Evaluation of antibody production and chIL6 levels after broiler vaccination with different Infectious bronchitis vaccine

Nashwan B. Kareem and Ammar M. Al-Aalim

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

nashwan.23vmp28@student.uomosul.edu.iq, 0009-0009-2207-3503

ammarmahmmod@uomosul.edu.iq, 0000-0001-5734-8815 , Corresponding author

Abstract

Background: The avian infectious bronchitis virus (IBV) is a widespread virus that specifically targets the upper respiratory tract, kidneys, and reproductive system, it has a huge economic impact on the poultry business, costing millions of dollars globally each year; there is a wide variety of IBV vaccines that protect against different serotypes of the virus but the absence of cross-protection between imported vaccine strains and field strains may explain the inability to implement an effective vaccination program against IBV.

Objective: the main aim was to evaluate the antibody production and IL6 level after broiler vaccination with two different IB vaccines.

Materials and Methods:

One hundred and sixty-one-day-old Ross 308 broiler chicks were divided into four groups (n=40), both live vaccine (Nobilis IB Ma5 (classical) and 4/91(variant serotype) strain were used to vaccinate different groups at 1 and 14 days, ELISA kit for both IB antibody and IL6 were used to estimate changes in this parameter in all experiment groups.

Results: a significant increase in antibody titer especially in the twenty-eighth-day post-vaccination in G3 (variant Nobilis 4/91 strain vaccine) and G4 (classical and variant vaccine), which amounted to (3765 ± 2.12, 2876.2 ± 1.92), while the second group (Classical Nobilis IB Mas5) recorded the highest antibody titers on the twenty-first day (2835.6 ± 3.50); also chIL6 also show continually increase with variations observed across the different vaccinated groups until the end of the experiment, particularly in G2 at the fourteenth day which reach highest concentration (64.48± 3.12).

Conclusion :

We conclude that all types of vaccination used in this study can elevate antibody titer but the Variant Nobilis 4/91 strain vaccine gives equivalent protection when used alone or in combination with classical Nobilis IB Ma5 strain vaccine also the combined vaccines of IB Ma5 and IB 4/91 variants can provide strong protection without negative interaction between tow vaccine on immune response, the study of interleukin 6 gives strong indication as it important influence in targeting immunity from humoral to cellular after boosting dose.
Keywords: IBV, Broiler, Vaccine, Antibody, IL6

Introduction

The poultry industry is crucial to the human food supply due to its quick production and low-cost protein sources. It is also a key economic sector, particularly in developing nations[1,2]. IB has a huge economic impact on the poultry business, costing millions of dollars globally each year due to the slaughter of diseased chickens[3]. Infectious bronchitis (IB) is an acute and highly contagious respiratory disease of chicken [4]. Avian infectious bronchitis virus (IBV) is a widespread virus that specifically targets the upper respiratory tract, kidneys, and reproductive system in chickens [5,6].

The IBV virus is a specific type of enveloped RNA virus that is single-stranded, positive-sense, and non-segmented. It is classified within the genus gamma coronaviruses of the Coronaviridae family [3,7,8]. The disease is associated with different clinical signs, including respiratory symptoms such as nasal mucous discharge, gasping, conjunctivitis, coughing, lacrimation, sneezing, and increased bronchial mucus[9,10]. Effected flocks can experience a morbidity rate of up to 100%. Secondary bacteria also contribute to raising mortality rates. Estimates suggest that between 25 and 30 % of young chicks will die, especially with the virologic strains which may potentially reach up to 80% [11,12].

the control of infection with infectious bronchitis is based on providing a strong biosecurity program for managing poultry farms, and vaccination; Many vaccines and vaccination programs are available to immunize and protect chickens against IB infection including uses either live or killed vaccines wide worldwide use[13,14,15]. Worldwide, there is a wide variety of IBV vaccines that protect against different serotypes of the virus; often, a combination of vaccines is administered to provide greater protection against viruses that are prevalent in a particular area[16]. IBV vaccines utilize live attenuated or inactivated vaccines originating from classical or variant serotypes. In Iraq, Mass-type and variant vaccine strains were utilized to offer enhanced protection in poultry. The absence of cross-protection between imported vaccine strains and field strains may explain the inability to implement an effective vaccination program against IBV [17,18]. this study aimed to investigate antibody production and changes in IL6 levels after boiler vaccinated with two types of IB vaccine.

