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EVALUATION OF PROTECTIVE ANTIBODY LEVELS IN LAYING HENS FOLLOWING VACCINATION AGAINST AVIAN INFLUENZA, INFECTIOUS BRONCHITIS, AND NEWCASTLE DISEASE

Evaluasi Level Antibodi Protektif Pada Ayam Petelur Post Vaksinasi *Avian Influenza, Infectious Bronchitis,* dan *Newcastle Disease*

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Abstract

Avian Influenza (AI), Infectious Bronchitis (IB), and Newcastle Disease (ND) often infect broilers and layers. This study aimed to identify the immunological response of laying hens following vaccination against AI, IB, and ND. Laying hens received a multivalent vaccine containing AI, IB, and ND antigens at 18 and 45 weeks of age. Subsequently, antibody titers were assessed as a measure of the post-vaccination response. Blood samples were collected on days 7, 14, and 21 post – vaccination. Blood samples were collected from the brachial vein of laying hens that had been previously vaccinated. Data will be analyzed descriptively, and comparisons will be made in accordance with existing literature. The variables examined included AI, IB, and ND antibody titers. Results from the hemagglutination inhibition (HI) test for ND and AI antibodies demonstrated a notable increase in titers over the observation period. At day 0, the mean antibody titers for ND and AI were 2.5 and 4.6, respectively, suggesting that baseline antibody levels prior to the post-vaccination immune response were optimal. The ELISA IB test results were seropositive in all samples, both before and 21 days after vaccination. This indicates that all chickens already had antibodies against the IB virus. The

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study concluded that the highest protective antibody titers for AI, ND, and IB vaccines occurred between days 14 and 21 for AI and IB. Meanwhile, for ND, a decline in protection is observed after day 14, attributable to several factors including vaccine efficacy not guaranteeing complete protection, the timing of administration, the vaccine dose and route of administration, human error (particularly with injectable routes), and the specific strain used. Implementing biosecurity measures within poultry houses is crucial to prevent ND transmission.

Keywords: Antibody, Avian Influenza, Infectious Bronchitis, Laying Hen, Newcastle Disease, Teaching Factory

Abstrak

Avian Influenza (AI), *Infectious Bronchitis* (IB), dan *Newcastle Disease* (ND) sering menginfeksi ayam petelur dan ayam pedaging. Studi ini bertujuan untuk mengidentifikasi respons imun ayam petelur setelah vaksinasi AI, IB, dan ND. Ayam petelur menerima vaksin multivalent AI, IB, dan ND pada usia 18 dan 45 minggu. Selanjutnya, titer antibodi diuji sebagai respons pasca-vaksinasi. Sampel darah diambil pada hari ke-7, ke-14, dan ke-21 setelah vaksinasi. Sampel darah diambil dari vena brachialis ayam petelur yang sebelumnya divaksinasi. Data akan dianalisis secara deskriptif, dan perbandingan akan dilakukan sesuai dengan literatur. Variabel yang diamati adalah titer antibodi AI, IB, dan ND. Berdasarkan hasil uji HI untuk antibodi ND dan AI, terlihat peningkatan yang nyata pada titer antibodi selama periode pengamatan. Pada hari ke-0, rata-rata titer antibodi untuk ND dan AI masing-masing sebesar 2,5 dan 4,6, menunjukkan tingkat antibodi awal sebelum respons imun pasca-vaksinasi berkembang secara optimal. Hasil uji ELISA IB menunjukkan status seropositif pada semua sampel, baik sebelum maupun 21 hari setelah vaksinasi. Hal ini menunjukkan bahwa semua ayam memiliki antibodi terhadap virus IB. Kesimpulan dari studi ini adalah tingkat perlindungan tertinggi dari vaksin AI, ND, dan IB terjadi antara hari ke-14 dan ke-21 untuk AI dan IB. Sementara itu, untuk ND, setelah hari ke-14 terjadi penurunan yang disebabkan oleh beberapa faktor seperti efikasi vaksin yang tidak menjamin perlindungan 100%, waktu pemberian, dosis, dan rute pemberian vaksin, kesalahan petugas vaksin (untuk rute injeksi), dan jenis strain yang digunakan. Vaksinasi untuk menjaga kesehatan ayam petelur sangat direkomendasikan sebagai tindakan preventif biosecurity untuk ayam petelur.

Kata kunci: Antibodi, Influenza Burung, Bronkitis Infeksius, Ayam Petelur, Penyakit Newcastle, Teaching Factory

INTRODUCTION

Layer chickens face risks from various viral diseases, including Avian Influenza (AI), Infectious Bronchitis (IB), and Newcastle Disease (ND). Among these, AI is the most prevalent in poultry. Avian Influenza, caused by the influenza A virus subtype H5N1, is endemic in Indonesia and represents a significant threat, similar to other infectious viral diseases (Kencana *et al.*, 2017). Layer chickens are susceptible to AI, exhibiting symptoms such as upper respiratory tract infections and reproductive problems, which can result in morbidity and mortality rates reaching 100% (Yehia *et al.*, 2023; Tseng *et al.*, 2024). This disease has caused significant disruptions within the poultry industry, leading to substantial economic losses and impacting the supply chain of poultry products, as well as human food safety (Tseng *et al.*, 2024).

Infectious bronchitis virus is a highly contagious, acute respiratory disease in chickens (Triyatjaya *et al.*, 2024). Furthermore, IB virus can replicate in organs beyond the respiratory tract, including the kidneys and reproductive tract (Albassam *et al.*, 1986; Najimudeen *et al.*, 2020). This disease represents a significant challenge for chicken farmers, as it infects chickens

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of all ages. Beyond respiratory illness, IB virus reduces egg quality and production, and can lead to mortality, resulting in substantial economic losses due to associated symptoms, such as decreased farmer profitability (Bhuiyan *et al.*, 2021). Visible symptoms include nasal exudate, head swelling, frequent sneezing, and dyspnea. In poultry production, IB virus infection can cause abnormal egg shape, rough shell formation, and reduced egg production (Najimudeen *et al.*, 2020).

In addition to the two diseases discussed above, Newcastle Disease (ND) represents a critical concern in laying hen farming. This disease is highly contagious in poultry species and can affect all ages (Emilia *et al.*, 2015; Dharmayanti *et al.*, 2024). ND causes substantial economic losses for commercial poultry farmers worldwide (Charkhkar *et al.*, 2024). ND is an acute, contagious disease in poultry characterized by respiratory disorders, frequently followed by nervous disorders and diarrhea (Zhang *et al.*, 2023). Losses due to ND are attributable to high morbidity and mortality rates in poultry. Losses associated with ND are primarily due to high morbidity and mortality rates, which can reach 50–100% following infection with velogenic strains, 50% with mesogenic strains, and 30% with velogenic strain virus infections (Aboe *et al.*, 2006; Awuni, 2006; Enahoro *et al.*, 2021; Botchway *et al.*, 2022). Transmission of ND occurs directly between chickens within a single infected livestock group (Animal Health Australia, 2004). The virus is typically sourced from the excreta of infected chickens, disseminated through feed, drinking water, mucus, feces, or air contaminated with the virus, equipment, and personnel (Ansari & Dabas, 2025). The pathogenicity of ND is influenced by viral strain, route of infection, age, environmental factors, and the immune status of the host (El-Morshidy *et al.*, 2021). During illness, chickens shed significant amounts of virus in their feces (Guy, 1998). While avian influenza (AI) and infectious bronchitis (IB) often present with similar symptoms in broilers and layers, a key differentiator in layers is decreased egg production (Hassan *et al.*, 2020). Reduced egg production following infection may not always be recognized as a direct consequence of the disease (Rafique *et al.*, 2024).

