



Evaluating Humoral Immune Responses in Cobb Chicken Subjected to a Long Extensive Vaccination Program against Newcastle Diseases Virus

Abdel Kader A. Zaki^{1,2}, Abdulaziz S. Alolayqi³, Saleh M. Albarrak⁴

10.18805/IJAR.BF-1937

ABSTRACT

Background: Newcastle disease (ND) is a highly contagious viral disease that affects poultry. Evaluation of vaccine schedule is important since some programs have been failing to provide complete immunity against the disease. The current study aimed to evaluate antibody responses starting at week one of life.

Methods: The study included 300 Cobb chickens sourced from a local breeder. Chickens were vaccinated seven times. Thirty unvaccinated one-day-old chicks were raised separately and considered as control group. Blood samples were collected at 1, 3, 5, 8, 10, 15, 20, 40 and 60 weeks old. Ten of the chicks were slaughtered for spleen and bursa histological examination. The IgM, IgG and IgA levels were quantified using ELISA kits.

Result: The ND vaccine significantly boosted IgM and IgA levels during the first 8 weeks, indicating a strong early immune response. However, the effectiveness of the vaccine in stimulating long-term IgA and IgM production appears to wane over time. The IgG levels rapidly dropped ($P < 0.05$) after the initial peak (Week 9) suggesting that long-term immunity may not be effectively maintained without booster vaccinations. The bursa of the one-week-old chick showed areas of lymphocyte depletion and necrosis within the medulla. The bursal follicles of the 3, 5, 8, 10, 15 and 18-weeks-old chicks showed numerous well-defined structures indicating effective vaccination schedules. The most prominent feature of the bursa of 40 and 60-week-old chicken is the presence of necrosis and vacuolation within the follicles. Histology depicts the spleen of all week's study except for week 8 showed a well-organized lymphoid architecture with distinct white and red pulp areas. The results of this study highlight the importance of developing effective vaccination schedules and routes to enhance immunity against ND in layer chickens.

Key words: Chickens, Disease, Histology, Immunity, Newcastle vaccination.

INTRODUCTION

One of the most significant diseases impacting poultry industries is Newcastle Disease (ND). Avian orthoavulavirus-1 (AoAvV-1), formerly known as the ND virus (NDV), is the cause of ND. AoAvV-1 is a member of the order Mononegavirales, family Paramyxoviridae and genus Orthoavulavirus (Dimitrov *et al.*, 2019 and Deepthi *et al.*, 2023). The species, age and immunity of the host, as well as the strain of the virus AoAvV-1, can all affect how ND manifests clinically. It can vary from a disease with no symptoms to an infection that spreads quickly and has a 100% fatality rate. It has a significant financial impact on the global poultry market. Velogenic ND (vND), which can also affect the female reproductive, neurological, digestive, pulmonary and lymphatic systems, is characterized by high death rates (Al-Zuhary 2023).

The goal of hyper-immunization programs in commercial poultry production is to transfer passive immunity to the offspring so that they can acquire passive immunity after hatching. Depending on the breed type, endemic diseases and other local pressures, these vaccination schedules may vary from one location to another. Since there is now no effective treatment for ND, managing the condition relies on both adequate biosecurity to protect the birds from harmful viruses and the right

¹Department of Medical Biosciences, College of Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia.

²Department of Physiology, College of Veterinary Medicine, Cairo University Buraydah, Saudi Arabia.

³Department of Poultry Health (PHD), Alwatanya Breeder Company, Buraydah, Saudi Arabia.

⁴Department of Pathology and Laboratory Diagnosis, College of Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia.

Corresponding Author: Saleh M. Albarrak, Department of Pathology and Laboratory Diagnosis, College of Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia.

Email: salbarrak@qu.edu.sa

ORCID: 0000-0001-7616-0789; 0007-88020009; 0000-0003-4965-6142

How to cite this article: Zaki, A.K.A., Alolayqi, A.S. and Albarrak, S.M. (2025). Evaluating Humoral Immune Responses in Cobb Chicken Subjected to a Long Extensive Vaccination Program against Newcastle Diseases Virus. Indian Journal of Animal Research. 1-10. doi: 10.18805/IJAR.BF-1937.

Submitted: 08-12-2024 **Accepted:** 13-02-2025 **Online:** 12-05-2025

vaccinations to prevent the spread of viruses (Dimitrov *et al.*, 2017). However, to ensure chickens' effective protection against ND, the poultry industry requires carefully

planned immunizations with higher safety and effectiveness margins (Bello *et al.*, 2018). Large doses of immature vaccines are commonly administered in chicken husbandry environments, which might cause immunological stress (Li *et al.*, 2020). Therefore, it became necessary and effective to improve the body's ability to respond to the immune response to address the issue of vaccine immunization prevention in the poultry sector (Tian *et al.*, 2023). Animals or flocks' immunological status was often assessed using the enzyme-linked immunosorbent test (ELISA).

Both the generation of antibodies against NDV and cell-mediated immunity contribute to protection against infection with the NDV. An essential organ for the formation of lymphatic cells in birds is the Bursa Fabricius (BF), where B lymphocytes generate and release immunoglobulins (Igs). Bird serum has three antigenically distinct classes of Igs, two of which, IgM and IgA, are shared by mammals, while the third, IgY, differs from the IgG present in mammals. IgY is only present in eggs' vitellin, where it is crucial for the transfer of immunity (Criste *et al.*, 2020). IgY ($5-15 \text{ mg/mL}^{-1}$) is found in avian serum at higher concentrations than IgM ($1-3 \text{ mg/mL}^{-1}$) and IgA ($0.3-0.5 \text{ mg/mL}^{-1}$), according to Lestari *et al.* (2022). Other names for naturally passive acquired immunity in birds include maternal or vitellin immunity. The immunity from egg fluids to birds is a process like that of mammals which give the fetus transplacental passive immunity. Ig Y from the serum is transferred to the egg yolk by the birds while the egg is still in the ovary. IgM and IgA immunoglobulins enter the egg together with the oviduct secretions (Criste *et al.*, 2020).

