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Impact of Mycoplasma gallisepticum vaccines in performance and biochemical testes in broilers chickens

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***Abstract*---**This study aimed to evaluate two vaccines of *Mycoplasma gallisepticum* (killed and live CRDF) in broiler chicks, 130 one day old broiler chicks were randomly divided into four groups,30 birds each group., treated as follows:G1 : were vaccinated with alive FMG vaccine (eye drop one drop 0.03ml/dose) , G2: the chicks were vaccinated with killed MG (0.3ml s.c)vaccine,G3: vaccinated with killed MG and alive MG vaccine G4:. control group not vaccinated., Blood samples were taken from the chickens at 21 and 35 days of age to assess their performance development (body weight, weight gains, feed intake, and feed conversion coefficient) as well as their liver enzymes (ALT, AST) and kidney function (creatinine) following immunization. At 21 and 35 days old, the results of the liver enzymes AST, ALT, and kidney function (creatinine) were non-significantly different between the vaccinated groups and the non-vaccinated group (control). Additionally, at 35 days old, there is a non-significant increase in all groups compared to 21 days old. The results of body weight and weight gain showed non-significant differences between all groups that received the MG vaccine (G1, G2, G3) and the G4 control group (not vaccinated) in various time frames (1week, 2week, 3week, 4week, and 5week). However, the results of feed intake showed significant differences($P \leq 0.05$) in G2 compared with G4, G3, and While all groups' results for 3week, 4week, and 5week did not differ significantly($P \leq 0.05$) , The feed conversion ratio results showed at 1 week there was a significant differences ($P \leq 0.05$) in G2compared with G4 and G1 respectively, G3 was recorded lower significant ($P \leq 0.05$) . while at 2,3,4 and 5 weeks ,none of the groups' differences were statistically significant. From these result, it was concluded that the vaccinated with killed and alive MG vaccines had no negative effects

on liver and kidney function also not caused side effect on performance growth.

***Keywords*---**Mycoplasma gallisepticum vaccines, biochemical testes, broilers chickens.

Introduction

The most significant pathogen of poultry commercially is *Mycoplasma gallisepticum* (MG), one of the Mycoplasma species that affects hens the most (Hennigana *et al.,* 2012; OIE, 2019; Ferguson-Noel,2020). Highly pathogenic MG infects poultry, leading to enormous financial losses in commercial poultry farms across the world, including increased feed intake and reduced weight gain. (Seifi *et al.*,2012;Raviv and Ley, 2013; Shiferaw *et al.*,2022). A MG infection results in avian respiratory mycoplasmosis, which can cause infectious sinusitis in turkeys and chronic respiratory illness in chickens , both of these diseases have the potential to raise mortality and slow growth. (Prajapati *et al.*, 2018; Yadav *et al.*, 2021) MG can spread horizontally through direct or indirect touch as well as vertically through the egg (dust, aerosol, etc.) (Ferguson-Noel and others, 2020) A bird's susceptibility to other illnesses is increased by the presence of MG, and these infections collectively result in death, stunted development, and carcass condemnation (Kempf et al., 1992; Asway et al.,2009). Avian mycoplasmosis can be identified by polymerase chain reaction (PCR), isolation, identification, and antibody detection. (Qasem *et al.*,2015;Mera *et al.*,2019) By immunizing hens with live, attenuated MG strains, which only cause minor infections and provoke an immune response startlingly similar to that caused by wild-type strains, MG transmission can be decreased. (Matucc et al.,2020). MG is the most common illness in Iraq, and numerous researchers have successfully isolated it from broiler and layer farms that had respiratory indications of infection (Ibrahim et al., 2015; Ali and Ali,2019; Abed et al.,2021; Flayyh, 2021) There are two forms of vaccination: live vaccinations like the TS-11, 6/85, and F strains, as well as dead vaccinations (bacterins) (Kleven,2008; Jacob et al.,2015; Ishfaq et al.,2020) The goal of this investigation was to evaluate the effect of these vaccines on biochemical test and performance growth when vaccination of broiler chickens at one day old. In Iraq, there were no data and studies concerning the evaluation of two commercial vaccines against M. gallisepticum that are enter Iraq.

Materials and Methods

Preparation of poultry house

The experiment was conducted at animals house in the College of Veterinary Medicine Baghdad University. Prior to the experiment's start, the experiment house was washed, cleaned, and formalin-disinfected. brooders were used to manage the temperature.10 cm of wood mince litter was spread throughout the ground,the same breeding management applied to each group,feeders and watering cans have been cleaned and sterilized.as previously mentioned by Burnham *et al.*, (2002) complete rations and tap water were made available, and feed and water were given as needed.

