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RESEARCH ARTICLE

Immunological responses of single vaccination NDVAC 1174/08 vaccine on specific pathogen free chickens (SPF) and village chickens (VC)

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ABSTRACT

The ND vaccine 1174/08, developed by the Veterinary Research Institute (VRI), was evaluated for immune response in specific-pathogen-free (SPF) and village chickens (VC). No prior immunological data existed for VC; this study compared the antibody responses the two groups. Nine SPF (14 days old) and nine VC (20 days old), all antibody-negative for ND, were vaccinated with a single dose of NDVAC 1174/08 ($10^{6.5}$ EID_{50}/bird), with a control group receiving PBS. Serum samples were collected up to 49 days post-vaccination (dpv) and tested using the haemagglutination inhibition (HI) assay. No clinical signs were observed in vaccinated birds. SPF chickens showed a mean HI titre (log_2) of 5.17 at 14 dpv, while VC reached a titre of 4.0 by 21 dpv with a mean of 5.22. At 49 dpv, both groups maintained protective antibody levels: SPF (4.17) and VC (4.38), indicating that a single dose of NDVAC 1174/08 provided sustained immunity in both chicken types.

1. INTRODUCTION

The poultry industry plays a vital role in Malaysia's agri-food sector, contributing significantly to national food security, economic development, and rural livelihoods. It provides affordable protein to the country's diverse population, with chicken being the most consumed meat. Per capita consumption rose from 46.2 kg in 2021 to 48.0 kg in 2022, placing Malaysia among the top three countries globally and first in Asia in terms of poultry meat consumption (Abdurofi et al., 2017; Ferlito, 2020; DVS, 2022). However, the sustainability and growth of this sector are persistently challenged by infectious poultry diseases, particularly Newcastle Disease (ND).

ND is recognized globally as one of the most economically devastating avian viral diseases, caused by virulent strains of *Avian orthoavulavirus 1*, commonly referred to as Newcastle Disease Virus (NDV) (El-Yuguda & Baba, 2021; Dimitrov, 2023). Although NDV does not pose a direct risk to human health, the disease severely affects poultry productivity, causing acute respiratory symptoms, enteric disturbances, neurological signs, and high mortality rates. Consequently, NDV outbreaks result in substantial economic and social burdens for both commercial and backyard poultry producers worldwide (Dimitrov, 2023). In Indonesia, which has the second-largest poultry population after China, ND continues to cause major losses, despite control efforts (Dharmayanti et al., 2023). Similar patterns are observed in countries like Iran and Nepal, where ND remains endemic and accounts for a significant proportion of poultry deaths annually, even in the presence of routine vaccination (Sarcheshmei et al., 2016; Napit et al., 2023).

Historically, the first ND outbreak was recorded in 1926 on Java Island, Indonesia, followed by an outbreak in Newcastle-upon-Tyne, England (Dharmayanti et al., 2023). In Malaysia, ND was first reported in 1934 in Parit Buntar, Perak (Mahamud et al., 2021), and has since become endemic, affecting both vaccinated commercial flocks and unvaccinated indigenous village chickens (Shohaimi et al., 2015). Given its impact, ND is classified as a notifiable disease under Malaysia's Animal Act 1953 (revised 2006) and the Bird Disease Control and Prevention Regulations 2009. These designations reflect the threat ND poses to Malaysia's poultry industry, which is a major contributor to national food security and rural income generation.

Although vaccination is the cornerstone of ND prevention and control, outbreaks continue to occur in Malaysia.

Multiple NDV genotypes have circulated in Peninsular Malaysia over the years, including genotypes I, II, III, VI, VII, and VIII. In recent outbreaks, genotype VII—particularly subgenotypes VIIa and VIIh—has become the predominant strain, replacing the earlier VIId variant (Shohaimi et al., 2019). Genotype VII viruses are known for their high virulence, rapid transmission, and genetic divergence from conventional vaccine strains such as LaSota and V1, which belong to genotype II (Dimitrov et al., 2017). A notable outbreak occurred in Sabah in 2021, with mortality rates reaching 50% among affected chickens. Molecular characterization confirmed the involvement of genotype VII NDV strains, suggesting the possible emergence of novel sub-genotypes in the region (Syamsiah Aini et al., 2022).

Commercial poultry farms in Malaysia generally follow structured vaccination programs using both live attenuated (e.g., LaSota, B1, VG/GA) and inactivated NDV vaccines. Live vaccines, usually administered via drinking water or spray, induce rapid protective immunity, especially in broilers. Inactivated vaccines, delivered through injection, are typically reserved for layers and breeders due to their longer duration of protection. However, vaccination failures are still reported due to several factors, including improper administration, immunosuppressive diseases, and antigenic mismatch between vaccine and field strains (Syamsiah Aini et al., 2022). As a response to the limitations of conventional vaccines, Malaysia has initiated the development of genotype-matched vaccines. For example, Bello et al. (2020) developed a recombinant live attenuated vaccine (mIBS025) derived from a local genotype VII isolate (IBS025/13), which demonstrated superior immunogenicity and protection compared to LaSota.