Politeknik Negeri Lampung (Polinela), especially Animal Husbandry Department, has maintained Teaching Factory (TEFA) Laying Hens since 2020. Initially, the flock consisted of 200 hens, achieving a production percentage of 80% following commencement of maintenance; however, this subsequently declined to 30%. Daily egg production ranges from approximately 500 g to 2 kg. This is suspected because the chickens are infected with viral diseases such as AI, ND, or IBD. Within the TEFA Laying Hens program, a vaccination schedule is implemented; however, disease outbreaks during vaccination can indicate vaccination failure (Clements et al, 2008). Vaccination failure presents a challenge to the farm due to the infrequent evaluation of chickens' immune responses following vaccination (Bhuiyan *et al.*, 2021). Consequently, routine post-vaccination evaluations are necessary to assess the level of protection/immunity in laying hens (Geletu & Robi, 2024). This research aims to compare antibody titers for AI, IB, and ND in the TEFA Layer Polinela flock, examining changes in ND, IBD, and AI antibody titers before and after vaccination to inform future vaccination programs. These results are used viewing the ND IBD AI antibody titers based on the time before and after vaccination as a basis for carrying out further vaccination programs, and can help in confirming the diagnosis, and can also be used as a reference in carrying out medical actions, and can help determine the potential pathogenicity of viral diseases in both animals and humans, considering the existence of diseases in poultry that zoonotic are zoonotic.

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RESEARCH METHODS

Ethical Approval

This study did not require ethical approval for experimental animals because the study was conducted by veterinarians, from blood collection through technical implementation. The research adhered to animal welfare principles by minimizing stress and pain experienced by the chickens during sampling.

Study Object

This study was conducted at the Teaching Factory (TEFA) of Politeknik Negeri Lampung (Polinela) to assess the maintenance process and blood sampling procedures for laying hens. Twenty-four-week-old Isa Brown chickens, meeting clinical health criteria and exhibiting no signs of illness during the research, were utilized.

Study Design

This study utilized hemagglutinin (HA) and hemagglutinin inhibition (HI) tests, alongside enzyme-linked immunosorbent assays (ELISA), and observed layer hens in TEFA. A longitudinal, descriptive design was employed to evaluate changes in protective antibody levels in laying hens following vaccination against avian influenza, infectious bronchitis, and Newcastle disease. Serum samples were collected from the hens at multiple predetermined time points, including before vaccination (Day 0) and at days 7, 14, and 21 post-vaccination.

Study Variables

The independent variables in this study were the type of AI, IB, and ND vaccines and the time of serum sampling. The dependent variable was the immune response of laying hens following vaccination. The main parameters included: (1) AI antibody titer is calculated using the HI test beta method. Antibody titer was declared protective if >24 (WOAH, 2022); (2) The ND antibody titer was calculated using the HI test beta method. Antibody titer was declared protective if >64 (Oberländer et al, 2020); and (3) The IB antibody titer was measured using indirect ELISA. Protective titer was 834 (Ismail *et al.*, 2020). Antibody titer test results are determined by the presence or absence of agglutination reactions, indicated by dots dispersed at the bottom of the well (+). Conversely, the absence of agglutination is indicated by dots centered at the bottom of the well (-) (Costabile, 2010).

Data Collection

This study utilized layer chicken blood serum samples in the TEFA Polinela. Sampling was conducted four times, as detailed in Table 1. Following blood collection, testing was performed in the HI ND and HI AI test laboratories to determine antibody titers in laying hens. According to WOAH (2022), a protective antibody titer for both ND and AI is > 3 log 2.

Hemaglutination Assay (HA) of Newcastle Disease and Avian Influenza

A total of 0.025 mL of PBS is added to each well of the microplate using a micropipette or microdropper. The antigen suspension is then added to the first and second wells to be tested, followed by serial dilutions in doubling steps from the second to the eleventh well using a microdiluent. Next, add 0.025 mL of PBS to each well (1-12) and stir with a micro shaker. Following this, 0.05 mL of a 1% red blood cell suspension is added to each well, and the contents are gently sieved for 3 seconds. The microplate is incubated at room temperature for 1 hour, and red blood cell reactions are observed at 15-minute intervals. A positive reaction is indicated by crystal formation in the wells due to hemagglutination. The HA titer is determined by tilting the microplate at an angle of at least 45°. The HA titer of the virus is expressed as the

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reciprocal of the highest dilution that still results in complete agglutination. Typically, a HA titer of 4 HI units is used in the HI test (Ahmad *et al.*, 2014).

Hemagglutination Inhibition (HI) Test

Hemagglutination inhibition (HI) test was performed according to the WOAH (2024) procedure with modifications. The technique is as follows: 0.025 mL of PBS was added to each well of a microplate. Wells 1 and 2 were filled with 0.025 mL of serum, then serially diluted twofold from well 2 to well 10 using microdilution. From well 10, 0.025 mL of suspension was discarded. In wells 1 through 11, 0.025 mL of standard virus suspension (4 HAU) was added, while well 12 was filled with only 0.025 mL of PBS. The microplate was shaken for 30 seconds on a microshaker, then left to stand at room temperature for 30 minutes. A 1% erythrocyte suspension was added to wells 1 through 12, 0.025 mL each, then shaken for 30 seconds. The plate was then left at room temperature for one hour, with observations every 15 minutes. The HI test results were read when agglutination of erythrocytes was visible in well 11 and a sediment of erythrocytes was seen in well 12. The HI titer was determined by tilting the microplate and observing whether red blood cells settled (tear-shaped). The HI titer was identified as the highest serum dilution still capable of inhibiting erythrocyte agglutination. The antibody titer obtained after booster vaccination was calculated as the average from week 0 to week 3 (Ahmad *et al.*, 2014).

Indirect Enzyme-linked Immunosorbent Assay (ELISA) Test

Antigenicity testing involves reacting four antigen samples with ten positive IB antibodies. The indirect ELISA procedure begins by coating the IB virus antigen at a concentration of 2 µg/mL. One hundred microliters of the diluted antigen (1/100 dilution in coating buffer) are then added to each well of a microplate and incubated at 4°C for approximately 18 hours. The microplate is washed three times with washing buffer (physiological NaCl and 0.5 mL Triton X-100), each wash lasting 1 minute. Following washing, the microplate is blocked with a 4% milk blocking solution (100 µL per well) and incubated at 37°C for 1 hour. Subsequently, the microplate is washed three times with washing buffer (physiological NaCl and 0.5 mL Triton X-100), each wash lasting 1 minute. Vaccine-derived anti-IB virus antibodies are diluted 1/500 with blocking buffer and then added to each well (100 µL per well) and incubated at 37°C for 1 hour. The microplate is washed three times with washing buffer (physiological NaCl and 0.5 mL Triton X-100), each wash lasting 1 minute. Next, a conjugate (anti-chicken Ig G, labeled with alkaline phosphatase enzyme) diluted 1:10,000 in blocking buffer (100 µL per well) is added and incubated for 1 hour at 37°C. The microplate is washed three times with washing buffer (physiological NaCl and 0.5 mL Triton X-100), each wash lasting 1 minute. Then, 100 µL of p-nitrophenyl phosphate (p-NPP) substrate is added to each well, covered with aluminum foil, and incubated in the dark for 30 minutes. Immediately afterward, 50 µL of 3N NaOH is added to each well to stop the reaction. The results are then read using an ELISA reader at a wavelength of 405 nm. The Optical Density (OD) values are observed as the results in this study (Rahmahani *et al.*, 2022).