Materials And Method

Experimental Design:

The study included the use of 160 one-day-old Ross 308 broiler chicks. The chicks were divided into four groups (n=40), all groups rearing in the poultry hall of the laboratory animal house at the College of Veterinary Medicine / University of Mosul for the period from 15/10/2024 until 1 /12/2024 the chicks were vaccinated with the IB vaccine during days (1 and 14), respectively the G1 was kept without vaccination and considered as a control group while G2, G3 and G4 groups were vaccinated with the Classical IB, Variant IB, and with both Variant and Classical IB vaccine respectively.

Vaccine:

The Nobilis IB Ma5 (classical) and 4/91(variant serotype) strain is a live vaccine from the Netherlands company (MSD) that was used to vaccinate broiler chickens at (1, 14) days of age via the intranasal/ocular. The vaccines were dissolved in physiological saline (usually 75 ml per 2,500 doses) and administered via a standardized dropper. One drop should be placed a few centimeters high in one nostril or eye (According to the instructions of the vaccine manufacturer).

Collection of blood samples:

All blood samples were collected by heart puncture in 1, 7, 14, 21, and 28 days after vaccination in all groups, each blood sample was put in a gel tube and allowed to coagulate. Subsequently, the serum was

separated by centrifugation for 10 min/ 3000 rpm [19,20], and the collected serum was stored at -20℃ until required[21].

Estimation Of IB antibody:

An ELISA kit prepared by Bio Chek company/United Kingdom was utilized to detect the changes in antibody titer in all groups during the experiment time, the results of the kit were read using an ELISA reader at a wavelength (450 nm) within a maximum period of 15 minutes

Estimation Of Interleukin 6 (IL-6) level:

The Enzyme-Linked Immunosorbent Assay (ELISA) test provided by Sunlong©, China, was utilized to detect the changes in blood interleukin levels following the manufacturer's recommendations. The standard curve was produced following the dilution of the standard using the specified standard dilution in each kit, and it was utilized to determine the concentration of each test parameter.the result of the kit were read using an ELISA reader at a wavelength (450 nm) within a maximum period of 15 minutes

Statistical Analysis:

All results were analysed using Graph Prism 8, IBM SPSS Version 24 statistical programs employing a T-test, one-way ANOVA, and Duncan's multiple range test to explain significant differences among groups [22, 23].

Results

1- antibody levels between different groups:

the result of antibody titer on the first day of the trial, there were no statistically significant differences between any of the groups; however, on the seventh, fourteenth, twenty-first, and twenty-eighth days, there were significant disparities between all vaccinated and unvaccinated experimental groups which recorded the highest significant antibody titer the twenty-eighth-day post-vaccination in G3 (variant Nobilis 4/91 strain vaccine) and G4 (classical and variant vaccine), which amounted to (3765 ± 2.12, 2876.2 ± 1.92), while the second group (Classical Nobilis IB Mas5) recorded the highest antibody titers on the twenty-first day (2835.6 ± 3.50). The first group (no vaccine) showed a steady decrease in antibodies, reaching (557.2 ± 6.76) on the twenty-one day of the experiment, Fig .1, Table 1.