The experiment

This experiment was conducted to study the efficacy of MG vaccine in 120 chickes, 1day old broiler chicks ((ross308) breed from (Al-tajy hatchery) were used to carry out this experiment from 1 to 35 days and At the first day,divided randomly into equal four groups each group 30 chicks . At the first day,treated as follows:

The first group G1: the chicks were vaccinated with alive MG vaccine(CRDF) (eye drop one drop 0.03ml/dose)
The second group G2: the chicks were vaccinated with killed MG (0.3ml s.c) vaccine
The third group G3: the chicks were vaccinated with killed MG and alive MG vaccine
The fourth group G4: control group Not vaccinated

Liver enzymes and kidney function

At 21 and 35 days old, 10 blood samples were randomly drawn from each group to assess the levels of the liver enzymes Alanine Aminotransferase (ALT) and Aminotransferase (AST). Reitman and Frankel modified the colorimetric technique created by Tonhazy, White, and Umbreit to determine the activity in serum at 505 nm also to measure concentration of creatinine to evaluate kidney function were using of Creatinine and alkaline picrate undergo a colorimetric reaction (Jaffe reaction) that is kinetically monitored at 490 nm (490-510),according to Manufacturer:Anufacturer:Biolabo SA .

Performance growth parameter

Body weight

Chick's weight was calculated weekly by taking chicks randomly including 10 chicks from each group and to obtain average chicks weight the total weight of each group divided by the number of weighted chicks.

Weight gain

The weight gain was calculated using the formula below:
Body weight at the end of the week minus body weight at the beginning of the week equals weight gained over the week.

Feed Intake

The weight of the consumer feed at the end of the week =feed weight added throughout the week-the weight of the remaining feeder during the time..

Ratio of Feed Conversion (FCR)

"Is the consumption of each bird of feed to produce one unit of live weight (meat)" and was measured by applying the following equation (AL-Zubaydi, 2016).

$$FCR = \frac{\text{Finally average feed intake (g)}}{\text{Finally average weight gain (g)}}$$

Statistical analysis

The data were statistically analyzed using SAS (Statistical Analysis System - version 9.1).The two-way ANOVA and Least Significant Differences (LSD) post hoc test were performed to evaluate whether there were statistically significant differences between the means.Use P0.05 to assess statistical significance(SAS.2010).

Results

The Result of liver enzymes aspartate aminotransferase (AST) , alanine transaminase (ALT) and kidney function(creatinine) were show non-significant deference between vaccinated groups and not vaccinated group (control) at 21 and 35 day old , also at 35 day old there's non-significant increased in all groups compared with 21 day old as tables 1,2 and 3 respectively ,

Table(1); Results of effect of CRDF vaccine and killed vaccine of *Mycoplasma gallisepticum* on levels of liver enzymes (AST) (Mean ±SE) in serum

Groups/ AST	21day old	35 day old
G1	137.50±7.21 Aa	146.20±8.31Aa
G2	130.43±7.49 Aa	139.74±2.95Aa
G3	138.17±9.71Aa	151.54±7.40Aa
G4 control	120.21±6.20 Aa	130.55±4.52Aa
LSD	22.76	

Means with a different small letter in the same column are significantly different (P<0.05).
Means in the same row with different capital letters are substantially different (P<0.05).

Table(2); Results of effect of CRDF vaccine and killed vaccine of *Mycoplasma gallisepticum* on levels of liver enzymes (ALT) (Mean ±SE) in serum

Groups/ ALT	21day old	35 day old
G1	A28.02±3.08a	A33.35±2.37a
G2	A28.13±2.13a	A33.26±2.45a
G3	A29.92±2.20a	A33.79±2.80a
G4control	A27.41±1.57a	A31.49±1.55a
LSD	6.56	

Means in the same column with different tiny letters are substantially different (P 0.05).
Means in the same row with different capital letters are substantially different (P 0.05).