Since the ND virus (NDV) strains are still endemic and can infect birds that have not received enough vaccinations or have not been vaccinated, biosecurity and vaccination strategies alone will not be sufficient to eradicate these strains (Dimitrov *et al.*, 2019). Additionally, the conventional immunizations cannot prevent the multiplication and shedding of other NDV strains that are present in poultry birds and might potentially serve as a source of persistent infection (Bello *et al.*, 2018). New medications have been developed because of continuous research into more effective options, even though standard immunizations remain the foundation for controlling ND in poultry.

ND significantly impacts Saudi Arabia's commercial poultry sector. Poultry breeders have experienced significant financial losses as a result of outbreaks,

decreased egg production *and* increased mortality rates (Almubarak, 2019). In Saudi Arabia, there are several serious issues with ND transmission and control. First, the great density of poultry in the nation, which is composed of both commercial and backyard flocks' communities are close to one another. The danger of disease transmission rises as a result. Second, KSA is situated along important migratory bird routes, making it more vulnerable to the introduction of the NDV. Finally, vaccination campaigns and biosecurity measures have a significant impact on the efficacy of ND management. The goal of the current study was to ascertain the Cobb breed's overall IgM, IgA *and* IgY levels as well as any histological alterations in the spleen and bursa in response to a new, lengthy, robust immunization schedule against NDV.

MATERIALS AND METHODS

Feeding programs

The nutritional makeup of chicken feeding programs at various developmental stages is broken down in depth in Table 1. To satisfy the standard requirements for healthy and optimal growth, feed formulas are carefully designed to meet the individual nutritional demands of the birds at each stage of life: starter, grower, pre-breeder *and* laying. Diets were formulated for both the starter and finisher phases as outlined by Ajaykumar *et al.* (2024). The table displays each nutrient's standard limits, the test method used (ISO 12099:2017) *and* the feed's actual outcomes. As the birds mature, their nutritional requirements for the first five to sixteen weeks change slightly, requiring varying amounts of protein and energy. Slight alterations in nutrient levels are observed to meet the birds' nutritional needs during the 16-9-week period, which is centered on getting their bodies ready for reproduction (16-19-week period acts as a transitional stage).

For the birds general health, appropriate skeletal growth and reproductive fitness, it is imperative that they obtain the essential nutrition. To prepare the birds for the following phase, the emphasis here is on moderate protein and calorie levels. Birds start laying eggs during the productive phase, which lasts from 23 to 45 weeks. The nutritional makeup is modified to satisfy the high protein and energy requirements of egg production. Additionally, sustaining healthy bones and general well-being depends on the amounts of minerals like calcium and phosphorus. The later stage of the laying period (45th-60th week), birds

Table 1: Feeding program of poultry.

Nutrients	1-4 weeks	5-16 weeks	16-19 weeks	19-23 weeks	23-45 weeks	45-60 weeks
Moisture%	10.17	10.69	9.75	9.75	10	9.73
Fat%	3.09	3.58	3.51	3.51	4.08	3.53
Protein%	18.92	14.02	14.63	14.63	14.63	14.39
Fiber%	2.54	3.00	2.24	2.24	2.93	2.48
Ash%	6.22	5.89	7.06	7.06	10.51	10.66
Starch%	42.89	45.68	45.92	45.92	44.65	46.53

continue to lay eggs but may begin to exhibit signs of decreased output. The feed's nutritional makeup is modified to promote consistent egg production, body condition maintenance *and* general health as the birds get older.

Vaccination program

Assessing how effectively the hens' immune systems react to the repeated vaccines was the task. Three hundred chickens from the local Alwatanya breeder company's line were used in the investigation. A separate ground ranch 120 m long by 15 m wide) was utilized to house and incubate the chickens. The Newcastle disease virus (NDV) vaccination was administered to chickens fifteen times (Table 2). On the first day, the chicks received a coarse spray vaccination with the live VITABRON CEVA vaccine. The Gallimune-208 (MERIAL-S.A. S) killed vaccine was subcutaneously given to the chicks at days 7-11 at a dose of 15,000 per 12,000 birds (Fig 1). On day 12, the chickens received a dose of 15000 doses per 12,000 birds of live attenuated ND CLONE 30+Ma5 NOBILIS-MSD *via* coarse spray (large drops, used at young ages, because the chicks' respiratory system is not fully developed and there is no reaction to vaccination, the large drops are picked up in the mouth). On days 22 and 84, medium spray was utilized to apply live attenuated virus ND (LASOTA, 15000 dose /12000 birds) (a fine spray intended for later ages constituted of extremely small drops). It was safe for adults' birds *and* since their respiratory systems are fully developed, they can detect it with their eyes and nose. Both the Gallimune-208 (0.03 mg/chick) and Gallimune-407 (0.05 mg/chick) (MERIAL-S.A. S) vaccines were administered to the chickens on days 56-59 and 126. As a control group, thirty-one chicks that had not received any vaccinations were raised apart. The study's requirements were met by the

Cobb chickens, who were of a specific breed, within a given weight range, fed a standardized and controlled diet and free of any known illnesses or infections. Exclusion criteria of the study applied to the vaccinated and non-vaccinated chickens exhibiting abnormal behavior or signs of stress, with physical deformities or injuries, that have received recent *and* medication or treatments exposed to known contaminants or environmental hazards.