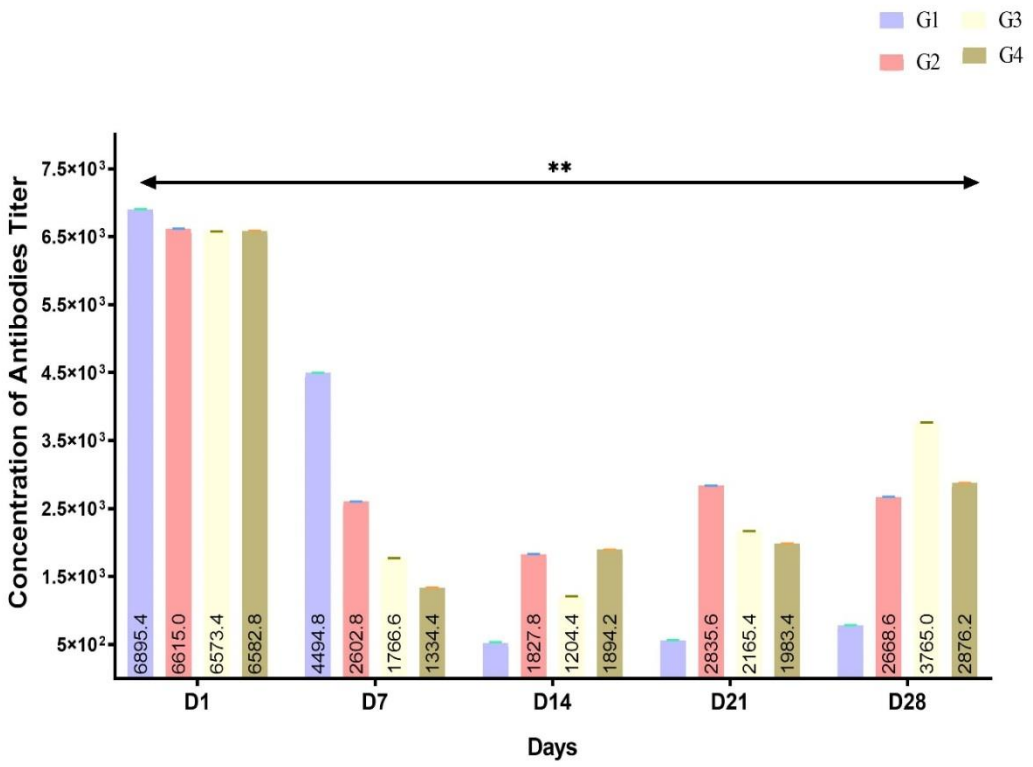

Fig. 1. Differences in the concentration of antibodies titer between groups of vaccinated animals

Table 1: Antibodies Titer Against IBV

Groups / Days	G1 (No Vaccine)	G2 (Classical Nobilis IB Mas5)	G3 (Variant 4/91 Strain Vaccine)	G4 (Classical And Variant Vaccine)
D1	6895.4 ± 8.14	6615 ± 2.23	6573.4 ± 3.20	6582.8 ± 2.16
D7	4494.8 ± 2.16	2602.8 ± 0.83	1766.6 ± 3.84	1334.4 ± 2.50
D14	520 ± 14.19 A	1827.8 ± 2.16	1204.4 ± 2.88	1894.2 ± 2.77
D21	557.2 ± 6.76 A	2835.6 ± 3.50	2165.4 ± 3.57	1983.4 ± 2.30
D28	782 ± 4	2668.6 ± 3.97	3765 ± 2.12	2876.2 ± 1.92

- All data represented the Mean ± SD of antibodies titer

- The similar Capital letter indicates no significant difference between days within groups at $p<0.05$
- The similar small letter indicates no significant difference between groups at $p<0.05$

2- Interleukin 6 levels between different groups

When comparing the levels of response to interleukin 6 in the various groups of the experiment, it was found that there were significant differences between the (G1 vs. G2, G1 vs. G3, G1 vs. G4)) but it was found that there were no significant differences between the (G2 vs. G3 and G4, G3 vs. G4) on the first day of the experiment, The seventh day of the experiment showed significant differences between the (G1 vs. G2, G1 vs. G3, G1 vs. G4, G2 vs. G3, G2 vs. G4) while in the fourteenth day of the experiment, statistically significant differences were recorded between the experimental groups (G1 vs. G2, G1 vs. G3, G1 vs. G4) ; On the twenty-first day of the experiment, statistically significant differences were recorded between the experimental groups (G1 vs. G2, G1 vs. G3, G1 vs. G4); finally on the twenty-eighth day of the experiment, statistically significant differences were recorded between the experimental groups (G1 vs. G2, G1 vs. G3, G1 vs. G4 ,), the Analysis of Fig (2) and table 2 shows that interleukin 6 levels continually increase until the end of the trial, particularly on the fourteenth day which reach highest concentration in G2 (64.48± 3.12) , with variations observed across the different vaccinated groups.

Table 2: Estimation of chicken interleukin 6

Groups / Days	G1 (No Vaccine)	G2 (Classical Nobilis IB Mas5)	G3 (Variant 4/91 Strain Vaccine)	G4 (Classical And Variant Vaccine)

D1	20.33 ±1.52 A,a	22.33 ± 2.52 B,b	24.66± 1.52 G,b	22.00± 2.65 Y,b
D7	20.33± 3.21 A,c	52.14 ±2.01C,d	36.35± 1.52 G,e	36.89± 3.37 I,e
D14	20.33 ± 4.16 A, f	63.48 ± 3.12 CD,g	54.14± 1.21 O,g	56.33± 3.50 I,g
D21	19.33 ± 1.15 A,h	48.02 ± 2.66 DE,i	44.59± 1.50 H,i	43.71± 1.23 IK,i
D28	19.66 ± 2.08 A,j	42.70 ± 1.12 EF,n	42.26±± 2.05 H,n	42.66± 3.05 IK,n

- All data represented the Mean ± SD of chIL6 in ug/ml
- The similar Capital letter indicates no significant difference between days within groups at $p<0.05$
- The similar small letter indicates no significant difference between groups at $p<0.05$