Data Analysis

The study data were measured and analyzed by comparison to established standards, specifically those provided by the World Organisation for Animal Health (WOAH). The results were subsequently presented descriptively. Antibody titers data were analyzed descriptively, including mean titer values and graphical representations illustrating changes in antibody titers before and after vaccination; therefore, statistical tests were not employed.

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RESULT AND DISCUSSION

Result

Hemagglutination Inhibition Test of Avian Influenza and Newcastle Disease

This study was conducted at TEFA Polinela using 24 Isa Brown laying hens with predetermined criteria. Blood was collected from the *brachial* vein using a 3 mL volume via a disposable 23G × 1 ¼ inch needle. The blood was stored in a collecting tube 3 mL and placed in an ice box, then transported to the laboratory for HI and ELISA tests. Blood sampling was performed four times: prior to vaccination (Day 0) and on days 7, 14, and 21 post-vaccination. Samples representing 10% of the total population were tested. The percentage increase in antibody titers against AI and ND in post-vaccination laying hens is shown in Figure 1.

Based on HI test results for Newcastle Disease (ND) and Avian Influenza (AI) antibodies, a noticeable increase in antibody titers was observed during the observation period. On day 0, the mean antibody titers for ND and AI were 2.5 and 4.6, respectively, indicating initial antibody levels prior to optimal immune response development following vaccination. On Day 7, the mean antibody titer for ND increased to 4.1 and AI to 5.95. This increase suggests the initiation of an immune response to the administered vaccine. Subsequently, on Day 14, the mean antibody titer for ND reached its peak value of 6.65, while the mean titer for AI increased to 6.25. These results indicate that the immune responses to ND and AI are in an optimal phase. On Day 21, the mean neutralizing antibody titer decreased slightly to 5.75, yet it remained at a protective level. Simultaneously, the mean antibody index titer continued to increase to 6.4, indicating a stable and sustained immune response.

ELISA Test of Infectious Bronchitis

Blood samples for IB ELISA testing were collected twice: once one day prior to vaccination and once 21 days post-vaccination. Ten samples were obtained from each chicken at each time point. The results of the IB ELISA test are shown in Figure 2. Based on the ELISA test results, all samples showed a seropositive status, both before and 21 days after vaccination. This indicates that all chickens already had antibodies against the IB virus, both from environmental exposure and in response to the vaccine. An increase in antibody titer was observed in five samples (No. 1, 2, 6, 7, and 10). Comparing antibody titer values before and 21 days after vaccination revealed an increase in titer in seven samples (No. 1, 2, 4, 5, 6, 7, and 10), indicating a robust immune response to the IB vaccine and its ability to stimulate antibody production. This increase indicates a strong immune response to the IB vaccine, demonstrating its ability to stimulate antibody production. In three samples (No. 3, 8, and 9), the antibody titer decreased. However, despite the decline, the titer still remained within the seropositive range.

Discussion

Avian influenza is a pathogenic and highly contagious disease, classified as HPAI and LPAI (Yoo *et al.*, 2022). The virus has been detected in ducks, chickens, and various minor poultry species, including quail, pheasant, partridge, and pigeons (Zhuang *et al.*, 2025). HPAI can cause mortality rates of up to 100% within 48 hours, particularly in chickens and turkeys (Song *et al.*, 2025). In laying hens, AI infection can lead to cessation of egg production within four days of infection (Kintz *et al.*, 2024). Routine vaccination remains the primary strategy for preventing and controlling illnesses caused by avian influenza (Azab *et al.*, 2023).

The results of the AI antibody titer are presented in Figure 1. The average antibody titer one day prior to vaccination was 4.6 log 2, which increased to 6.4 log 2 on the 21st day post-vaccination. Booster vaccination induces a more rapid increase in antibody titer formation

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compared to the initial vaccination, as memory cells are already established following the first dose, thereby accelerating antibody production in subsequent doses (Mandala *et al.*, 2023).

In this study, the Harbindo AI Plus vaccine was administered via intramuscular injection into the pectoral muscle. Figure 1's results demonstrate that antibody titers against AI increased from one day prior to vaccination through 21 days post-vaccination. The average antibody titers observed on days 0, 7, 14, and 21 were 4.6, 5.95, 6.25, and 6.4, respectively. These observed differences in antibody formation are related to the animal's response to antibody production. This response may be influenced by factors such as the animal's health condition, environmental conditions, the viral load, and the duration of exposure to the virus/disease (Wang *et al.*, 2022). The administered vaccine offers protection against field exposure. Variations in immune responses following vaccination are influenced by several factors, including potential differences in antigenic properties among vaccine viruses, antigen quality, and adjuvant content (Montgomery & Larbi, 2025). The antibody titer in field chickens should not be zero, as they are susceptible to infectious diseases. Conversely, antibody titers that are too high are also not recommended because they can render the vaccine ineffective, as they may be mistaken for infection (Agustin and Ningtyas, 2021).

The success of poultry vaccination programs hinges on two critical factors: the antigenic similarity between vaccine virus strains and circulating HPAIV strains, and rigorous monitoring of vaccinated herds (EFSA, 2024; Piesche *et al.*, 2025). Utilizing vaccines that antigenically match circulating field viruses offers superior efficacy and reduces the risk of emerging virus escape variants. Close-mesh surveillance aims to detect HPAI circulating stealthily in vaccinated herds that appear clinically healthy (Lewis *et al.*, 2021).

Newcastle disease (ND) is a highly contagious viral disease affecting poultry and impacts poultry production (Okwor *et al.* 2012; Mao *et al.* 2022; Akhtar *et al.* 2023; Putri *et al.*, 2024). It has become one of the endemic poultry diseases in Indonesia. ND infects birds through oropharyngeal secretions, fecal matter, or by inhaling contaminated dust or aerosols containing the virus. Four pathotypes of ND are recognized, categorized by the severity of the disease they induce in chickens: asymptomatic enteric, lentogenic, mesogenic, and velogenic. The most virulent velogenic strains cause acute, lethal infections, often characterized by hemorrhagic lesions in the intestines, as well as neurological and respiratory issues, resulting in morbidity and mortality rates of up to 100% (Pestka *et al.*, 2014 ; Eerde *et al.*, 2023). Vaccination is a method used in commercial farms to reduce ND cases (Eerde *et al.*, 2023). However, ND-infected poultry still occurs. According to OIE (2009), between 1500 and 8000 chickens were infected with the ND virus each month in Indonesia during 2007. In 2009 and 2010, clinical ND outbreaks were reported in vaccinated commercial birds, with mortality rates ranging from 70% to 80% (OIE 2009).