Blood samples

Blood samples were taken from the chickens at different ages at weeks 1, 3, 5, 8, 10, 15, 18, 20, 40 and 60. All of the chicks and hens were bled through the wing vein and a 3 mL heparinized syringe with a 23-gauge needle was used to collect 2 mL of blood. The blood for every one of the hens were centrifuged at 3500 r.p.m for half an hour at room temperature. Before being analyzed, the plasma samples were taken and kept at a temperature of -20°C until analysis.

Collection of bursa and spleen for histological examination

After the blood samples were taken, 10 of the chicks were slaughtered. Spleen and bursa from the sacrificed birds were isolated and kept in a 10% formalin solution for histological examination.

Quantification of Immunoglobulins levels

With quantitative ELISA kits purchased from Sunglung Biotech co., LTD, the amounts of total IgY, IgA *and* IgM in the plasma of the dams and chicks were ascertained in accordance with the manufacturer's instructions. Catalogue number: IgG/SL0033Ch, IgM/SL0034Ch and IgA/SL003Ch.

Three copies of the samples were examined. For every plate, a standard curve that illustrates the relationship between the standards' concentration and absorbance value was created. These curves typically follow a

Table 2: Vaccination program of poultry.

Age	Vaccine	Type	Route
Day 1	IB+ND VITABRON CEVA	Live	Coarse spray
Days 7-11	Gallimune - 208 (MERIAL-S.A. S)	Killed	S/C Injection
Day 12	ND CLONE 30+Ma5 NOBILIS- MSD 15000 dose /12000 birds	Live	Coarse spray
Day 22	ND LASOTA - ZOETIS. 15000 dose /12000 birds	Live	Medium spray
Days 56-59	ND Gallimune 208 (MERIAL-S.A. S) 0.3 mg dose/bird+ Gallimune 407 (MERIAL-S.A. S) 0.5 mg /bird	Killed	I/M Injection
Day 84	ND LASOTA vaccine -ZOETIS 15000 dose /12000 birds	Live	Medium spray
Day 126	ND Gallimune 208 (MERIAL-S.A. S) 0.3 mg dose/bird +ND Gallimune 407 (MERIAL-S.A. S) 0.5 mg.	Killed	I/M Injection
Week 21	ND CLONE 30+MA5 -MSD	Live	Medium spray
Week 25	ND CLONE 30+MA5 - MSD	Live	Medium spray
Week 29	IB+ND (VITABRON -CEVA)	Live	Medium spray
Week 33	ND CLONE 30+MA5 - MSD	Live	Medium spray
Week 38	ND CLONE 30+MA5 - MSD	Live	Medium spray
Week 44	ND CLONE 30+MA5 - MSD	Live	Medium spray
Week 50	ND CLONE 30+MA5 - MSD	Live	Medium spray
Week 56	ND CLONE 30+MA5 - MSD	Live	Medium spray

logarithmic scale and their equations allow for the interpolation of immunoglobulin concentrations from OD values. The CV for inter-assay precision is less than 12% for all three assays, indicating good consistency across different assay runs. A CV of less than 12% is typically considered acceptable in clinical and research settings for immunoassays, ensuring that the results are reliable across different batches of tests. Additionally, the provided catalog numbers and details about assay range (0.3 ug/ml-20 ug/ml, 0.3 ug/ml-18 ug/ml and 30 ug/ml-1800 ng/ml) and sensitivity (0.06 ug/ml, 0.06 ug/ml and 80 ng/ml for IgG, IgM and IgA respectively) give us a comprehensive understanding of the assay's performance characteristics. The standard log-dose response curves represented by XY equation for IgA, IgG and IgM were $X \text{ ng/ml} = 86.67 + 2996 y \text{ OD}$; $X \text{ ng/ml} = -0.8467 + 15.35 y \text{ OD}$ and $X \text{ ng/ml} = 0.166 + 26.55 y \text{ OD}$ respectively.

Statistical analysis

Data values will be represented as means standard errors. For each measured parameter, a straightforward one-way analysis of variances (ANOVA) test will be conducted. The Mann-Whitney test will be used in post hoc analysis to compare the positive control to other experimental groups and the negative control to the positive control at $p < 0.05$, $p < 0.01$ and $p < 0.001$ that indicated a statistical difference.

RESULTS AND DISCUSSION

Concentrations of circulating IgM

Table (3) show data on the IgM levels in the plasma of Cobb chickens that were vaccinated with a long vaccination program against ND. The IgM levels are measured from Week 1 (W1) to Week 60 (W60), with a comparison between vaccinated and non-vaccinated chickens. The data demonstrated significant differences in the IgM response between the two groups in the earlier weeks post-vaccination, which gradually decrease over time. At W 1, vaccinated chickens display a significantly higher IgM concentrations (9.896 ng/ml) compared to non-vaccinated chickens (3.106 ng/ml). This significant difference ($p < 0.01$)

indicates that the Newcastle vaccine triggers a strong early immune response, as expected from the role of IgM in primary immunity. At week 3, the IgM levels in vaccinated chickens remained high (9.916 ng/ml), while non-vaccinated chickens show negligible change from week 1 (3.070 ng/ml). At week 5, the vaccinated group maintained significantly higher IgM levels (6.803 ng/ml) compared to the non-vaccinated group (3.120 ng/ml). At week 8, IgM levels in the vaccinated group remained elevated (6.830 ng/ml), while the non-vaccinated chickens exhibited very low IgM levels (4.426 ng/ml). The significant difference ($p < 0.01$) shows that the vaccine continues to stimulate immune activity, likely due to either sustained immune memory or continuous pathogen exposure. At week 10, the vaccinated group retained a strong IgM response reaching to 6.655 ng/ml, while the non-vaccinated group remained near the baseline 4.194 ng/ml. The significant difference ($p < 0.01$) suggests that vaccination provides longer-lasting protection than natural exposure alone. By week 15, IgM levels in the non-vaccinated group increase to 3.650 ng/ml, possibly due to natural exposure to pathogens. The vaccinated group shows higher IgM levels (3.784 ng/ml), but the difference is not statistically significant. At week 18, both groups show an increase in IgM levels, with vaccinated chickens maintaining higher levels (7.360 ng/ml). However, the absence of statistical significance suggests that natural exposure has allowed non-vaccinated chickens to develop comparable immunity. By week 20, both groups exhibit similar IgM levels, with no significant difference between them. At Week 40, there is still no significant difference in IgM levels between the two groups ($T\text{-value} = 0.67$), suggesting that the vaccine-induced immune response has waned to the point where it is no longer detectable in comparison to the non-vaccinated group. By the end of the study at week 60, the vaccinated group shows slightly higher IgM levels (3.778 ng/ml) than the non-vaccinated group (2.660 ng/ml). However, the difference is not statistically significant.