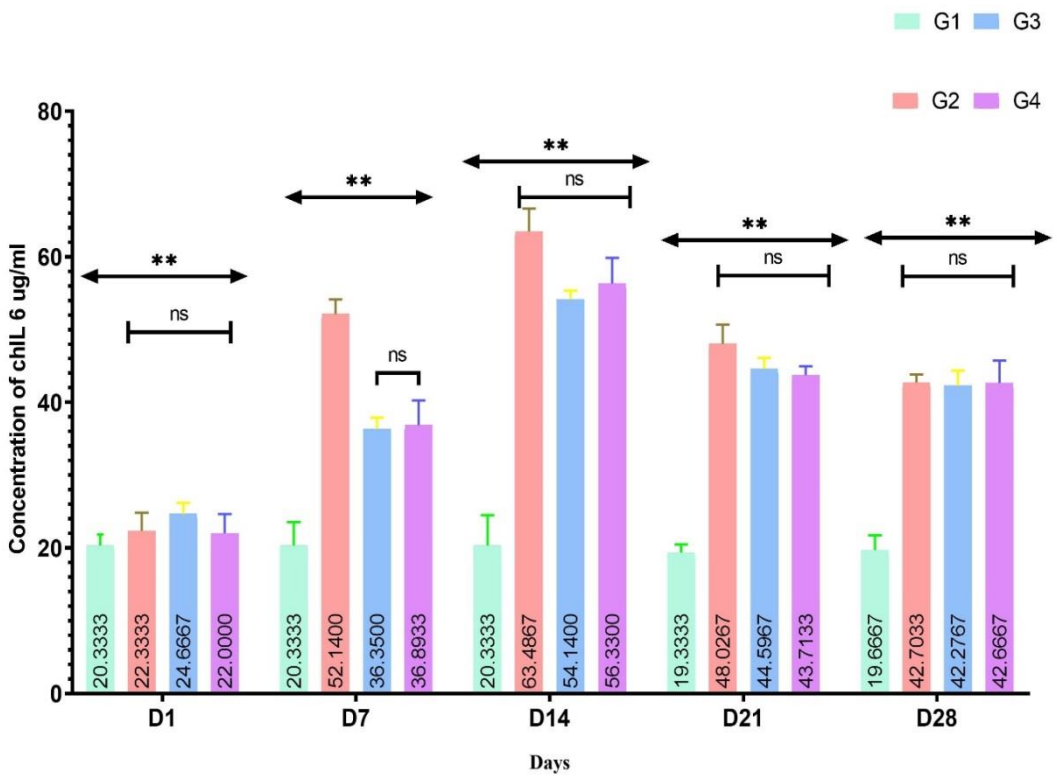

Fig.2. Difference in the concentration of chIL6 in ug/ml in vaccinated animal's groups

Discussion

The poultry industry is vital to the human food supply due to its rapid production method, which provides affordable protein sources. This makes it the most significant sector in the economy, particularly in developing nations. Numerous efforts have been made to boost broiler resistance to infection by vaccination since chicken health is seen as a major concern in the poultry business. Several diseases impact broiler farms, leading to significant economic losses[1,6,24]. The results showed no significant difference between all groups during the first day. This is due to the presence of a high level of antibodies coming from the egg (the mother), which works to protect the chicks during the first days of their life, and which agrees with [21,25,26,27,28], where we showed that the high level of antibodies in the first days of a chick's life responsible for the presence of immunity that works to protect it from pathogens until its immune system develops.

The results demonstrated that there was a significant increase in the level of antibodies to the infectious bronchitis virus within days in each of the working groups. while this increase did not appear in the control groups (unvaccinated), and the increase appeared in the workgroups as the days of the experiment progressed. Then it decreased on the seventh day of the experiment, and in the unvaccinated control group I, the decrease continued until the end of the experiment, and which agree with [28,29,30,31]. Subsequently, following the second vaccination at 14 days post-administration (doa), an elevation in antibody titers was noted until 28 doa (day of administration) in groups, II III and IV, with statistically significant differences in antibody levels between these vaccinated groups ($P > 0.05$), which agree with [21,27,28,31,32] that that difference is due to the stimulation obtained by vaccination with a different type of vaccine that contain different virus strain and serotypes.