While vaccination programs are well-established in commercial operations, the same cannot be said for village chickens (VCs) and backyard poultry. VCs are commonly reared in rural and peri-urban areas for subsistence, traditional, and ceremonial purposes (Tenza et al., 2024). In the East Coast of Peninsular Malaysia, VCs are popular due to their hardiness, disease resistance, and preferred taste, although their productivity remains lower compared to commercial breeds (Nematbakhsh et al., 2021; Ha et al., 2017). These chickens are typically raised under low-input systems, with minimal investments in housing, feeding, and veterinary care. Consequently, they are more vulnerable to infectious diseases like ND, particularly in the absence of routine vaccination and proper biosecurity (Leow et al., 2015).

Backyard poultry systems in many parts of the world—such as Ethiopia, Nepal, and various rural regions in Malaysia—often lack structured disease prevention strategies. Studies have shown that backyard chickens can serve as reservoirs or amplification hosts for virulent NDV strains due to their close interaction with other domestic and wild avian species and the absence of adequate vaccination (Abdi et al., 2016; Enahoro et al., 2021; Hussain et al., 2019; Brown & Bevins, 2017). This poses a significant risk not only to village flocks but also to nearby commercial operations. Surveillance efforts in Malaysia have already recovered virulent NDVs from village chickens and even from fighting cocks, underscoring the role of non-commercial poultry in disease persistence (Syamsiah Aini et al., 2021).

Accurate monitoring of immune response post-vaccination is essential to evaluate vaccine effectiveness and guide immunization programs. The hemagglutination inhibition (HI) test remains the gold standard for detecting NDV-specific antibodies and is recommended by the World Organisation for Animal Health (WOAH/OIE). It is cost-effective, relatively simple, and widely used. Enzyme-linked immunosorbent assay (ELISA) is another commonly used method, especially for high-throughput analysis. The virus neutralization test (VNT), although more sensitive, is less frequently used due to its complexity and requirement for live virus (Choi et al., 2015; Steensels et al., 2024).

In 2008, a Newcastle disease virus strain (NDV 1174/2008) was isolated from an unvaccinated duck in Perak, Malaysia, and identified as a lentogenic strain based on F gene sequencing and pathogenicity testing (Suriani M. N. et al., 2016). This local isolate was selected as a candidate for live vaccine development due to its safety profile and potential immunogenicity. Subsequent studies evaluated its protective efficacy against Malaysian genotype VII challenge strains, demonstrating promising results. This study aims to evaluate and compare the immune response elicited by oral administration of the local NDV 1174/2008 vaccine strain in specific-pathogen-free (SPF) chickens and village chickens (VCs). The goal is to determine whether NDV 1174/08 can serve as an effective vaccine candidate for broader application in both commercial and backyard poultry settings in Malaysia.

2. MATERIALS AND METHODS

2.1. Ethical statement

With reference number IACUC-DVS-008-2022, all experimental procedures were carried out in accordance with the Institutional Animal Care and Use Committee (IACUC) of the Department of Veterinary Services (DVS) approval.

2.2. NDVAC 1174/08 vaccine preparation

The NDVAC 1174/08 vaccine used in this study was sourced from the Veterinary Research Institute (VRI) in Ipoh, Perak, Malaysia. This vaccine is a live, lyophilized preparation derived from the lentogenic Newcastle disease virus strain 1174/08, a local isolate. It is produced by propagating the virus

in the allantoic sac of embryonated specific-pathogen-free (SPF) chicken eggs, followed by harvesting the allantoic fluid for vaccine formulation. The NDVAC 1174/08 vaccine is specifically designed for the control and prevention of Newcastle disease in chickens. Its production strictly complies with internationally recognized standards, including those established by the World Organisation for Animal Health (WOAH, 2021) and the United States Food and Drug Administration's Code of Federal Regulations, Title 9 (9CFR, 2024), ensuring vaccine safety, potency, and quality.

2.3. Experimental house preparation

An experimental house was fumigated with one percent formalin. Drinkers, feeders, and buckets were among the equipment that were cleaned, sanitised, and brought into the house.

2.4. Experimental design and vaccination procedure

The experiment was conducted using SPF chickens at the Viral Vaccine Chicken Experiment Facility, VRI, in Ipoh, and VC chickens at a farm in Teluk Intan, Perak. A total of nine SPF chickens and nine ND antibody-negative VC chickens, aged 14 and 20 days respectively, were orally vaccinated with a single dose of $10^{6.5}$ / chicken. The freeze-dried vaccine was reconstituted with 500 mL of distilled water and administered orally at a dose of 1 mL per bird to both the SPF and VC groups, excluding the control group. The vaccine was administered as soon as possible, and during the vaccination process, it was kept in an ice container to maintain its stability. The unvaccinated control group received phosphate-buffered saline (PBS) via the same oral route.

2.5. Blood sample collection

Blood sampling was conducted on the chickens at weekly intervals up to 49 days post-vaccination. Samples were obtained via either the brachial (wing) vein or the jugular vein, using sterile 1 ml syringes fitted with 24G needles. Each sample was clearly labelled and positioned at an angle at room temperature to promote proper clotting. Upon completion of the sampling process, the blood samples were placed into a plastic container and promptly transported to the Viral Vaccine Section, VRI Ipoh, for further analysis.