Concentrations of circulating IgG

Table (4) provide data on the levels of Immunoglobulin G (IgG) in the plasma of Cobb chickens, vaccinated with a

Table 3: IgM levels in vaccinated and non-vaccinated cobb chickens over time.

IgM (ng/ml)	Non-vaccinated	Vaccinated	T value
W1	3.106±0.277	9.896±0.102**	22.86
W3	3.070±0.550	9.916±0.296**	10.97
W5	3.120±0.304	6.803±0.094***	11.57
W8	4.426±0.243	6.830±0.321**	5.97
W10	4.194±0.294	6.655±0.157**	7.38
W15	3.650±0.016	3.784±0.140	2.12
W18	3.207±0.262	7.360±1.700	1.51
W20	3.027±0.289	3.508±0.130	0.57
W40	2.939±0.250	3.649±0.094	0.67
W60	2.910±0.239	3.778±0.129	1.74

IgM concentrations (ng/mL) measured at different weeks post-vaccination. Data are presented as means±SE. Significant differences ($p < 0.01$ and $p < 0.001$) are indicated with asterisks (* and ** Respectively).

long vaccination program against the Newcastle disease (ND). Notably, the table shows that in the early stages of the vaccination program, there are significant increases in IgG levels in vaccinated chickens compared to their non-vaccinated counterparts. However, the significance of this difference decreases over time, especially after Week 15. At week 1, the vaccinated chickens exhibit a much higher IgG concentration (114.000 µg/ml) compared to the non-vaccinated group (29.79 µg/ml). By week 3, the IgG levels in both groups increase, with the vaccinated group reaching 142.800 µg/ml and the non-vaccinated group rising to 63.80 µg/ml. At week 5, the vaccinated group's IgG levels remained high (142.650 µg/ml) with minimal change from week 3. Meanwhile, the non-vaccinated group's IgG levels declined to 51.68 µg/ml. The highly significant difference ($p < 0.001$) suggests that the vaccine continues to provide strong immune protection. By week 8, the IgG levels in both groups are nearly identical (49.43 µg/ml in non-vaccinated chickens vs. 48.680 µg/ml in vaccinated chickens). At week 10, the vaccinated group exhibits slightly higher IgG levels (55.860 µg/ml) than the non-vaccinated group (36.73 µg/ml). However, the difference was not statistically significant. At week 15, the vaccinated group maintained higher IgG levels (50.532 µg/ml), while the non-vaccinated group showed a continued decline to 24.82

µg/ml. Although the vaccinated group retained some immune advantage, the difference was no longer statistically significant. By week 18, the vaccinated chickens exhibit higher IgG levels (52.197 µg/ml) than the non-vaccinated group (27.31 µg/ml). At week 20, the non-vaccinated group shows a slight increase in IgG levels (40.84 µg/ml), surpassing the vaccinated group (35.000 µg/ml). By week 40, both groups display reduced IgG levels, with the non-vaccinated group at 17.24 µg/ml and the vaccinated group at 15.40 µg/ml. At the end of the study (week 60), the non-vaccinated group showed higher IgG levels (35.60 µg/ml) than the vaccinated group (12.048 µg/ml).

Concentrations of circulating IgA

Table (5) illustrate the impact of vaccination on the levels of circulating IgA (ng/ml) in the plasma of Cobb chickens, vaccinated with a long vaccination program against NDV with data spanning week 1 (W1) to week 60 (W60). At Week 1, IgA levels in the vaccinated chickens were significantly higher than in the non-vaccinated group (3.178ng/ml and 2.656ng/ml respectively). By Week 3, the difference in IgA levels between the vaccinated and non-vaccinated chickens remained highly significant (3.165 ng/ml). At Week 5, the IgA levels in vaccinated chickens remained significantly

Table 4: IgG levels in vaccinated and non-vaccinated cobb chickens over time.

IgG (ug/ml)	Non-vaccinated	Vaccinated	T value
W1	29.79±7.83	114.000±12.30***	5.78
W3	63.80±20.50	142.800±14.10**	3.17
W5	51.68±7.89	142.650±1.15***	11.42
W8	49.43±9.02	48.680±6.72	0.07
W10	36.73±6.18	55.860±8.12	1.88
W15	24.82± 6.43	50.532±0.40*	3.99
W18	27.31±3.22	52.197±0.42***	7.66
W20	40.84±7.82	35.000±16.0	0.33
W40	17.24±5.80	15.400±2.09	0.30
W60	35.60±18.10	12.048±0.38	1.30

IgG concentrations (ng/mL) measured at different weeks post-vaccination. Data are presented as means±SE. Significant differences ($p < 0.05$, $p < 0.01$ and $p < 0.001$) are indicated with asterisks (*, ** and *** Respectively).

Table 5: IgA levels in vaccinated and non-vaccinated cobb chickens over time.