A significant increase was observed in the vaccinated groups, especially on the twenty-first and twenty-eighth days of the experiment, The serum from the third group, which got the (strain 4/91 vaccine variant) booster dose at 14 days old, recorded the highest level of antibody titer on the twenty-eighth day of the experiment, which agree with [28,29,31,33,35,36,37] that high level of antibodies raise after booster dose of vaccine to reach high level.

the chicken groups vaccinated with both Nobilis® IB MA5 and Nobilis® IB 4/91, administered separately and concurrently at 1 and 14 days, responded with an enhanced antibody. Chickens show elevated IBV-specific antibody levels compared to those vaccinated on Day 1 which showed a clear increase in the level of antibody titer as the days progressed, this is consistent with the results obtained before [25,28,38,39]. When they studied the IB vaccine and its effects on antibodies in broiler chickens, they discovered that the vaccine was effective in terms of boosting antibodies and/ or working with other vaccine programs. When administered concurrently, there is no indication of any interference between Ma5 and 4/91, according to the manufacturer's directions [40]. Attenuated IBV vaccines, encompassing classical and/or variant serotypes, are employed in numerous nations based on their necessity to safeguard avian populations against wild-circulating IBVs within established regulations [25]. It was reported earlier that the combined vaccines of IB Ma5 and IB 4/91 variants can provide strong protection against protection well enough against the heterologous field IBV variant strain [41,42,43]. Similar propaganda was recorded in group 4 which received two types of IBV vaccine (classical and variant) and expressed elevated antibody titer.

The results showed low-level elevated interleukin 6 on day 1 may have originated from the first chick stress that occurs after hatching due to thermal changes and transport; rather than IL 6 considered as pro-inflammatory acute face protein, many studies reported that heat shock stress responsible for elevated IL-6 levels to induce protection mechanisms for tissue [44,45] there are many methods for activation and release of IL6 but the heat shock transcription factor 3 in chicken is responsible on the increased level of IL6 after environmental stress [46] this explanation agrees with results obtained by Al-Zghoul and his collagenous when studying the effect of post-hatching thermal manipulation and post hach heat shock stress on chicken IL6 expression and serum level [47].

Our results recorded a significant increase in the level of interleukin 6 within days in each of the groups of experience, especially in the vaccinated compared to an unvaccinated group, As it is known, interleukin 6 is one of the proinflammatory cytokines that are secreted by all immune cells, specially activated macrophages, dendritic cells, and various lymphocytes, as well as epithelial cells when secreted it activates inflammatory cells, [48, 49,50], leading to increased immune response that occurs in poultry with a change in the immune response towards humoral immunity after the occurrence of vaccine boosters, and accompanied by an increase in cellular proliferation as a result of the response to antigens upon successive stimulation which agree with [28,51,52] on them work on Cytokine and acute-phase protein response following vaccination against infectious bronchitis in broilers; The second, third and fourth groups showed an increase in the serum level of interleukin 6, especially on the fourteenth day, all groups gave a similar fluctuation pattern to those of the first vaccination and started to decrease from day 28 in the vaccinated groups which agree with [28,51].
In our understanding after 14 days the Interleukin 6 level will decrease (after booster dose in all vaccinated groups) this pattern may be due to using different types of IB vaccines that leak adjuvant induce different patterns of APPs responses between first and second vaccine administration and lead to medium level of stimulation of cytokine by inducing by short live Antigen-presenting cells by first vaccine followed by lower level after booster dose which may be due to stress responses and /or induced immune competence. Which agrees with [28,51,53].

Conclusion

We conclude that all types of vaccination used in this study can elevate antibody titer but the Variant Nobilis 4/91 strain vaccine gives equivalent protection when used alone or in combination with classical Nobilis IB Ma5 strain vaccine also the combined vaccines of IB Ma5 and IB 4/91 variants can provide strong protection without negative interaction between tow vaccine on immune response, the study of interleukin 6 gives strong indication as it important influence in targeting immunity from humoral to cellular after boosting dose.

Acknowledgements

The authors appreciate all the support the Department of Microbiology/College of Veterinary Medicine/ Mosul University provided in completing this manuscript.

Funding statement

This study did not receive any funding support**.**

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

All animals were treated and euthanized humanely by neck dislocation, and all blood was drawn in ways that were not harmful to the animals, according to the instructions provided by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, and according to book number UM.VET.2024.029 on 7/15/2024.

References

1. Attia, Y. A., Rahman, M. T., Hossain, M. J., Basiouni, S., Khafaga, A. F., Shehata, A. A., & Hafez, H. M. (2022). Poultry production and sustainability in developing countries under the COVID-19 crisis: Lessons learned. Animals, 12(5), 644.

2. Al-Dabhawe, A. H., Kadhim, H. M., & Samaka, H. M. (2013). Molecular detection of infectious bronchitis virus and it is relation with avian influenza virus (H9) and Mycoplasma gallisepticum from different geographical regions in Iraq. *Iraqi Journal of Veterinary Sciences*, *27*(2), 97-101.