Table(3); Results of effect of CRDF vaccine and killed vaccine of *Mycoplasma gallisepticum* on Levels of creatinine (Mean ±SE) in serum

Groups/ Creatinine	21day old	35 day old
G1	0.44±0.03 Aa	0.43±0.03 Aa
G2	0.41±0.02 Aa	0.35±0.04 Aa
G3	0.46±0.05 Aa	0.45±0.04 Aa
G4control	0.35±0.03 Aa	0.32±0.02 Aa
LSD	0.14	

Means in the same column with different tiny letters are substantially different (P 0.05).
Means in the same row with different capital letters are substantially different (P 0.05).

For all groups, the mean BW was shown in Table (4)., results showed non-significant differences between all groups that were vaccinated with MG vaccine (G1, G2, G3) and G4 control group (not vaccinated) in different periods (1week, 2week, 3week, 4week and 5week) , The body weight gains results Table (5), revealed no differences that were significant at level (P 0.05). between all groups that were vaccinated with MG vaccine (G1, G2, G3) and(G4) control group (not vaccine) in the average weight gain through different periods (1week, 2weeks, 3weeks, 4weeks and 5weeks,The feed intake results Significant differences (P 0.05) were displayed in Table 6. in G2(275.00±5.37) compared with G4(260.90±5.81) ,G3(250.00±3.94) andG1(250.00±3.94) respectively. also 2week there was significant differences in G3(500.00 ±5.37) and G1(500.00±6.66) compared with G2(477.50±7.04),G4 (475.00 ±6.54) respectively. While in 3,4,5 weeks revealed no differences that were significant (P≤0.05 in all groups,The feed conversion ratio results Table (7), showed at 1 week there was a significant differences (P≤0.05) in G2(1.94±0.03) compared with G4(1.83±0.02) and G1(1.82±0.02) respectively, while G3 was recorded lower significant at level (P≤0.05) . while at 2,3,4 and 5 weeks there was non significant differences in all groups

Table(4); Results of effect of CRDF vaccine and killed vaccine of *Mycoplasma gallisepticum* on body weight (gram;Mean±SE)

	G1	G2	G3	G4	LSD
One day	45.60±1.20 A*	44.40±1.21 A	45.00±1.20 A	45.40±1.23 A	3.48
One week	182.50±2.81 A	186.00±3.71 A	184.00±3.05 A	187.10±3.174 A	9.19
Two week	421.00±7.37 A	415.20±8.02 A	431.00±7.77 A	420.00±6.83 A	21.6
Three week	748.00±13.400 A	743.00±14.62 A	746.00±13.84 A	750.00±14.98 A	40.8
Four week	1070.00±28.08	1085.00±26.92	1080.00±23.805	1090.00±23.82 A	73.8

	A	A	A		
Five week	1515.00±48.87 A	1530.00±53.85 A	1535.00±32.53 A	1550.00±37.41 A	126

*Non Significant (P≤0.05)

Table(5); Result of effect of CRDF vaccine and killed vaccine of *Mycoplasma gallisepticum* on weight gain (gram;Mean±SE)

	G1	G2	G3	G4	LSD
One week	136.90±2.93 A*	141.60±3.77 A	139.00±3.59 A	141.70±3.09 A	9.647
Two week	238.50±8.33 A	229.20±9.26 A	247.00±8.69 A	233.90±8.93 A	25.27
Three week	327.00±13.50 A	327.80±11.65 A	315.00±18.46 A	330.00±17.00 A	44.16
Four week	322.00±36.56 A	342.00±28.98 A	334.00±22.07 A	340.00±28.69 A	84.69
Five week	445.00±49.72 A	445.00±50.94 A	455.00±31.66 A	460.00±43.21 A	99.63

Non-Significant (P≤0.05)

Table(6); Result of effect of CRDF vaccine and killed vaccine of *Mycoplasma gallisepticum* on feed intake (gram;Mean±SE)

	G1	G2	G3	G4	LSD
One week	250.00±4.47 C	275.00± 5.37 A	250.00±3.94 C	260.90±5.81 B	14.21
Two week	500.00±6.66 A	477.50±7.04 B	500.00 ±5.37 A	475.00 ±6.54 B	18.45
Three week	645.00±6.98 A	652.80±6.97 A	650.50±3.65 A	645.50±6.19 A	17.51
Four week	650.00±9.62 A	670.00±5.91 A	655.00±8.75 A	658.00±7.07 A	20.75
Five week	900.50 ±6.68 A	910.50 ±7.04 A	905.00 ±4.83 A	890.00 ±5.00 A	21.13

Mean with different capital letters (A,B,C)in same column differed signifigant(P≤0.05)