The study results indicated antibody titers on days 0, 7, 14, and 21 were 2.5 log 2, 4.1 log 2, and 6.65 log 2, respectively, but on the 21st day, it decreased to 5.75 log 2. Previous research has demonstrated that vaccination provides excellent protection against ND and reduces mortality; however, vaccination alone is insufficient to prevent viral spread and transmission, and cannot eliminate the endemicity of Newcastle disease in Indonesia. These findings align with previous observations of declining antibody titers after day 14. This decline is attributable to several factors, including the fact that vaccine efficacy does not guarantee complete protection; specifically, the timing of administration, the antigen (including formulation, adjuvant, and dose), the route of administration (Putri *et al.*, 2022), and the strain type used (Haque & Haque, 2024). Additionally, it is important to implement biosecurity measures in the poultry house as a preventive effort against ND transmission. (Piesche *et al.*, 2025).

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Prior research demonstrated that both primary and secondary lentogenic ND vaccinations elicited robust immune responses and protection against a velogenic NDV challenge, resulting in significant reductions in viral replication and shedding, and elevated antibody levels (Mindaye *et al.*, 2025). This indicates that a faster and higher induction of protective antibody titers after vaccination followed by infection shows the potential for better protection from the ND vaccine against infection. (Mindaye *et al.*, 2025). The vaccine used is VOLVAC-AC PLUS ND IB EDS KV. Vaccines are specifically formulated to stimulate immune responses, providing protection against highly infectious strains of NDV prevalent across diverse geographic regions (Dimitrov *et al.*, 2017; Haque & Haque, 2024). Common vaccine categories include live-attenuated, inactivated, and recombinant vector vaccines (Andey *et al.*, 2024). Inactivated vaccines are recognized for their ability to elicit strong humoral and cell-mediated immune responses, offering efficient defense. Typically, live-attenuated ND vaccines are administered via eye drops, aerosol, or drinking water, facilitating rapid and straightforward administration (Guérin *et al.*, 2024).

Live attenuated Newcastle disease (ND) vaccine, produced through reverse genetics, elicited high levels of T-cell proliferation, interferon-γ (IFN-γ), and antibody production. Newcastle live vaccine induced IgY and IgA anti-NDV antibody responses in vaccinated birds (Ike *et al.*, 2021). Furthermore, the live vaccine triggered a robust type I interferon response, including IFN-α and IFN-β, in chickens prior to or following NDV infection. In contrast, the inactivated ND vaccine resulted in elevated interleukin-6 (IL-6) and IFN-γ levels in vaccinated birds. Vaccination with the attenuated ND vaccine (Nobilis ND LaSota; Cevac Vitapest L) and the inactivated vaccine (Nobilis Newcavac) led to the production of anti-NDV IgY, IgM, and IgA antibodies (Bodman-Harris *et al.*, 2024).

Infectious Bronchitis

Infectious bronchitis (IB) is a multisystemic disease primarily affecting the respiratory system, but also impacting the urogenital system and causing kidney problems and reduced egg production, resulting in significant economic losses in intensive farming (Cavanagh, 2007). IB is one of the three major diseases in the poultry industry, including in laying hens (Maletic *et al.*, 2025). The virus responsible for IB belongs to the family *Coronaviridae*, genus *Gammacoronavirus*, and order Nidovirales. Fewer than 30 IB virus serotypes are known worldwide, differing in virulence or pathogenicity toward the respiratory organs, kidneys, and oviduct (Ziebuhr *et al.*, 2000). The vaccination program is carried out as an immunoprophylaxis effort on farms. Generally, vaccines are either live or killed (Maletic *et al.*, 2025).

Vaccination enhances the body's immunity, drawing upon prior viral infections or previous vaccinations (Darbyshire & Peters, 1984; Greenwood 2014). Vaccines stimulate an immune response in poultry, mirroring the effects of direct infection. The humoral immune response plays a very important role in stimulating a certain level of immune response. Humoral response generally increases after vaccination. However, it can gradually decline or be delayed depending on current infection exposure on the farm (Bhuiyan *et al.*, 2021). Testing before and after vaccination resulted in an average antibody titer significantly higher than the established protection level of > 853. This can occur due to the vaccine's relatively high efficacy, supported by several factors. Factors include vaccine manufacturer guidelines, vaccine storage process, time of vaccination, and vaccine expiration date. Vaccines should be administered under veterinary supervision, with ongoing monitoring of livestock health during poultry vaccination (Birhane and Fesseha, 2020).

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In this study, the vaccination results presented in Figure 2 demonstrate that all vaccinated chickens exhibited protective antibody levels; however, antibody titers decreased in samples 3, 8, and 9 following vaccination. Despite this decrease, the titers remained at protective levels. This decline was likely due to the high antibody titers prior to vaccination, resulting in a decrease observed by day 21 post-vaccination. This phenomenon may be attributed to the existing antibody levels in laying hens on the day of challenge and subsequent protection against the challenge strain (Geletu & Robi, 2024). Poultry vaccination is a standard practice for egg-laying chicken entrepreneurs. However, the laying hen industry faces challenges due to increasing egg demand, particularly in Indonesia (Bodman-Harris *et al.*, 2024). Several studies have shown that implementing a progressive vaccination program, involving successive booster vaccinations with virulent strains, can protect against disease and low mortality rates following virulent NDV infection. This is provided that vaccinated birds maintain high antibody titers. In this study, all vaccinated chickens developed protective antibodies, and a positive correlation was observed between the presence of these titers on the day of testing in laying hens and protection against the challenging strain (Geletu & Robi, 2024).

Figure 2 compares antibody titer values before and 21 days after vaccination, revealing an increase in titers in seven samples (samples 1, 2, 4, 5, 6, 7, and 10). This increase suggests a robust immune response to the IB vaccine, indicating its ability to stimulate antibody production. A decrease in antibody titer was observed in 3 samples (samples number 3, 8, and 9). However, this decrease in titer still falls within the protective category; it may be caused by several factors, including high initial IB antibody titers before vaccination, physiological antibody decline (antibody decay), and individual variations in immune response. The trend of increasing IB antibodies in poultry aged 63–73 weeks is notably elevated, particularly in laying hens (Meher *et al.*, 2017; Meher *et al.*, 2021). Live attenuated vaccine is one of the vaccines that is very important for preventing IB disease. The vaccines administered in this study included drinking water vaccines for IB and a combination vaccine via intramuscular injection containing AI, ND, and IB, which produced protective immunity with a more stable average antibody level (Meher *et al.*, 2021). The layer chickens at TEFA are vaccinated using a live vaccine that has a sufficiently high antibody titer. The immune response initially triggered by a live vaccine provides enhanced and more comprehensive immunity. This is in accordance with previous research that states the effectiveness of two distinct vaccination strategies in inducing a robust immunological response in laying chickens was evaluated. The initial method employed live-attenuated IB vaccines, whereas the subsequent method included both live-attenuated and inactivated IB vaccines. Serum antibody levels against IBV were assessed at three and ten weeks post-immunization. The vaccination strategy combining live-attenuated and inactivated vaccines demonstrated superior efficacy in eliciting both systemic and localized immune responses in the vaccinated chickens. Inactivated vaccines are frequently used in conjunction with live vaccines in commercial poultry immunization (Buharideen *et al.*, 2021).

These findings align with a previous study that developed an inactivated avian influenza H5 vaccine and evaluated antibody responses in chickens vaccinated with three doses (0.2, 0.5, and 0.7 mL) via intramuscular (IM) and subcutaneous (SC) routes. Woziri *et al.* (2021) and Parvin *et al.* (2024) reported higher antibody levels in chickens vaccinated via the SC route compared to those vaccinated via the IM route 21 days post-vaccination. In this study, the protective efficacy of the prepared IB vaccine from local isolates ranged from 63% to 93%, depending on dose and route, following challenge with a homologous IBV strain.