3. Gurjar, R. S. (2013). *Cell-Mediated Immunity After Ocular Ark-Type Infectious Bronchitis Virus Vaccination* (Doctoral dissertation, Auburn University).

4. Khudeir, M. A., Alsultan, A. T., & Khudhair, Y. I. (2024). Novel CRISPR/Cas13-based assay for diagnosis of avian infectious bronchitis. *Iraqi Journal of Veterinary Sciences*, *38*(1), 63-69.

5. Mohammed, M. H., Hair-Bejo, M., Zahid, A., Alazawy, A., Ahad, E. A., & Hasoon, M. F. (2013). Adaptation of infectious bronchitis virus in primary cells of the chick embryo chorioallantoic membrane. *Iraqi Journal of Veterinary Sciences*, *27*(1), 33-37.

6. Seger, W., GhalyanchiLangeroudi, A., Karimi, V., Madadgar, O., Marandi, M. V., & Hashemzadeh, M. (2016). Genotyping of infectious bronchitis viruses from broiler farms in Iraq during 2014-2015. *Archives of virology*, *161*(5), 1229-1237.

7. Alam, W., Khan, S., Abbas, G., Haseeb, F., Saleem, M. I., Hussain, I., ... & Abbas, M. (2023). ANTIBODY TITERS AGAINST NEWCASTLE DISEASE AND INFECTIOUS BRONCHITIS VIRUSES IN BROILER BREEDERS RAISED UNDERFLOOR VERSUS CAGE SYSTEM. *Pakistan Journal of Science*, *75*(4).

8. Hasan II, Rasheed ST, Jasim NA, Shakor MK. Pathological effect of infectious bronchitis disease virus on broiler chicken trachea and kidney tissues. Vet World. 2020 Oct;13(10):2203-2208. doi: 10.14202/vetworld.2020.2203-2208. Epub 2020 Oct 21. PMID: 33281357; PMCID: PMC7704328.

9. Yaba, Z. H., Abdulrahman, N. R., & Hassan, S. (2023). Molecular and Pathological Investigation of Infectious Bronchitis in Broiler Flocks in Sulaimani Province, Iraq. *Al-Anbar Journal of Veterinary Sciences*, *16*(2).

10. Gallardo, R. A. (2011). *Avian Infectious Bronchitis Coronavirus Variation and Selection*. Auburn University.

11. Ali, M. H., & Allawe, A. B. (2023). Phylogenetic analysis of the infectious bronchitis virus in Iraqi farms. *History of Medicine*, *9*(1), 1162-1167.

12. Ramakrishnan, S., & Kappala, D. (2019). Avian infectious bronchitis virus. *Recent Advances in Animal Virology*, 301-319.

13. Zhao, J., Zhao, Y., & Zhang, G. (2023). Key aspects of coronavirus avian infectious bronchitis virus. *Pathogens*, *12*(5), 698.

14. Falchieri, M., Coward, V. J., Reid, S. M., Lewis, T., & Banyard, A. C. (2024). Infectious bronchitis virus: an overview of the “chicken coronavirus”. *Journal of Medical Microbiology*, *73*(5), 001828.

15. Cavanagh, D. (2007). Coronavirus avian infectious bronchitis virus. *Veterinary research*, *38*(2), 281-297.

16. Mark W. Jackwood, Randi Clark, Sunny Cheng & Brian J. Jordan (2020) Protection following simultaneous vaccination with three or four different attenuated live vaccine types against infectious bronchitis virus, Avian Pathology, 49:4, 335-341, DOI:10.1080/03079457.2020.1748173

17. Kim, B. Y., Lee, D. H. , Jang, J. H. , Lim, T. H. , Choi, S. W. , Youn, H. N. Park, J. K. , Lee, J. B. , Park, S. Y. , Choi, I. S. , Song. C. S. 2013. Cross-Protective Immune Responses Elicited By A Korean Variant Of Infectious Bronchitis Virus. Avian Dis. 57,667–670.

18. Toro, H., Van Santen, V. L. Ghetas, A. M. Joiner, K. S. 2015. Cross-Protection By Infectious Bronchitis Viruses Under Controlled Experimental Conditions. Avian Dis. 59, 532–536.

19. Al-Aalim, A.M., Al-Baroodi, S.Y. Ahmed, I.M. and Al-Attar, M.Y. Detection of antibodies level for goat brucellosis in Mosul, Iraq. *Iraqi Journal of Veterinary Sciences*, **23**(1), 33-36 (2009).

20. Isihak, F. A., Ismail, H. K., & Wahid, A. A. (2021). Comparison study between the efficacy of immune complex and conventionally live vaccine against Gumboro disease in broilers. *Iraqi Journal of Veterinary Sciences*, *35*(4), 627-632.