Table(7); Result of effect of CRDF vaccine and killed vaccine of *Mycoplasma gallisepticum* on Feed conversion ratio (gram;Mean±SE)

	G1	G2	G3	G4	LSD
One week	1.82±0.02 AB	1.94±0.03 A	1.79±0.03 B	1.83±0.02 AB	0.08
Two week	2.09±0.05 A	2.08±0.05 A	2.02±0.04 A	2.03±0.06 A	0.16
Three week	1.97±0.05 A	1.98±0.05 A	2.07±0.11 A	1.95±0.10 A	0.25
Four week	2.01±0.27 A	1.97±0.21 A	1.96±0.14 A	1.93±0.17 A	0.59
Five week	2.02±0.32 A	2.04±0.44 A	1.98±0.16 A	1.93±0.31 A	0.61

Mean with different letters (A,B,C)in same column differed significantly (P≤0.05).

Discussion

The current study found that liver enzymes (ALT and AST) levels in the blood, as well as kidney function (creatinine), did not significantly differ between the vaccinated groups and the non-vaccinated group (control) at 21 and 35 days old (Coles, 1986). The liver contains large amounts of these enzymes, and damage to these organs increased plasma membrane permeability, releasing the enzymes into the circulatory system and raising enzyme levels in the blood. (Ramazzotto and Carlin, 1978; Rzuqi, 2018). 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(Cray *eta*l.,2008),in the current study the results of liver enzymes and kidney function were conferred that CRDF and killed vaccines of MG not caused damage in liver or kidney because not produced significant increased of these enzymes in serum and these results agree with Asway *et al.*,(2009) which suggested using the F-strain live vaccine of MG. to immunize hens against mycoplasmosis since it boosted the immune system without having any negative side effects. Creatinine is a waste product created by the partial conversion of creatine and phosphocreatine. As a result, the quantity of creatinine generated each day depends on factors such as muscle mass (and body weight), age, sex, food, and activity, and it does not significantly change day to day. Due to poor feed conversion ratio (FCR) of broilers and downgrading of carcasses of air sacculitis condemnations at processing, MG is a serious poultry pathogen that has a negative economic impact on the poultry industry. (Nneoma, 2016). This lowers carcass quality and exposes sick birds to additional infections (Ishfaq *et al.*, 2019). Broilers lost 20% to 30% of their body weight, experienced a(5–10%) mortality rate, a (10–20%) decline in food conversion efficiency, and a 10% to 20% mortality rate, according to slaughter statistics from Brazil's Federal Inspection Service.(Nascimento *et al.*,2005). After one week, two weeks, three weeks, four weeks, and five weeks, The hens' body weight (BW) and weight increase did not differ noticeably from one another. These results supported those of studies by Burnham *et al.*, (2002) and Peebles *et al.* (2012) and demonstrated that handling the birds and the side effects of vaccination had no negative effects on their bodies. In addition, feed intake revealed significant and non-significant differences at 1 week, with increases significant in G2 and G4 compared to G3

and G1 respectively. G3 and G1 levels increased significantly at 2 weeks compared to G2 and G4, respectively, whereas there were no significant changes at 3 weeks, 4 weeks, or 5 weeks. Overall feed. The results of feed conversion ratio significant and non significant differences, at1week there was showed increase significant G2 ,G4 and G1respectivaly, while G3 recored lower significant ,also showed the results in 2week, 3week, 4week, 5week non significant differences in all groups. in total conversion ratio recorded the result non significant in all groups,this results agreement with (Viscione *et al.* ,2008; Vance *et al.* ,2008;Jacob ,2014).

References

Abed, A.A,. Al-Iedani, A.A and Neamah, A.J(2021). Sequencing based phylogenetic analysis of local *Mycoplasma gallisepticum* of broiler chickens in Al-Dewaniyah Province / Iraq. Ann. Rom. Soc. Cell Bio, 25(5):2719–2738.

Ali,B. H. ,Ali,A.J. and Yosif,E.H.(2020)"Isolation and Molecular characterization of *Mycoplasma synoviae* from infected chickens with respiratory signs".The Iraqi Journal of Agricultural Science,51(5):1321–1328.

Ali,E.J...and Ali, B. H. (2019). Inflammatory reaction against *Mycoplasma gallisepticum* infection in broiler. The Iraqi Journal of Agricultural Science, 50(4), 1432-1438.