In general, AI, ND, and IB vaccinations provide the highest level of protection on days 14 and 21 (Nielsen *et al.*, 2023). Vaccination-induced immunity should replicate that induced by natural infection and activate both humoral and cellular immune responses (Arunachalam,

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2024). Furthermore, vaccines can be modified to enable serological differentiation between vaccinated and infected birds, distinguishing infected animals from vaccinated ones (Nielsen *et al.*, 2023).

Immune System of Chickens Against Viral Diseases

Chickens possess two complementary immune systems: innate and adaptive. These, along with proteins, cells, and other molecules, cooperate to block pathways leading to viral infections (Kapczynski *et al.*, 2013; Ike *et al.*, 2021). Because the viruses causing IB and ND are persistent, the chicken immune response has been extensively studied to understand their roles in viral immunity and to find markers for effective vaccine development. Although the chicken immune system shares similarities with those of mammals, notable differences exist (Ike *et al.*, 2021). For example, the avian immune system features heterophils as equivalents to mammalian neutrophils, but chickens lack functional eosinophils and lymph nodes. Despite the lymphatic system being divided into primary and secondary organs (Lu *et al.*, 2023), chickens possess only two primary lymphoid organs: the thymus and bursa. Their susceptibility to viral infection is partly determined by the major histocompatibility complex (MHC I and II) genotype, which influences humoral and cell-mediated responses by binding and presenting antigens to T lymphocytes (Ike *et al.*, 2021).

CONCLUSION AND RECOMMENDATION

Conclusion

Conclusion of this study is that the highest protective levels of AI, ND, and IB vaccinations occur between days 14 and 21 for AI and IB. However, for ND, protection declines after day 14, likely due to factors including vaccine efficacy not guaranteeing complete protection, the timing and dosage of administration, the route of administration, potential human error (particularly with injectable routes), and the specific strain type utilized. Implementing biosecurity measures within poultry houses is therefore crucial to prevent ND transmission.

Recommendation

Vaccination to maintain laying hens is highly recommended as part of the biosecurity program for layers.

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REFERENCES

Aboe, P. A., Boa-Amponsem, K., Okantah, S. A., Dorward, P. T., & Bryant, M. J. (2006). Free-range village chickens on the Accra plains, Ghana: Their contribution to households. *Tropical Animal Health and Production, 38*(3), 223–234. https://doi.org/10.1007/s11250-006-4357-9

Ahmad, U., Ahmed, I., Othman, F., & Yoke, Y. (2014). Effectiveness of haemagglutination test in detection of Newcastle disease virus (NDV) AF2240 strain from harvested allantoic fluids of infected chicken eggs. *International Journal of Biomedical Research*, 2–5. https://doi.org/10.7439/ijbr

Agustin, A. L. D., & Ningtyas, N. S. I. (2021). Titer antibody of Newcastle disease in layer chicken in Narmada District, West Lombok. *Jurnal Medik Veteriner, 4*(1), 98–103. https://doi.org/10.20473/jmv.vol4.iss1.2021.98-103

Volume 18 No. 2: 347-363
April 2026
https://doi.org/10.24843/bulvet.2026.v18.i02.p01

Akhtar, T., Shahid, S., Asghar, A., Naeem, M. I., Aziz, S., & Ameer, T. (2023). Utilisation of herbal bullets against Newcastle disease in poultry sector of Asia and Africa (2012–2022). *International Journal of Agriculture and Biosciences, 12*(1), 56–65. https://doi.org/10.47278/journal.ijab/2023.044

Albassam, M. A., Winterfield, R. W., & Thacker, H. L. (1986). Comparison of the nephropathogenicity of four strains of infectious bronchitis virus. *Avian Diseases, 30*, 468–476.

Andey, T., Soni, S., & Modi, S. (2024). Conventional vaccination methods: Inactivated and live attenuated vaccines. In *Advances in vaccination technologies for infectious and chronic diseases* (pp. 37–50). https://doi.org/10.1016/B978-0-443-18564-9.00030-8

Animal Health Australia. (2004). *Disease strategy: Newcastle disease (Version 3.0)*. Australian Veterinary Emergency Plan (AUSVETPLAN).

Ansari, S., Patel, A., Dabas, R., & Pradhan. (2025). *Newcastle disease: Strategies for effective management* (pp. 96–110).

Arunachalam, A. B. (2024). Vaccines induce homeostatic immunity, generating several secondary benefits. *Vaccines, 12*(4), 396. https://doi.org/10.3390/vaccines12040396

Awuni, J. A., Coleman, T. K., & Sedor, V. B. (2006). Comprehensive approach to the improvement of rural poultry production in Ghana. In *Improving farmyard poultry production in Africa: Interventions and their economic assessment* (pp. 84–89). FAO/IAEA.

Azab, A. A., Yehia, N., Makhareta, M., Samir, M., Shoukry, A., Abd, A., Mohamed, E., Alhag, S. K., Alwabli, A. S., El-Saadony, M. T., El-Tarabily, K. A., & Soliman, M. A. (2023). Evaluation of inactivated avian influenza virus and Newcastle disease virus bivalent vaccination program against newly circulated H5N8 and NDV strains. *Poultry Science*, 1–15. https://doi.org/10.1016/j.psj.2023.102952

Bhuiyan, M. S. A., Amin, Z., Rodrigues, K. F., Saallah, S., Shaarani, S. M., Sarker, S., & Siddiquee, S. (2021). Infectious bronchitis virus (Gammacoronavirus) in poultry farming: Vaccination, immune response and measures for mitigation. *Veterinary Sciences, 8*(11), Article 273. https://doi.org/10.3390/vetsci8110273

Birhane, N., & Fesseha, H. (2020). Vaccine failure in poultry production and its control methods: A review. *Biomedical Journal of Scientific & Technical Research, 29*, 22588–22596. https://doi.org/10.26717/BJSTR.2020.29.004827

Bodman-Harris, O., Rollier, C. S., & Iqbal, M. (2024). Approaches to enhance the potency of vaccines in chickens. *Vaccines, 12*(12), 1–34. https://doi.org/10.3390/vaccines12121337

Botchway, P. K., Amuzu-Aweh, E. N., Naazie, A., Aning, G. K., Otsyina, H. R., Saelao, P., Wang, Y., Zhou, H., Walugembe, M., Dekkers, J. C. M., Lamont, S. J., Gallardo, R. A., Kelly, T. R., Bunn, D., & Kayang, B. B. (2022). Host response to successive challenges with lentogenic and velogenic Newcastle disease virus in local chickens of Ghana. *Poultry Science, 101*(11), 102138. https://doi.org/10.1016/j.psj.2022.102138

Buharideen, S. M., Hassan, M. S. H., Najimudeen, S. M., Niu, D., Czub, M., Gomis, S., & Abdul-Careem, M. F. (2021). Immune responses in laying hens after an infectious bronchitis vaccination of pullets: A comparison of two vaccination strategies. *Vaccines, 9*(5), 531. https://doi.org/10.3390/vaccines9050531

Cavanagh, D. (2007). Coronavirus avian infectious bronchitis virus. *Veterinary Research, 38*, 281–297. https://doi.org/10.1051/vetres:2006055