IgA (ng/ml)	Non-vaccinated	Vaccinated	T value
W1	2.656±0.029	3.178±0.010***	17.03
W3	2.570±0.051	3.165±0.007***	11.65
W5	2.619 ±0.043	3.006±0.004***	9.04
W8	1.676±0.019	2.932±0.037***	37.58
W10	2.694±0.007	2.921±0.037***	7.67
W15	2.650±0.016	2.875±0.056*	4.01
W18	2.957±0.015	2.772±0.071	2.40
W20	2.776±0.041	2.652±0.019	2.68
W40	2.688±0.012	2.604±0.059	1.08
W60	2.660±0.016	2.651±0.047	0.36

IgA concentrations (ng/mL) measured at different weeks post-vaccination. Data are presented as means±SE. Significant differences ($p < 0.05$ and $p < 0.001$) are indicated with asterisks (*and *** Respectively).

elevated (3.006 ng/ml), though the T-value has decreased slightly. Week 8 shows a very large difference, with vaccinated chickens having much higher IgA levels (2.932 ng/ml) compared to non-vaccinated chickens (1.676 ng/ml). At Week 10, the IgA levels in the vaccinated group 2.921 ng/ml remained significantly higher than those of the non-vaccinated group (2.694 ng/ml), although the T-value is lower compared to Week 8. By Week 15, there is still a significant difference in IgA levels between the vaccinated and non-vaccinated groups (2.772 ng/ml and 2.957 ng/ml respectively), but this difference becomes smaller as the chickens age. In Week 18, there is no longer a significant difference between the groups. By Weeks 20

to 60, the IgA levels in vaccinated and non-vaccinated chickens converge. The differences are no longer statistically significant *and* the T-values indicate a diminishing effect of the vaccine on IgA production over time.

Histology of Bursa Fabricius (BF) of vaccinated and non-vaccinated chicks

The tissues of BF were stained with hematoxylin and eosin (Fig 1). The BF of a 1-week-old chicks showed areas of lymphocyte depletion and necrosis within the medulla of some bursal follicles. The tissue sections of the 3-week-old chicken showed numerous well-defined bursal follicles,

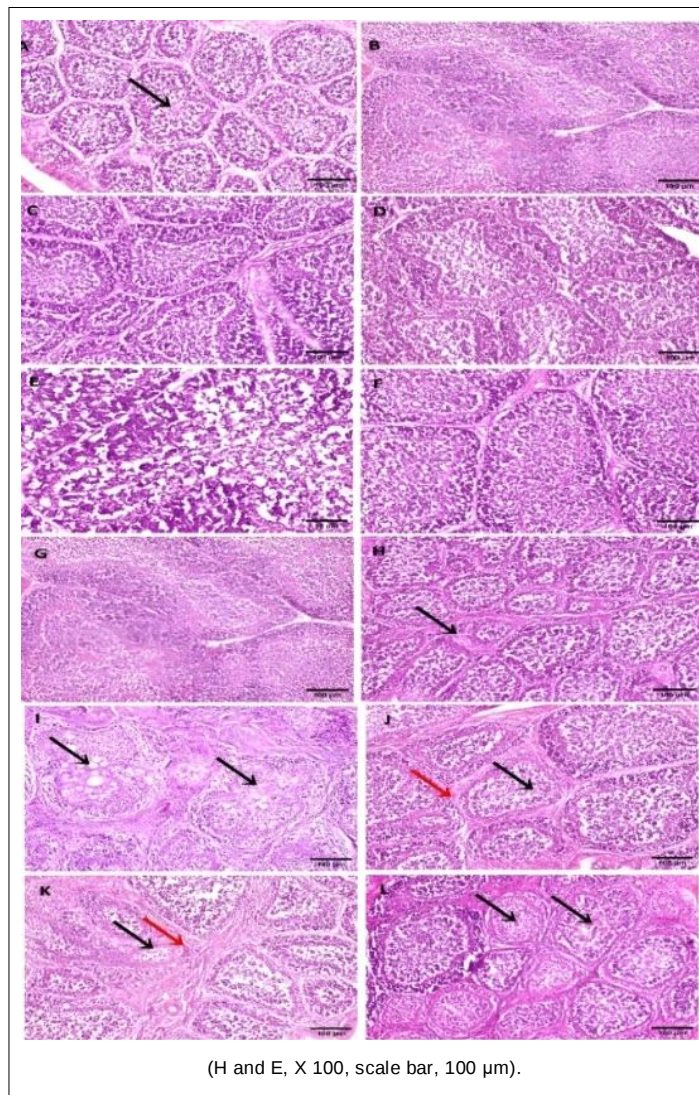


Fig 1: (A): Photomicrograph of bursa of chicken (1 week) showing slight lymphocytic necrosis and depletion in the medulla of some bursal follicles (black arrow). (B, C, D, E, F and G): Photomicrographs of bursa of chicken (3,5,8,10,15 and 18 weeks) showing normal bursal follicles without histopathological lesions. (H, I and J): Photomicrograph of bursa of chicken (20, 40 and 60 weeks) showing slight lymphocytic necrosis in some bursal follicles (black arrow). (K and L): Photomicrograph of bursa of non-vaccinated chicken (2 and 13- weeks) showing lymphocytic necrosis caseated material (black arrow) and heterophils infiltration (red arrow).

which are the primary functional units of the BF. The follicles appear to have a uniform size and distribution, suggesting a healthy and developing BF. The BF of a chicken at 5, 8, 10, 15 and 18 weeks old, exhibited a well-preserved and organized structure. The lymphocytes within the follicles appear to be intact and without any signs of necrosis, inflammation, or other cellular damages. The BF of 20- and 40-weeks-old chicken contained some follicles showing signs of lymphocytic necrosis and the follicles appeared dark and necrotic. The bursal follicles of a 60-week-old chicken showed a reduction in the number of lymphocytes (depletion) and evidence of lymphocyte necrosis. The histology highlights the presence of increased connective tissue (fibrosis) between the follicles. The histology of the BF of a non-vaccinated chicken (2 weeks old), showed areas of lymphocytic necrosis and

depletion. Heterophils infiltration and the presence of inflammatory cells were noticed. The histology the BF of a 13-week-old non-vaccinated chicken showed a reduction in the number of lymphocytes (lymphocyte depletion) and evidence of lymphocyte death (necrosis). Within the affected areas, the formation of caseated material can be observed.

