PPLO (pleuro pneumonia like organism) broth and agar media were used for preparation of inoculum as well as viable count of the organism. Field strain grown in the broth medium for (24-48) hours at 37°C, and diluted with PPLO broth to provide viability of 10^6 colony-forming units (cfu)/ml. The dose of inoculum was 0.2 ml (10^6) colony forming units (CFU) per chick according to (32).

Design of the experiment

One hundred (at one day old) broiler chicks (Rose 308) was obtained from breed from hatchery were used to carry out this experiment was conducted at period 1 to 60 days . At the first day, five chicks(1-day-old) from the birds were selected for slaughtered and examined for pathological lesions as well as for the presence of mycoplasmas. No pathological lesions characteristic of mycoplasma infection was found and cultivation for mycoplasmas was negative, and divided randomly into equal five groups each group 20chicks which were treated as follows:

The first group: the chicks were inoculated Intra Venous (I.V) with *Mycoplasma gallisepticum* isolate

The second group: the chicks were inoculated Intra Ocular (I.O) *Mycoplasma gallisepticum* isolate

The third group: the chicks were inoculated Intra Nasal (I.N) infection with *Mycoplasma gallisepticum* isolate

The fourth group: The chicks were inoculated Intra Tracheal I.T infection with *Mycoplasma gallisepticum* isolate

The fifth group: The chicks did not inoculate with *Mycoplasma gallisepticum* MG isolate

All groups were infected at two weeks old with 0.2 ml of 10^6 (colony forming units per chick) of *Mycoplasma gallisepticum* (local strain) isolated from chicken infected with respiratory sign and confirmation by PCR and submitted in Gene bank database and have accession number:ID: MG846120.1,blood samples were collected from jugular vein at 1,2,3 and 4 weeks post infection to measure (IL1,TNF, I FG and IgG) and using sandwich-ELISA method.Blood samples were collected and let to clot ; sera were separated and stored at -20 until used for the tested by commercial ELISA kits, China. Wuhan Fine Biotech. CO., the tests were carried out according to the manufacturer's assay protocol and catalog number The data were analyzed statically using the Microsoft Program (29) and LSD. (LEAST Significant Differences) values were used to show level of significance

RESULTS AND DISCUSSION

The result of the present study showed a significant differences($P\leq0.05$) among all infected groups compared with control group in IL-1 concentrations explained in different periods (1,2,3 and 4weeks) after infection the highest mean concentrations showed in G4(I.T) in1,2,3 and 4weeks (17.45±1.34), (19.69±0.47), (51.59±0.35) and (32.72±1.14), respectively followed with another infected groups while the less level in the G5(control) which was(6.13±0.73 (9.64±0.40), (18.36±) and (15.14±0.56) respectively as show in table(1)also presence of significant difference at level ($P\leq0.05$) between all infected groups and control group in IFG concentrations at different period (1,2,3 and 4weeks) post infection the highest means level was present in (G4) was (20.33±1.70), (28.82±0.37), (51.44±1.12) and(36.60±1.0) respectively with a significant difference($P\leq0.05$) followed by another infected groups while the less concentration in the G5(control)which was(9.87±0.62). (14.53±0.50) **,** (15.54±0.6)and(11.76±0.5) Table 2. The result of TNF concentration explained in different period (1,2,3 and 4weeks) post infection, the highest mean appeared in (G4) (68.99±0.60), (96.00±1.41), (120.26±1.76) and (90.52±1.67) respectively while the less level was in the G5(control) 26.69±0.65, 41.44±1.03) ,(44.51±1.51)and (45.01±1.61).Table 3, In the present study,the IL-1 , TNF and interferon gamma concentration in all the infected groups with MG were higher than control group with significant differences in periods 1,2,3,4 weeks post infection these results was in concurrence with several studies invitro have demonstrated the induction of pro-inflammatory cytokines during *M.*gallisepticum infection , Majumder *et al*.,(15) was studied in vitro of conditioned medium from TECs(tracheal epithelial cells) and exposed to the virulent Rlow of MG strain induced macrophage chemotaxis to a much higher degree than the non virulent Rhigh strain of MG also co culture of chicken macrophages (HD-11) with TECs exposed to live mycoplasma revealed the upregulation of several proinflammatory genes associated with macrophage activation, including interleukin-1β (IL-1β), IL-6, IL-8, CCL20, macrophage inflammatory protein 1β (MIP-1β), CXCL-13, and Rantes, also (6) showed that in vitro infection of monocytes and macrophages caused induction of specific chemokines when *M. gallisepticum* lipid associated membrane proteins or LAMPs interact with chicken tracheal epithelial cells; Mycoplasmas express lipoproteins (LP) that interact with pattern recognition receptors (PRRs) including toll like receptors (TLRs) and NOD-like receptors this lead to induction of proinflammatory cytokines in chickens(14) Mycoplasmal lipoproteins have also been shown to play a role in macrophage activation or by activating the NLRP3**(** NACHT, LRR and PYD domains-containing protein 3) resulting in the induction of IL-1β (31)Mycoplasma infection often leads to extracellular release of ATP and activation of inflammasomes via ligation of ATP to P2X7 receptors and subsequent release of IL-1β, thereby contributing to the inflammatory response,(25,10) Macrophages and Monocytes have also been found to produce chemokines and cytokines upon infection to live mycoplasma or mycoplasma lipid associated membrane proteins (LAMPs) (27,28,12) chemokines and cytokines includes TNF-α, IL-1β, IL-6, MIP-1β(17).