21. Rasheed, B. Y., Taha, D. K., & Ahmed, I. M. (2023). Detection of Ornithobacterium rhinotracheale antibodies in broiler by ELISA in Mosul city. *Iraqi Journal of Veterinary Sciences*, *37*(3), 739-743.

22. Denis, D.J. *Applied Univariate, Bivariate, and Multivariate Statistics: Understanding Statistics for Social and Natural Scientists, With Applications in SPSS and R. 2ed edition*. John Wiley & Sons publisher. USA. (2021).

23.Al-kalo, O. A., & Al-Aalim, A. M. (2024). The Effect of Oils Additive on Some Immunological Cytokines Newcastle Disease-Vaccinated Broiler Chickens. Egyptian Journal of Veterinary Sciences, 1-7.

24. Bande F, Arshad SS, Omar AR, Hair-Bejo M, Mahmuda A, Nair V. Global distributions and strain diversity of avian infectious bronchitis virus: A review. Anim Health Res Rev. 2017;18(1):70-83.

25. Eid, A. A., Mahmoud, A. M., Hamouda, E. E., Metwally, M., Ezz-Eldin, R. M., & ElBakrey, R. M. (2024). The efficacy of simultaneous successive classic and variant infectious bronchitis virus vaccines versus circulating variant II Egyptian field virus. *Open Veterinary Journal*, *14*(1), 90.

26. Meher, M. M., Hossaın, M., & Haıder, G. (2022). Detection of MDA titer of ınfectious bronchitis virus and comparison of antibody titer produced by two different ınfectious bronchitis vaccine. *Journal of Poultry Research*, *19*(2), 44-51.

27. Ishara Muhammadu Isham, F. (2023). Infectious bronchitis virus (IBV) infection in chickens: Some aspects of pathogenesis and diagnosis. https://hdl.handle.net/1880/117728.

28. Anousheh, D., Allymehr, M., Talebi, A., & Asri-Rezaei, S. (2024). Cytokine and acute-phase proteins response following vaccination against infectious bronchitis in broilers. *Veterinary Medicine and Science*, *10*(5), e1586.

29. Pohuang, T., Tanasatian, S., & Sasipreeyajan, J. (2016). Efficacy of different vaccination programs of live 4/91 strain against Thai QX-like infectious bronchitis virus in broiler chickens. *The Thai Journal of Veterinary Medicine*, *46*(3), 419-425.

30. Cardoso, W. M., Gomes, L. P., Romão, J. M., Salles, R. P. R., Teixeira, R. S. C., Sobral, M. H. N. R., ... & Oliveira, W. F. (2006). Antibodies specific to infectious bronchitis in broilers in Ceará state, Brazil. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *58*, 327-332.

31. Chhabra, R., Forrester, A., Lemiere, S., Awad, F., Chantrey, J., & Ganapathy, K. (2015). Mucosal, cellular, and humoral immune responses induced by different live infectious bronchitis virus vaccination regimes and protection conferred against infectious bronchitis virus Q1 strain. *Clinical and Vaccine Immunology*, *22*(9), 1050-1059.

32. J. J. De Wit (2000) Detection of infectious bronchitis virus, Avian Pathology, 29:2, 71-93, DOI: 10.1080/03079450094108.

33. FallahMehrababdi, M. H., Ghafouri, S., Ghalyanchilangeroudi, A., Hosseini, H., Khaltabadi Farahani, R., Maghsoudloo, H., ... & Aghaiyan, L. (2018). Evaluation of the Antibody Titration in Vaccinated Chickens Against Infectious Bronchitis Virus Using Three Commercial ELISA Kits. *Iranian Journal of Virology*, *12*(2), 13-18.

34. Buharideen, S. M. (2021). Host responses in laying hens following infectious bronchitis vaccination: Comparison of two vaccination strategies.

35. AL-Zuhariy, M. T. (2017). Improved vaccine strategies of infectious bronchitis disease to reduce shedding of virulent virus from infected birds. *Kufa Journal For Veterinary Medical Sciences*, *8*(1), 50-57.

36. Okino, C. H., Alessi, A. C., Montassier, M. D. F. S., Rosa, A. J. D. M., Wang, X., & Montassier, H. J. (2013). Humoral and cell-mediated immune responses to different doses of attenuated vaccine against avian infectious bronchitis virus. *Viral immunology*, *26*(4), 259-267.

37. Liu, S., Zhang, X., Wang, Y., Li, C., Liu, Q., Han, Z., ... & Tong, G. (2009). Evaluation of the protection conferred by commercial vaccines and attenuated heterologous isolates in China against the CK/CH/LDL/97I strain of infectious bronchitis coronavirus. *The Veterinary Journal*, *179*(1), 130-136.