Alkhatib, M. M., & Mohmmad, M. F. (2022). Effect of adding sunflower seeds in broiler diet on serum lipids profile and some productive performance . *International Journal of Health Sciences*, *6*(S4), 8392–8405. https://doi.org/10.53730/ijhs.v6nS4.10575

Al-Zubaydi, J. H. (2016). Slope stability analysis of AL-Qadhi Cliff in AL-Qadissiya, Iraq. Iraqi Bulletin of Geology and Mining, 12(1), 53-64.

Asway , A.M.E.,Shlaby,H.A. ,Deeb, K.H. and Shalaby ,M.H.M.A. (2009) Serological studies on *Mycoplasma gallisepticum* in chickens. Animal Health Research Institute Volume 55, Issue 120, Page 1-24.

Burnham, M. R.; Branton, S.L.; Peebles,E.D. Lott, B. D. and Gerard, P. D. (2002) Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve weeks of age on performance and egg characteristics of commercial egg-laying hens. Poult. Sci. 81:1478–1485

Cole, B.C., Ward, J.R. and Martin, C.H. (1986) Hemolysin and peroxide activity of Mycoplasma species. J. Bacteriol., Vol. 95(6): 2022-2033.

Cray, C., Gautier, D., Harris, D. J., & Arheart, K. L. (2008). Changes in clinical enzyme activity and bile acid levels in psittacine birds with altered liver function and disease. Journal of Avian Medicine and Surgery, 22(1), 17–24.

Farfán, R. F. M., Zambrano, T. Y. M., Sosa, V. M. D., & Zambrano, R. A. M. (2021). Design and construction of a thermoelectric system for high performance computers. *International Journal of Physical Sciences and Engineering*, *5*(2), 25–33. https://doi.org/10.29332/ijpse.v5n2.1359

Ferguson-Noel, N., Armour, N. K., Noormohammadi, A. H., El-Gazzar, M.and Bradbury, J. M. (2020). Mycoplasmosis. Diseases of poultry, 907-965.

Flayyih,A.N.(2021).Detection of Antibodies to Mycoplasma gallisepticum by ELISA Test in Broiler chickens infected with chronic respiratory Disease in Nineveh Governorate.Higher diploma in Science of Veterinary Medicine /Poultry disease

Hennigan, S., Driskell, J., Ferguson-Noel, N., Dluhy, R., Zhao, Y., Tripp, R. and Krause, D.(2012). Detection and differentiation of avian mycoplasmas by

surface-enhanced Raman spectroscopy based on a silver nanorod array. Appl. Environ. Microbiol.78, 1930–1935. [Google Scholar] [CrossRef] [PubMed][Green Version]

Ibrahim, Z. Y.; Ali, B. H.; Saeed, N. M. and Rashid. P. M. A. (2015).Molecular and Clinical Study of Casea Ous Bronchopneumonia in Broilers. Indian Journal of Research Volume : 4 ,- 2250-1991.

Ishfaq, M., Hu, W., Khan, M.Z, Ahmad, I., Guo, W. and Li, J. (2020). Current status of vaccine research, development, and challenges of vaccines for Mycoplasma gallisepticum. Poult Sci. Sep;99(9):4195-4202. doi: 10.1016/j.psj.2020.06.014. Epub 2020 Jun 27. PMID: 32867963; PMCID: PMC7598112

Jacob R., Branton, S.L., Evans, J.D., Leigh, S.A and Peebles, E.D. (2015). Effects of different vaccine combinations against Mycoplasma gallisepticum on the internal egg and eggshell characteristics of commercial layer chickens 1,2,3. Poult. Sci;94:912–917. [PubMed] [Google Scholar].

Jacob, R., Branton, SL., Evans, J.D., Leigh, S.A. and Peebles, E.D. (2014).Effects of live and killed vaccines against Mycoplasma gallisepticum on the performance characteristics of commercial layer chickens. Poult Sci.

Kempf, I., Gesbert, M.,Bennejean, G. and Cooper, A.C. (1992): Efficacy of danofloxacin in the therapy of experimental mycoplasmoses in chicks. Research in Vet. Sci., 53 (2): 257-259.

Kleven, S. H. (2008). Control of avian mycoplasma infections in commercial poultry. Avian diseases, 52(3), 367-374.

Matucci, A., Stefani, E., Gastaldelli, M., Rossi, I., Grandi, G., Gyuranecz ,M. and Catania, S.(2020). Molecular Differentiation of Mycoplasma gallisepticum Outbreaks: A Last Decade Study on Italian Farms Using GTS and MLST. Vaccines (Basel).8(4):665. doi: 10.3390/vaccines8040665. PMID: 33182244; PMCID: PMC7712042.