Volume 18 No. 2: 347-363
April 2026
https://doi.org/10.24843/bulvet.2026.v18.i02.p01

Charkhkar, S., Bashizade, M., Sotoudehnejad, M., Ghodrati, M., Bulbuli, F., & Akbarein, H. (2024). The evaluation and importance of Newcastle disease's economic loss in commercial layer poultry. *Journal of Poultry Sciences and Avian Diseases, 2*(1), 1–4. https://doi.org/10.61838/kman.jpsad.2.1.1

Clements, C. J., Nshimirimanda, D., & Gasasira, A. (2008). Using immunization delivery strategies to accelerate progress in Africa towards achieving the Millennium Development Goals. *Vaccine, 26*(16), 1926–1933. https://doi.org/10.1016/j.vaccine.2008.02.032

Costabile, M. (2010). Determining the reactivity and titre of serum using a haemagglutination assay. *Journal of Visualized Experiments*, (35), 1752. https://doi.org/10.3791/1752

Darbyshire, J. H., & Peters, R. W. (1984). Sequential development of humoral immunity and assessment of protection in chickens following vaccination and challenge with avian infectious bronchitis virus. *Research in Veterinary Science, 37*, 77–86.

Dharmayanti, N. I., Nurjanah, D., Nuradji, H., Suyatno, T., & Indriani, R. (2024). Newcastle disease virus: The past and current situation in Indonesia. *Journal of Veterinary Science, 25*(1), e3. https://doi.org/10.4142/jvs.23022

Dimitrov, K. M., Afonso, C. L., Yu, Q., & Miller, P. J. (2017). Newcastle disease vaccines—A solved problem or a continuous challenge? *Veterinary Microbiology, 206*, 126–136. https://doi.org/10.1016/j.vetmic.2016.12.019

Eerde, V., Rimstad, E., Bock, R., Su, H., Branza-Nichita, N., Yakovlev, I. A., & Clarke, J. L. (2023). Plant-made vaccines against viral diseases in humans and farm animals. *Frontiers in Plant Science*, 1–15. https://doi.org/10.3389/fpls.2023.1170815

EFSA Panel on Animal Health and Animal Welfare (AHAW), & European Union Reference Laboratory for Avian Influenza. (2024). *Vaccination of poultry against highly pathogenic avian influenza—Part 2: Surveillance and mitigation measures*. EFSA.

El-Morshidy, Y., Abdo, W., Elmahallawy, E. K., El-Dayem, G. A. A., El-Sawak, A., El-Habashi, N., Mosad, S. M., Lokman, M. S., Albrakati, A., & Asa, S. A. (2021). Pathogenesis of velogenic genotype VII.1.1 Newcastle disease virus isolated from chicken in Egypt via different inoculation routes: Molecular, histopathological, and immunohistochemical study. *Animals, 11*(12), Article 3567. https://doi.org/10.3390/ani11123567

Emilia, A., Setiyaningsih, S., & Soejoedono, R. (2015). Isolasi dan karakterisasi biologik virus Newcastle disease. *Jurnal Kedokteran Hewan, 9*(1). https://doi.org/10.21157/j.ked.hewan.v9i1.2789

Enahoro, D., Galie, A., Abukari, Y., Chiwanga, G. H., Kelly, T. R., Kahamba, J., Massawe, F. A., Mapunda, F., Jumba, H., Weber, C., Dione, M., & Kayang, B. B. (2021). Strategies to upgrade animal health delivery in village poultry systems: Perspectives of stakeholders from Northern Ghana and Central Zones in Tanzania. *Frontiers in Veterinary Science, 8*, 1–15.

Geletu, A. S., & 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), 1–8. https://doi.org/10.1002/vms3.1428

Greenwood, B. (2014). The contribution of vaccination to global health: Past, present and future. *Philosophical Transactions of the Royal Society B: Biological Sciences, 369*, 20130433.

Guérin, J. L., Balloy, D., Pinson, M., Jbenyeni, A., & Delpont, M. (2024). Vaccination technology in poultry: Principles of vaccine administration. *Avian Diseases, 67*, 489–494. https://doi.org/10.1637/aviandiseases-D-23-99997

Volume 18 No. 2: 347-363
April 2026
https://doi.org/10.24843/bulvet.2026.v18.i02.p01

Guy, J. S. (1998). Virus infections of the gastrointestinal tract of poultry. *Poultry Science, 77*(8), 1166–1175. https://doi.org/10.1093/ps/77.8.1166

Haque, M. A., & Haque, E. (2024). Determination of immunogenicity of an inactivated ND vaccine developed experimentally with Newcastle disease virus (genotype VII.2) local isolates of Bangladesh. *Frontiers in Immunology*, 1–9. https://doi.org/10.3389/fimmu.2024.1482314

Hassan, M. S. H., & Abdul-Careem, M. F. (2020). Avian viruses that impact table egg production. *Animals, 10*(10), 1747. https://doi.org/10.3390/ani10101747

Ike, A. C., Ononugbo, C. M., Obi, O. J., Onu, C. J., Olovo, C. V., Muo, S. O., Chukwu, O. S., Reward, E. E., & Omeke, O. P. (2021). Towards improved use of vaccination in the control of infectious bronchitis and Newcastle disease in poultry: Understanding the immunological mechanisms. *Vaccines, 9*(1), 1–25. https://doi.org/10.3390/vaccines9010020

Ismail, M., Tan, S. W., Hair-Bejo, M., & Omar, A. (2020). Evaluation of the antigen relatedness and efficacy of a single vaccination with different infectious bronchitis virus strains against a challenge with Malaysian variant and QX-like IBV strains. *Journal of Veterinary Science, 21*, e76. https://doi.org/10.4142/jvs.2020.21.e76

Kapczynski, D. R., Afonso, C. L., & Miller, P. J. (2013). Immune responses of poultry to Newcastle disease virus. *Developmental and Comparative Immunology, 41*, 447–453.

Kencana, G. A. Y., Nyoman, S., Daniel, R. B. N., & Agatha, S. L. T. (2017). Respons imun ayam petelur pascavaksinasi Newcastle disease dan egg drop syndrome. *Jurnal Sain Veteriner, 35*(1), 81–90.

Kencana, G. A. Y., Nyoman, S., Mesakh, P. S., Arini, N. H., Steffi, O., Syamsidar, & Aprillia, K. (2015). Respons antibodi terhadap penyakit tetelo pada ayam yang divaksin tetelo dan tetelo–flu burung. *Jurnal Veteriner, 16*(2), 289–290.