Table 1. IL-1 concentration (pg/ml) in serum after experimental infection with MG in chickens for different periods

periods / groups	1week	2week	3week	4week
G1IntraVeinous infection with MG	C 9.76±0.66	B 17.37±0.60	C 31.39±0.83	C 16.89±0.89
G2 Intraocular infection with MG	BC 11.22±0.74	D 11.70±0.42	B 44.76±0.91	B 28.62± 0.68
G3IntraNasal infection with MG	B 13.20±0.73	C 14.63±0.95	C 31.80±0.59	C 16.83± 0.36
G4IntraTracheal infection with MG	A 17.45±1.34	A 19.69±0.47	A 51.59±0.35	A 32.72± 1.14
G5/Control	D 6.136±0.73	D 9.64±0.40	D 18.36±0.58	C 15.14± 0.56

Means having with the different capital letters (A, B, C) in same column significantly differences(P<0.05

Table 2. IFG concentration (pg/ml) in serum after experimental infection with MG in chickens for different periods

Period / Group	1week	2week	3week	4week
G1 Intra Venous infection with MG	B 14.77±0.88	A 27.48±1.02	B 41.01±0.89	B 29.40±1.32
G2 Intra ocular infection with MG	C 12.65±1.37	C 16.66±1.03	D 27.72±0.69	C 21.58±0.46
G3/ Intra nasal infection with MG	C 12.29±1.02	B 24.39±1.43	C 32.39±1.03	C 21.72±0.49
G4/ Intratracheal infection with MG	A 20.33±1.70	A 28.82±0.37	A 51.44±1.12	A 36.60±1.07
G5/Control	D 9.87±0.62	C 14.53±0.50	E 15.54±0.6	D 11.76±0.544

Means with different capital letter in the same column significantly differences (P<0.05)

Table 3. TNF concentration (pg/ml) in serum after experimental infection with MG in chickens for different periods

Periods / Group	1week	2week	3week	4week
G1: Intravenous infection with MG	C 43.38±1.28	C 56.73±1.41	B 83.44±1.59	B 77.94±1.77
G2: Intra ocular infection with MG	D 34.09±0.66	B 72.58±0.50	C 70.12±1.30	C 59.02±1.29
G3: Intra nasal infection with MG	B 63.97±1.22	D 50.01±1.14	B 84.33±1.64	C 57.10±1.93
G4:Intratracheal infection with MG	A 68.99±0.60	A 9o.00±1.41	A 120.26±1.76	A 90.52±1.67
G5: control	E 26.69±0.65	E 41.44±1.03	D 44.51±1.51	D 45.01±1.61

Means with different capital letter in the same column significantly different (P<0.05).

The Table 4. showed presence of significant difference at level (P≤0.05) between all infected groups and control group in IgG concentrations in different period (2,3 and 4) weeks post infection the highest mean in (G4) which were (2.35±0.33), (4.74±0.24) and (2.08±0.43) respectively while the less concentrations in the G5(control)which were(1.13±0.31), (1.24±0.29) and(1.03±0.21) Table 4. This results comparable with many Authors used ELISA test were revealed increased level of immunoglobulin(IgG) in serum after infection with *Mycoplasma gallisepticum* (2,8,9) Others studies measure IgG pattern of mucosal immune responsiveness was observed for all three immunoglobulins isotypes measured (IgG, IgA and IgM anti-*M. gallisepticum)*, although IgA was the predominant immunoglobulins type present in nasal, tracheal and lung washes

(20) also The result similar to the results of (11)who showed that inoculated birds showed positive reactors 2 weeks earlier than the vaccinated. birds, possibly because the MG inoculum originated from a pathogenic isolate. The chemokines and inflammatory cytokines initially produced by epithelial cells upon interaction with *M. gallisepticum* lead to non-specifically stimulate B and T cells (24). B cells to be the pre-dominant cell types in tracheal submucosa of chicken after *M. gallisepticum* infection as early as 1 day post infection with various species of Mycoplasmas were found to elicit responses from all type of immunoglobulins classes including IgA, IgM, IgD, IgE and IgG (7). In the current study all routes of experimental infection induce production high concentration of IgG in serum than control group this result were consistent with the findings of many authors used different method of infecting with *Mycoplasma gallisepticum* including intratracheal and eye drop (29,3) also(4)detected positive reactors of *M. gallisepticum* by commercial ELISA kit from 6 to 7 weeks of age after challenge of chickens at third week of age with *M.gallisepticum* (10^6CFU/bird) via eye drop. Our findings suggested that infection of broiler with local strain of *Mycoplasma gallisepticum* at two weeks was able to induce inflammation response after 1week post infection

Table 4. IgG concentration (ng/ml) in serum after experimental infection with MG in chicken for different periods

Period / Groups	2weeks	3weeks	4weeks
G1 intravenous infection with MG	B 1.33±0.25	A 4.58±0.31	B 1.97±0.21
G2 intra ocular infection with MG	B 1.35±0.26	B 3.70±0.40	B 1.32±0.36
G3 intra nasal infection with MG	AB 1.49±0.33	B 3.23±0.36a	B 1.61±0.13
G4 intra tracheal infection with MG	A 2.35±0.33	A 4.74±0.24	A 2.08±0.43
G5/Control	B 1.13±0.31	C 1.24±0.29	B 1.03±0.21

Means with different capital letter in the same column significantly different (P<0.05).

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