Mera, U., and Mudasir, H.(2019). Prevalence of Mycoplasma gallisepticum (MG) antibodies in chicken in Sokoto, Nigeria. EAS J Vet Med Sci.1(5):58–59. [Google Scholar].

Nascimento, E.R.; Pereira V.L.A.; Nascimento M.G.F. and Barreto M.L. (2005). Avian mycoplasmosis update. Brazilian Journal of Poultry Science. Volume 7/No.1/pages 01-09.

Nneoma, O. (2016). Avian Mycoplasmosis: A Review IOSR Journal

Peebles, E.D. (2008). Effects of 6/85-strain *Mycoplasma gallisepticum* inoculation alone at ten weeks of age or in conjunction with F-strain *Mycoplasma gallisepticum* inoculation overlays at twenty-two or forty-five weeks of age on the performance of commercial egg-laying hens. Poult. Sci. 87:588–593.

Peebles, E.D. and Branton, S.L. (2012). "*Mycoplasma gallisepticum* in the performance and egg characteristics of commercial egg-laying hens. Poult. Sci. 87:588–593.

Prajapati, N., Subhashree, J., Siju,S.,Manjunath G.B., Reddy, R. Yogisharadhya and Patil, S.S. (2018). Prevalence of Mycoplasma gallisepticum and Mycoplasma synovae in Poultry-India Perspective Int.J.Curr.Microbiol.App.Sci 7(5): 2213-2220.

Qasem, J.A., Al-Mouqati, S.A., Al-Ali, E.M. and Ben-Haji, A. (2015). Application of Molecular and Serological Methods for Rapid Detection of Mycoplasma

gallisepticum Infection (Avian mycoplasmosis). Pak. J. Biol. Sci, 18, 81–87. [Google Scholar] [CrossRef]

Ramazzotto, L. J. and Carlin, R.(1978). Effects of DMSO on SGOT during hypothermia in adrenalectomized rats. Lifescience. 22: 329-336.

Raviv, Z.and Ley D.H.(2013). *Mycoplasma gallisepticuminfection.* In Diseases of Poultry, 13thEd. Swayne DE. editor.Wiley-Blackwell, Ames, Iowa, pp877–893.

Rzuqi, M. A. 2018. Effect of Anti-Riboflavin factor in the residues of the tomato paste industry on the Physiological and Biochemical traits of broilers. The Iraqi Journal of Veterinary Medicine, 41(2):24–30.

Seifi, B. S . and Shirzad, M.R. (2012). Seroprevalence and risk factors of Mycoplasma gallisepticum infection in Iranian broiler breeder farms. Int J Animal Vet Adv;4:45–48. [Google Scholar].

Shiferaw, J., Shifara, F., Tefera, M., Feyisa, A. and Tamiru, Y. (2022). Seroprevalence and Associated Risk Factors of Mycoplasma gallisepticum Infection in Poultry Farms of Hawasa and Bishoftu, Central Ethiopia. Veterinary Medicine: Research and Reports, 13, 101.

Statical Analysis System,User،s Guide.(2010) Statical.Version 9.th ed .SAS.Inst.Inc.Cary.N.C.USA.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, *5*(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938

Vance, A.M., Branton, S.L., Collier, S.D., Gerard, P.D. and Peeble, E.D. 2008. Effects of time-specific F-strain *Mycoplasma gallisepticum* inoculation overlays on prelay ts11-strain *Mycoplasma gallisepticum* inoculation on performance characteristics of commercial laying hens. Poult. Sci;87(2):655–660.

Viscione, K. A.,Branton, S. L., Vance, A. M., Gerard, P. D.,Whitmarsh, S. K. and Peebles, E.D. 2008. Effects of 6/85-strain *Mycoplasma gallisepticum* inoculation alone at ten weeks of age or in conjunction with F-strain *Mycoplasma gallisepticum* inoculation overlays at twenty-two or forty-five weeks of age on the performance of commercial egg-laying hens. Poult. Sci. 87(1):588–593.

Yadav, J.P., Tomar, P., Singh,Y. and Khurana S.K. (2021). Insights on *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in poultry: a systematic review. Anim. Biotechnol;10:1–10. doi: 10.1080/10495398.2021.1908316. [PubMed] [CrossRef] [Google Scholar].