Histology of spleen of vaccinated and non-vaccinated chicks

The histology depicts the spleen of a 1, 5, 10, 15, 18, 20, 40 and 60 -weeks -old chickens (Fig 2). The images show a well-organized lymphoid architecture with distinct white pulp and red pulp areas. There were no visible signs of inflammation, necrosis, or other pathological changes within the spleen tissue. This indicates a healthy and normal appearance of the organ. On the contrary, the histological

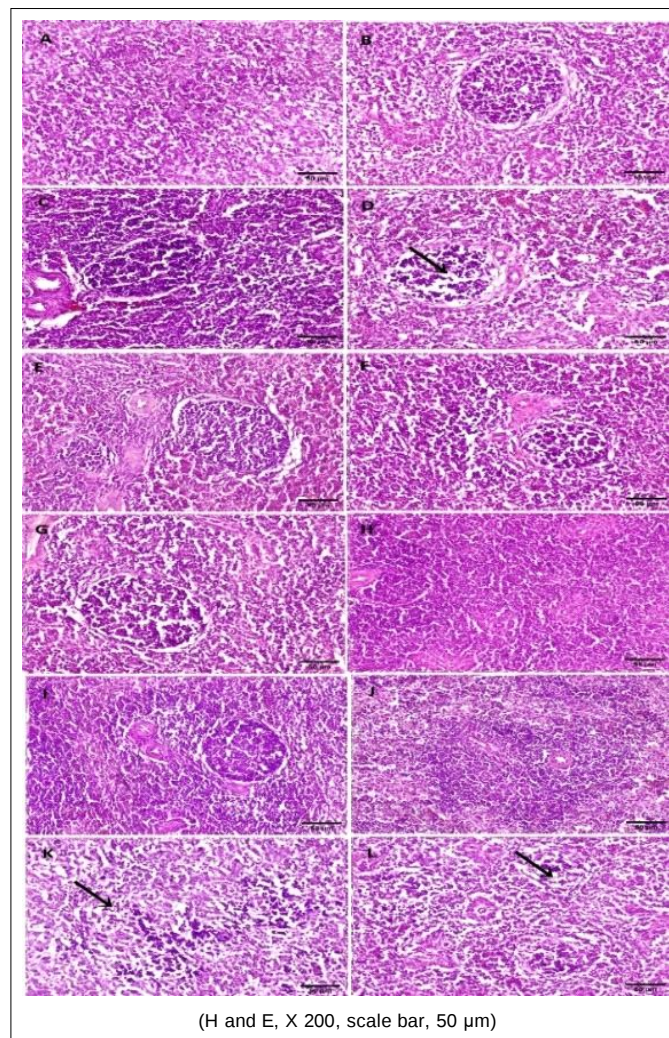


Fig 2: (A, B, C, E, F, G, H, I and J): Photomicrographs of spleen of chicken (1, 3, 5, 10, 15, 18, 20, 40 and 60 weeks) showing normal histological structure of splenic follicles without histopathological lesions. (D): Photomicrograph of spleen of chicken (8 weeks) showing slight lymphocytic depletion (black arrow). (K and L): Photomicrograph of spleen of chicken (non-vaccinated, 2 and 13-weeks) showing lymphocytic necrosis and depletion in the white pulp (black arrow).

examination of the spleen of the 3 and 8-weeks-old chicken showed areas of mild lymphocytic necrosis and depletion. The lymphoid follicles in the affected areas appeared less well-defined and organized compared to normal follicles. The histology of the spleen of a 2-week-old non-vaccinated chickens showed areas of lymphocytic necrosis and depletion and a significant reduction in the number of lymphocytes. The lymphoid follicles in the affected areas appear disorganized and less well-defined compared to normal follicles. On the other hand, the spleen of a 13-week-old non-vaccinated chicken indicated the presence of reticular cell hyperplasia. The lymphoid follicles within the white pulp appear enlarged and less well-defined compared to a normal spleen.

In this study we monitored the levels of circulating IgM in the Cobb chickens vaccinated against ND from week 1 to week 60. The data showed that the IgM levels of the vaccinated chickens were significantly higher than the IgM levels of the non-vaccinated chickens at weeks 1, 3, 5, 8 and 10. The IgM levels of the vaccinated chickens peaked at week 10 and then declined slightly over the next few weeks. The IgM levels then remained relatively stable from weeks 20 to 60. The circulating levels of both IgM and IgG in poultry are a sign of humoral immunity being modulated and their adaptive immune responses being stimulated (Du *et al.*, 2017). After the initial interaction with an antigen, specific IgM responses rise; responses decline with a subsequent exposure (Martinez *et al.*, 2018). The IgM levels of the non-vaccinated chickens also increased over time, but not as significantly as the IgM levels of the vaccinated chickens. This suggests that the non-vaccinated chickens may have been exposed to other microorganisms in the environment, which would have boosted their immune response. The results of the study suggest that the Newcastle vaccine is effective in induce IgM production in Cobb chickens.

IgG is another type of antibody that is found in primarily in the blood. It is essential for the body protection against infections. Our results showed the circulating levels of IgG in the chickens vaccinated against ND from week 1 to week 60. The data showed that the IgG levels of the vaccinated chickens were significantly higher than the IgG levels of the non-vaccinated chickens at weeks 1, 3, 5, 15 and 18. The IgG levels of the vaccinated chickens peaked at week 3 and then declined slightly over the next few weeks. The IgG levels then remained relatively stable from weeks 10 to 60. The vaccine appears to be sufficient in inducing IgG production in the first few weeks after vaccination. The overall results of the study suggest that the Newcastle vaccine is effective in maintaining a protective immune response against ND.