Kintz, E., Trzaska, W. J., Pegg, E., Perry, W., Tucker, A. W., Kyriakides, A., Antic, D., Callaghan, K., & Wilson, A. J. (2024). The risk of acquiring avian influenza from commercial poultry products and hen eggs: A qualitative assessment. *Microbial Risk Analysis, 27–28*, 100317. https://doi.org/10.1016/j.mran.2024.100317

Lewis, N., Banyard, A., Essen, S., Whittard, E., Coggon, A., Hansen, R., Reid, S., & Brown, I. H. (2021). Antigenic evolution of contemporary clade 2.3.4.4 HPAI H5 influenza A viruses and impact on vaccine use for mitigation and control. *Vaccine, 39*. https://doi.org/10.1016/j.vaccine.2021.05.060

Lu, M., Lee, Y., & Lillehoj, H. S. (2023). Evolution of developmental and comparative immunology in poultry: The regulators and the regulated. *Developmental & Comparative Immunology, 138*, 104525. https://doi.org/10.1016/j.dci.2022.104525

Maletić, J., Jezdimirović, N., Spalević, L., Milovanović, B., Vasić, A., Kureljusić, J., & Kureljusić, B. (2025). Pathological and molecular investigation of infectious bronchitis in broilers: Analyzing the impact of biosecurity lapses. *Frontiers in Veterinary Science, 12*, 1548248. https://doi.org/10.3389/fvets.2025.1548248

Mandala, G., Tangkonda, E., & Sanam, M. (2023). Respon vaksinasi ND ayam IPB D1 yang dipelihara pada lingkungan lahan kering. *Jurnal Kajian Veteriner, 11*, 125–133. https://doi.org/10.35508/jkv.v11i2.8249

Mao, Q., Ma, S., Schrickel, P. L., Zhao, P., Wang, J., Zhang, Y., Li, S., & Wang, C. (2022). Review detection of Newcastle disease virus. *Frontiers in Veterinary Science, 9*, 936251. https://doi.org/10.3389/fvets.2022.936251

Volume 18 No. 2: 347-363
April 2026
https://doi.org/10.24843/bulvet.2026.v18.i02.p01

Meher, M. M., Jahan, N., & Afrin, M. (2021). Assessment of antibody titer and lymphoid organs weight following Newcastle disease vaccination and feed-supplementation of vitamin C, probiotics and antibiotic-growth-promoters in Japanese quails. *Macedonian Veterinary Review*, *44*(2), I–IX. https://doi.org/10.2478/macvetrev-2021-0016

Meher, M. M., Rahman, M., Akter, M. R., & Rahaman, S. (2017). Detection of avian infectious bronchitis virus and its specific antibody in different ages layer birds in Dinajpur District of Bangladesh. *5*(11), 2502–2507.

Mindaye, M., Akalu, M., Bayisa, B., & Abayneh, T. (2025). Evaluation of Vero and chicken embryo fibroblast cell-based Newcastle disease vaccine efficacy in chickens. *Poultry Science*, *104*(10), 105624. https://doi.org/10.1016/j.psj.2025.105624

Montgomery, L., & Larbi, A. (2025). Monitoring immune responses to vaccination: A focus on single-cell analysis and associated challenges. *Vaccines*, *13*(4), 420. https://doi.org/10.3390/vaccines13040420

Najimudeen, S. M., Hassan, M. S. H., Cork, S. C., & Abdul-Careem, M. F. (2020). Infectious bronchitis coronavirus infection in chickens: Multiple system disease with immune suppression. *Pathogens*, *9*(10), 779. https://doi.org/10.3390/pathogens9100779

Nielsen, S. S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E., Drewe, J. A., Garin-Bastuji, B., Gonzales Rojas, J. L., Herskin, M., Michel, V., Miranda Chueca, M. Á., Padalino, B., Roberts, H. C., Spoolder, H., Stahl, K., Velarde, A., Winckler, C., & Viltrop, A. (2023). Vaccination of poultry against highly pathogenic avian influenza—Part 1: Available vaccines and vaccination strategies. *EFSA Journal*, *21*, Article 8271. https://doi.org/10.2903/j.efsa.2023.8271

Oberländer, B., Failing, K., Jüngst, C. M., Neuhaus, N., Lierz, M., & Möller Palau-Ribes, F. (2020). Evaluation of Newcastle disease antibody titers in backyard poultry in Germany with a vaccination interval of twelve weeks. *PLOS ONE*, *15*(8), e0238068. https://doi.org/10.1371/journal.pone.0238068

OIE. (2009). NDV in Indonesia.

Okwor, E., Eze, D., & M, U. (2012). Newcastle disease virus shedding among apparently healthy commercial chickens and its epidemiological importance. *Pakistan Veterinary Journal*, *32*, 354–356.

Parvin, M. K., Haque, M. E., Haque, M. A., Kamal, M. M., Sadekuzzaman, M., Hayat, S., Rahman, M. T., Siddique, M. P., Nahar, S. S., Khasruzzaman, A. K. M., Hossain, M. T., & Islam, M. A. (2024). Immunogenicity and protective efficacy of an inactivated infectious bronchitis virus vaccine candidate from a local isolate of Bangladesh. *Journal of Advanced Veterinary and Animal Research*, *11*(3), 592–600. https://doi.org/10.5455/javar.2024.k809

Pestka, D., Stenzel, T., & Koncicki, A. (2014). Occurrence, characteristics and control of pigeon paramyxovirus type 1 in pigeons. *Polish Journal of Veterinary Sciences*, *17*(2), 379–384.

Piesche, R., Cazaban, C., Frizzo da Silva, L., Ramírez-Martínez, L., Hufen, H., Beer, M., Harder, T., & Grund, C. (2025). Immunogenicity and protective efficacy of five vaccines against highly pathogenic avian influenza virus H5N1, clade 2.3.4.4b, in fattening geese. *Vaccines*, *13*(4), 1–18. https://doi.org/10.3390/vaccines13040399

Putri, D. D., Poetri, O. N., Candra, A. A., & Soejoedono, R. D. (2022). Production of hyperimmune serum against genotype VII Newcastle disease virus in rabbits with several

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https://doi.org/10.24843/bulvet.2026.v18.i02.p01

applications. *Journal of Advanced Veterinary and Animal Research*, *9*(2), 211–220. https://doi.org/10.5455/javar.2022.i586

Rafique, S., Jabeen, Z., Pervaiz, T., Rashid, F., Luo, S., Xie, L., & Xie, Z. (2024). Avian infectious bronchitis virus (AIBV) review by continent. *Frontiers in Cellular and Infection Microbiology*, *14*, 1325346. https://doi.org/10.3389/fcimb.2024.1325346

Rahmahani, J., Akbar, D. I., & Suwarno, S. (2022). Comparison of antigenicity between local and Massachusetts strains of infectious bronchitis virus using indirect ELISA test. *Jurnal Medik Veteriner*, *5*(1), 28–33. https://doi.org/10.20473/jmv.vol5.iss1.2022.28-33

Song, W., He, F., Deng, Z., Hu, M., Fang, K., Cheng, W., Wu, J., Wang, X., Fan, G., Kong, L., Zhou, Y., Li, K., Abudunaibi, B., Kang, X., Xiang, T., Li, H., & Chen, T. (2025). Avian influenza virus dynamics in poultry and the environment: An eight-year longitudinal study in the southwestern Poyang Lake region of China. *Infectious Disease Modelling*, *10*(4), 1126–1137. https://doi.org/10.1016/j.idm.2025.06.002

Triyatjaya, Y., Hermawan, F., Zega, D., Pratiwi, U., & Khairani, S. (2024). Diagnosis of infectious bronchitis in a local chicken. *ARSHI Veterinary Letters*, *8*, 15–16. https://doi.org/10.29244/avl.8.1.15-16

Tseng, I. S., Pan, B. Y., Feng, Y. C., & Fang, C. T. (2024). Re-evaluating efficacy of vaccines against highly pathogenic avian influenza virus in poultry: A systematic review and meta-analysis. *One Health*, *18*, 100714. https://doi.org/10.1016/j.onehlt.2024.100714

Wang, Z. H., Ke, F., Gui, J. F., & Zhang, Q. Y. (2022). Environmental factors and their threshold affecting the survival of five aquatic animal viruses in different animal cells. *Viruses*, *14*(11), 2546. https://doi.org/10.3390/v14112546

WOAH. (2023). *Avian influenza vaccination: Why it should not be a barrier to safe trade*.