38. Shao, G., Chen, T., Feng, K., Zhao, Q., Zhang, X., Li, H., Lin, W., & Xie, Q. (2020). Efficacy of commercial polyvalent avian infectious bronchitis vaccines against Chinese QX-like and TW-like strain via different vaccination strategies. *Poultry Science*, *99*(10), 4786–4794. https://doi.org/10.1016/j.psj.2020.06.062.

39. Zegpi, R. A. (2019). *Novel Infectious Bronchitis Virus Vaccines and Immune Responses* (Doctoral dissertation, Auburn University).

40. Intervet International, B.V. 2003. Scientific discussion, live attenuated avian infectious bronchitis virus variant strain 4-91. Boxmeer, The Netherlands: EMEA, pp: 1–9. Available via https://www.ema.europa.eu/en/documents/ scientific-discussion/nobilis-ib-4-91-eparscientific-discussion_en.pdf.

41. Bhuiyan, M. S. A., Amin, Z., Bakar, A. M. S. A., Saallah, S., Yusuf, N. H. M., Shaarani, S. M., & Siddiquee, S. (2021). Factor influences for diagnosis and vaccination of avian infectious bronchitis virus (Gammacoronavirus) in chickens. *Veterinary Sciences*, *8*(3), 47.

42. Sasipreeyajan, J., Pohuang, T. and Sirikobkul, N. 2012. Efficacy of different vaccination programs against Thai QX-like infectious bronchitis virus. Thai. J.Vet. Med. 42(1), 73–79.

43. Shirzad, M.R., Asasi, K. and Mohammadi, A.2012.Efficacy of vaccination programmes using two commercial live infectious bronchitis vaccines against a field IRFIB 32 strain. Bulg. J. Vet. Med. 15(4), 260–272.

44. Leon, L. R. (2007). Heat stroke and cytokines. *Progress in brain research*, *162*, 481-524.

45. Phillips, N. A., Welc, S. S., Wallet, S. M., King, M. A., & Clanton, T. L. (2015). Protection of intestinal injury during heat stroke in mice by interleukin-6 pretreatment. *The Journal of Physiology*, *593*(3), 739-753.

46. Prakasam, R., Fujimoto, M., Takii, R., Hayashida, N., Takaki, E., Tan, K., ... & Nakai, A. (2013). Chicken IL-6 is a heat-shock gene. *FEBS letters*, *587*(21), 3541-3547.

47. Al-Zghoul, M. B., Saleh, K. M., & Ababneh, M. M. K. (2019). Effects of pre-hatch thermal manipulation and post-hatch acute heat stress on the mRNA expression of interleukin-6 and genes involved in its induction pathways in 2 broiler chicken breeds. *Poultry science*, *98*(4), 1805-1819.

48. Velazquez-Salinas, L., Verdugo-Rodriguez, A., Rodriguez, L. L., & Borca, M. V. (2019). The role of interleukin 6 during viral infections. *Frontiers in microbiology*, *10*, 1057.

49. Gabay, C. (2006). Interleukin-6 and chronic inflammation. *Arthritis research & therapy*, *8*, 1-6.

50. Rattanasrisomporn, J., Tantikositruj, C., Thiptara, A., Kitpipit, W., Wichianrat, I., Kayan, A., & Boonkaewwan, C. (2022). Pro-inflammatory cytokine release from chicken peripheral blood mononuclear cells stimulated with lipopolysaccharide. *Veterinary World*, *15*(4), 885.

51. Fernando, F. S., Okino, C. H., Silva, K. R., Fernandes, C. C., Gonçalves, M. C. M., Montassier, M. F. S., Vasconcelos, R. O., & Montassier, H. J. (2015). Increased expression of interleukin-6 related to nephritis in chickens challenged with an Avian infectious bronchitis virus variant. *Pesquisa Veterinaria Brasileira*, *35*(3), 216–222. https://doi.org/10.1590/S0100-736X2015000300002.

52. Wigley, P., & Kaiser, P. (2003). Avian cytokines in health and disease. *Brazilian Journal of Poultry Science*, *5*, 1-14.

53. Okino, C. H., Santos, I. L. D., Fernando, F. S., Alessi, A. C., Wang, X., & Montassier, H. J. (2014). Inflammatory and cell-mediated immune responses in the respiratory tract of chickens to infection with avian infectious bronchitis virus. *Viral Immunology*, *27*(8), 383–391. https://doi.org/10.1089/vim.2014.0054.