IgA is a type of antibody that is essential for mucous immunity, such as the nose, mouth and intestines. IgA plays a role in protecting the body from infection. It plays a major role in mucosal immunity, providing the first line of defense against infections (Abbas *et al.*, 2021). During the early

weeks (Weeks 1-10), the vaccine provides a significant advantage in boosting IgA levels. The results indicated a strong IgA response within the first 10 weeks, with vaccinated chickens showing consistently higher IgA levels than their non-vaccinated counterparts. This demonstrated that the Newcastle vaccine effectively stimulates mucosal immunity during the critical early phase. As time progressed (Weeks 15-20), the difference between the two groups started to diminish. By weeks 18, 40 and 60, there was no significant difference between the two groups, indicating that both vaccinated and non-vaccinated chickens now rely on natural immune mechanisms. These findings highlight the temporary nature of vaccine-induced immunity and emphasizes the importance of booster vaccinations to maintain long-term protection. The study also highlights the adaptive capability of non-vaccinated chickens, can develop over time, although they remain vulnerable during the early stages without vaccination. IgA is also an important Ig involved in humoral immunity. Their content can reflect the level of humoral immunity in chickens. The data of the current study offers valuable insights for poultry management practices, helping farmers and veterinarians make informed decisions about vaccination schedules to ensure optimal protection against ND.

Several studies (Sedeik *et al.*, 2022) have indicated that vaccination programs using a progressive strategy that uses booster shots may provide protection against clinical illness and mortality after virulent NDV infection if vaccinated birds continue to have high antibody levels. All the vaccinated chickens in this investigation developed adequate antibody responses. Evaluation of vaccine efficiency is important in poultry farms and companies Geletu and Robi (2024). Despite the advances in immunization programs, ND remains a significant threat to poultry industries worldwide; however, the infection can be managed through the effective implementation of vaccination programs (Martinez *et al.*, 2018).

Suitable immunization techniques should be looked for (Mebrahtu *et al.*, 2018). Measurement of serum IgM and IgG by ELISA was recently done with parallel results (Attia *et al.*, 2020). Different NDV vaccines programs are carried out in poultry farms for different reasons. These comprise the avoidance of encouraging stress in chicks through excessive treatment and to reduce vaccination costs (Ball *et al.*, 2019). The detection of antibodies before elevation due to vaccination might be due to maternal antibodies following a natural decline in the unvaccinated group as previously mentioned (Martinez *et al.*, 2018). The results of the current investigation were in agreement with Landman *et al.* (2017) who observed high antibody titers with a nearly similar program (Landman *et al.*, 2017).

In the present study, the BF of a 1-week-old chicken showed areas within the medulla of some bursal follicles where there is a lymphocyte depletion and lymphocyte necrosis. After that, the image of BF of chicken at 3, 5, 8, 10, 15 and 18 weeks old show numerous well-defined

bursal follicles, which are the primary functional units of the BF. This indicates a lack of any significant immune system abnormalities or diseases in these weeks-old chicken. The BF of a chicken of a 20, 40 and 60 -weeks-old chicken present some of the follicles are showing signs of lymphocytic necrosis and the follicles that appear dark and necrotic. These changes are indicative of immune system dysfunction. The histology of the BF of a non-vaccinated chicken (2 and 13 weeks old), showed areas of lymphocytic necrosis and depletion. heterophils infiltration and the presence of inflammatory cells were noticed. This histological image reflects immune suppression or damage in the bursa, which is critical for the development of the immune system in young chickens and highlights the detrimental effects of the absence of vaccination or potential exposure to pathogens. To regulate humoral immunity, chicken BF grows rapidly after hatching and achieves a massive size at the age of one to four months (Abdul-Aziz 2016). A large number of lymphoid or bursal follicles that give the B lymphocytes that produce antibodies (Cooper, 2015 and Abdul-Aziz, 2016). Cooper (2015) reviewed the development of the concept that the lymphoid follicles are a key organ in immunology. This histology of BF demonstrated how the immune system of birds is complicated and influenced by various factors including age and immunization program (Attia *et al.*, 2020). At the early age of live in the vaccinated groups, some follicles appeared with mildly reduced lymphocytes and it might be triggering the immune response as previously reported by Cooper (2015).

Histological examination of the spleen of a 1, 3, 5, 10,15, 18, 20, 40 and 60 -weeks -old chicken show a well-organized lymphoid architecture with distinct white pulp and red pulp areas. There are no visible signs of inflammation, necrosis, or other pathological changes within the spleen tissue. This indicates a healthy and normal appearance of the organ. On the contrary, the histological examination of the spleen of 8-week-old chicken revealed areas of mild lymphocytic necrosis and depletion. The histology of the spleen of a 2 and 13 weeks-old non-vaccinated chicken showed areas of lymphocytic necrosis and depletion and a significant reduction in the number of lymphocytes. These organs (BF and spleen) are widely recognized as being crucial avian lymphoid organs and serving as primary immunological sites for induction of immunological responses against antigens (Attia *et al.*, 2020).

CONCLUSION

The results of this study are important because they provide evidence that the prolonged Newcastle vaccination is an effective tool for protecting against NDV in chickens.

ACKNOWLEDGEMENT

The Researchers would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (QU-APC-2025).