WOAH. (2024). *Manual of diagnostic tests and vaccines for terrestrial animals* (13th ed., Chapter 3.3.14: Newcastle disease).

Woziri, A., Meseko, C., Nasir, F., Abdulkarim, K., Fasina, F. O., Adamu, J., & Abdu, P. (2021). Impact of dose and route of administration on antibody responses of chickens inoculated with inactivated avian influenza H5 vaccine. *Microbes and Infectious Diseases*, 0–0. https://doi.org/10.21608/mid.2021.72759.1149

Yehia, N., Salem, H. M., Mahmmod, Y., Said, D., Samir, M., Mawgod, S. A., Sorour, H. K., AbdelRahman, M. A. A., Selim, S., Saad, A. M., El-Saadony, M. T., El-Meihy, R. M., Abd El-Hack, M. E., El-Tarabily, K. A., & Zanaty, A. M. (2023). Common viral and bacterial avian respiratory infections: An updated review. *Poultry Science*, *102*(5), 102553. https://doi.org/10.1016/j.psj.2023.102553

Yoo, D., Lee, K., Chun, B.-C., Lee, H., Park, H., & Kim, J. (2022). Preventive effect of on-farm biosecurity practices against highly pathogenic avian influenza (HPAI) H5N6 infection on commercial layer farms in the Republic of Korea during the 2016–2017 epidemic: A case-control study. *Preventive Veterinary Medicine*, *199*, 105556. https://doi.org/10.1016/j.prevetmed.2021.105556

Zhang, D., Ding, Z., & Xu, X. (2023). Pathologic mechanisms of the Newcastle disease virus. *Viruses*, *15*(4), 864. https://doi.org/10.3390/v15040864

Zhuang, Y. R., Liu, Y., Li, Q. F., Zhang, H. Q., Wang, C. Y., Ding, L. Y., Liu, A. Q., He, J., Meng, R., & Yu, L. G. (2025). An intelligence framework for the early warning of avian influenza in Chinese laying farms. *Poultry Science*, *104*, 105822.

Volume 18 No. 2: 347-363
April 2026
https://doi.org/10.24843/bulvet.2026.v18.i02.p01

https://doi.org/10.1016/j.psj.2025.105822

Ziebuhr, J., Snijder, E. J., & Gorbalenya, A. E. (2000). Virus-encoded proteinases and proteolytic processing in the Nidovirales. *Journal of General Virology*, *81*(4), 853–879. https://doi.org/10.1099/0022-1317-81-4-853

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Table

Table 1. Sampling Time of laying hen

Sampling Time	Description	Total
Day 0	Before vaccination	20 samples
Day 7	Seventh day post-vaccination	20 samples
Day 14	Fourteenth day post-vaccination	20 samples
Day 21	Twenty-first day following vaccination	20 samples

Figures

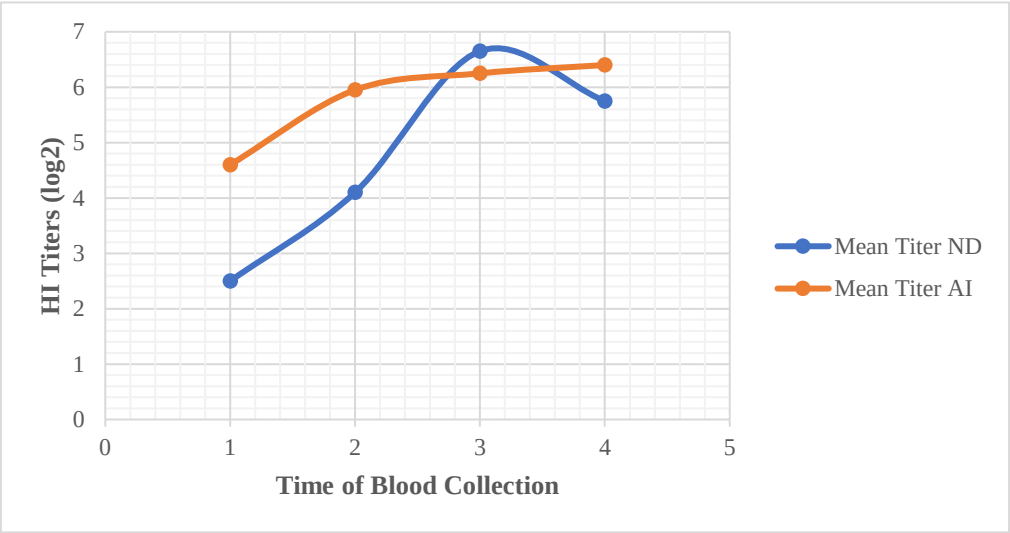

Figure 1. Haemagglutination Inhibition of AI and ND Antibody Titers

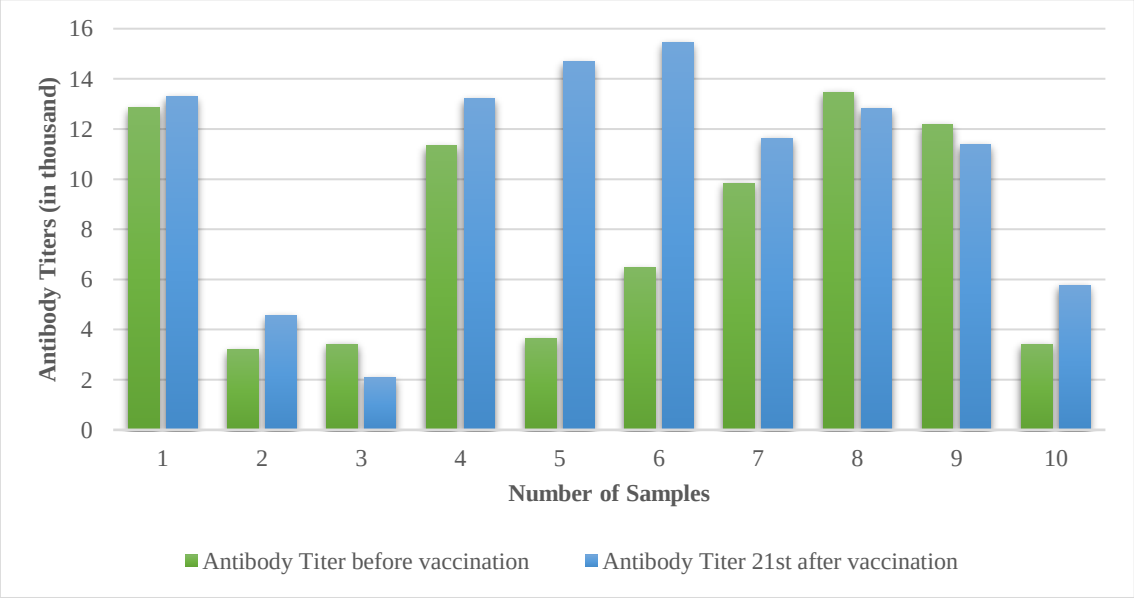

Figure 2. Comparison of IB antibody titers using ELISA

Terakreditasi Nasional Sinta 4, berdasarkan Keputusan Direktur Jenderal Pendidikan Tinggi, Riset, dan Teknologi No. 158/E/KPT/2021