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee (QU - No 24-12-01).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

- Abbas, A. (2021). Immuno-modulatory effects of *Lactobacillus* in *Salmonella Gallinarum* infected broiler chicks. *Pakistan Journal of Science*. 73: 77-87.
- Abdul-Aziz, T. (2016). *Avian Histopathology*. 4th Edition Paperback -January. 1: 423.
- Ajaykumar, R., Harishankar, K., Rangasami, S. R., Saravanakumar, V., Yazhini, G., Rajanbabu, V. and Premalatha, K. (2024). Growth performance, quantitative analysis and economics of broiler chickens as influenced by herbal dietary additives as alternative growth booster. *Indian Journal of Animal Research*. 58(7): 1139-1147. doi: 10.18805/IJAR.B-5326.
- Al-Zuhariy, M. T. (2023). Evaluation of the best vaccinal routes against Newcastle in the production stage of laying hens. *Iraqi Journal of Agricultural Sciences*. 54: 748-754.
- Almubarak, A.I. (2019). Molecular and biological characterization of some circulating strains of Newcastle disease virus in broiler chickens from Eastern Saudi Arabia in (2012-2014). *Veterinary World*. 12:1668-1676.
- Attia, Y.A., Al-Khalaifah, H., Abd El-Hamid, H.S., Al-Harthi, M.A. and El-Shafey, A.A. (2020). Effect of different levels of multienzymes on immune response, blood hematology and biochemistry, antioxidants status and organs histology of broiler chicks fed standard and low-density diets. *Frontiers in Veterinary Science*. 6: 510.
- Ball, C., Forrester, A., Herrmann, A., Lemiery, S. and Ganapathy, K. (2019). Comparative protective immunity provided by live vaccines of Newcastle disease virus or avian metapneumovirus when co-administered alongside classical and variant strains of infectious bronchitis virus in day-old broiler chicks. *Vaccine*. 37:7566-7575.
- Bello, M.B., Yusoff, K., Ideris, A., Hair-Bejo, M., Peeters, B.P. and Omar, A.R. (2018). Diagnostic and vaccination approaches for Newcastle disease virus in poultry: The current and emerging perspectives. *BioMed Research International*. 1: 7278459.

- Cooper, M.D. (2015). The early history of B cells. *Nature Reviews Immunology*, 15: 191-197.
- Criste, A., Urcan, A.C. and Corcionivoschi, N. (2020). Avian IgY antibodies, ancestors of mammalian antibodies-production and application. *Romanian Biotechnological Letters*. 25: 1311-1319.
- Deepthi, B., Ratnamma, D., Pushpa, R. N., Isloor, S., Veeregowda, B.M. and Satyanarayana, M.L.(2023). Molecular characterization of velogenic avian avulavirus type 1 isolated from apparently healthy emu birds: Implications to viral maintenance and spread. *Indian Journal of Animal Research*. 57(5): 558-564. doi: 10.18805/IJAR.B-4350.
- Dimitrov, K.M., Abolnik, C., Afonso, C.L., Albina, E., Bahl, J., Berg, M. et al. (2019). Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infection, Genetics and Evolution*. 74: 103917.
- Dimitrov, K.M., Afonso, C.L., Yu, Q. and Miller, P.J. (2017). Newcastle disease vaccines-A solved problem or a continuous challenge?. *Veterinary Microbiology*. 206: 126-136.
- Du, X., Chen, L., Huang, D., Peng, Z., Zhao, C., Zhang, Y. and Liu, G. (2017). Elevated apoptosis in the liver of dairy cows with ketosis. *Cellular Physiology and Biochemistry*. 43: 568-578.
- Geletu, A.S. and Robi, D.T. (2024). Evaluation of the immune response of layer chickens to Newcastle disease virus vaccines using the new vaccination regimens. *Veterinary Medicine and Science*. 10(3): e1428.
- Landman, W. J. M., Vervaeke, C., Remon, J. P., Huyge, K. and Van Eck, J. H. H. (2017). Primary Newcastle disease vaccination of broilers: comparison of the antibody seroresponse and adverse vaccinal reaction after eye-nose drop or coarse spray application *and* implication of the results for a previously developed coarse dry powder vaccine. *Pathology*. 46: 451-461.
- Lestari, D., Khaerunnisa, I., Murtini, S., Ulupi, N., Gunawan, A. and Sumantri, C. (2022). Variation and Association of Avian α -Defensin 2 Gene with the Concentration of Immunoglobulin Y and the Titer of Newcastle-Disease Antibody in IPB-D1 Chicken. *Tropical Animal Science Journal*. 45: 121-127.
- Li ShuYun, L. S., You GuoJin, Y. G., Du JiTeng, D. J., Xia Jing, X. J., Wen YiPing, W. Y., Huang XiaoBo, H. X. et al. (2020). A Class I lentogenic newcastle disease virus strain confers effective protection against the prevalent strains. *Biologicals*. 63: 74-80.
- Martinez, J. C. S., Chou, W. K., Berghman, L. R. and Carey, J. B. (2018). Evaluation of the effect of live LaSota Newcastle disease virus vaccine as primary immunization on immune development in broilers. *Poultry Science*. 97: 455-462.
- Mebratu, K., Teshale, S., Esatu, W., Habte, T. and Gelaye, E. (2018). Evaluation of spray and oral delivery of Newcastle disease I2 vaccine in chicken reared by smallholder farmers in central Ethiopia. *BMC Veterinary Research*. 14: 1-7.
- Sedeik, M. E., Awad, A. M. and El-Shall, N. A. (2022). Heterologous prime-boost vaccination programs against Newcastle disease virus genotype VII in chickens. *Comparative Immunology, Microbiology and Infectious Diseases*. 87: 101836.
- Tian, Y., Han, J., Wen, J., Wu, Y., Zhang, R., Xu, X. and Man, C. (2023). Regional immunity of chicken adipose tissue responds to secondary immunity induced by Newcastle disease vaccine *via* promoting immune activation and weakening lipid metabolism. *Poultry Science*. 102:102646.