2.6. Serological assay

Blood samples were kept at room temperature until serum separation occurred, which typically took about 2 to 3 hours. After separation, the serum was carefully collected and then inactivated by heating at 56 °C for 30 minutes before further testing. These serum samples were subsequently used to determine antibody titers against Newcastle Disease Virus (NDV) through the haemagglutination inhibition (HI) test, following the protocols outlined in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (WOAH, 2021).

3. RESULT AND DISCUSSION

The study evaluated the hemagglutination inhibition (HI) antibody response to the NDVAC 1174/08 vaccine in Specific Pathogen-Free (SPF) chickens and Village Chickens (VCs) over a 49-day post-vaccination period (Table 1). HI antibody titres showed a progressive increase in both groups, with notable differences in the timing and consistency of the response. SPF chickens exhibited a rapid immune reaction, achieving protective HI titres ($\log_2 \geq 4$) by 14 days post-vaccination, with a mean titre of 5.17. In contrast, VCs showed a delayed response, with no detectable HI antibodies at 14 days, but reaching protective levels by 21 days post-vaccination, recording a mean titre of 5.22. By 49 days post-vaccination, both groups maintained protective immunity following a single vaccine dose; SPF chickens had a mean titre of 4.17, while VCs had a slightly higher mean titre of 4.38. However, VCs displayed greater fluctuations in antibody levels over time compared to SPF birds. These variations likely reflect underlying biological and environmental differences between the two groups, such as genetic diversity, pathogen exposure, and management conditions. Importantly, no HI antibodies were detected in the unvaccinated control group throughout the study. The findings underscore the need for field vaccination programs to account for the delayed protection in VCs, potentially requiring temporary confinement to reduce disease exposure during the vulnerable period. Additionally, future research should explore adjuvant formulations to accelerate immune responses in VCs and determine optimal booster intervals suited to smallholder poultry production systems.

Table 1: Mean HI Antibody Titer ($\log_2$) of SPF and Village Chickens (VC) at Different Days Post Vaccination (dpv)

Mean HI Antibody Titer, $\log_2$		
Day Post Vaccination (dpv)	SPF Chicken	Village Chicken
3	0.00	0.00
5	0.00	0.00
7	2.67	0.00
14	5.17	3.56
21	5.33	5.22
28	4.50	5.22
35	5.00	5.25
42	5.17	4.13
49	4.17	4.38

The current study demonstrated that both specific-pathogen-free (SPF) and village chickens (VCs) vaccinated with NDV 1174/08 developed progressively increasing hemagglutination inhibition (HI) antibody titers, with peak responses observed at 3 weeks post-vaccination. This antibody response was sustained for at least 7 weeks, indicating that the NDV 1174/08 strain elicited protective immunity across different chicken types. These findings are in agreement with Kim et al. (2024), who reported peak HI titers at 4 weeks in vaccinated

birds. The slight difference in peak timing may be attributable to differences in host age, breed, immune status, or vaccine administration parameters such as dosage and route. A single dose is sufficient to initiate a short-term protective response in VC, but it may not be enough for long-term protection. A booster dose (possibly around 4–6 weeks after the first) is recommended for sustained immunity, especially in environments with high disease pressure.

Contrary to studies suggesting that older chickens typically mount stronger antibody responses due to more mature immune systems (Abdoshah et al., 2022), our results showed that even 5-month-old chickens achieved protective titers (≥4 $\log_2$), consistent with findings by Oberländer et al. (2020). This suggests that age may not be as critical a factor as previously thought, particularly when appropriate vaccine strains and administration methods are used. Sarker et al. (2021) demonstrated that LaSota-based vaccines produce higher HI titers than Mukteswar or B1 strains, possibly compensating for age-related immunological immaturity. Such differences emphasize the need to evaluate vaccine strain immunogenicity, especially in younger birds.

Future studies should explore the biological underpinnings of age-dependent vaccine responses. While older birds may benefit from immunological memory, younger birds might require booster doses or multivalent vaccines for optimal protection. Comparative trials involving multiple age cohorts and challenge models would provide valuable insights. The efficacy of NDV vaccination has been widely documented in controlling Newcastle disease outbreaks, reducing both disease incidence and mortality (Bessell et al., 2020). Nevertheless, despite widespread vaccination, complete protection is not always achieved, particularly in endemic areas. Vaccine failures have been attributed to multiple factors, including improper handling or administration, suboptimal vaccination schedules, immunosuppressive infections, and mismatches between vaccine strains and circulating field viruses (Sultan et al., 2021; Sarcheshmei et al., 2016).

Shahar et al. (2018) reported that conventional live attenuated vaccines (e.g., LaSota, B1) are effective in mitigating clinical disease but cannot fully prevent virus replication or shedding due to antigenic differences between vaccine and field strains. The genetic drift of NDV, particularly the emergence of genotype VII in Southeast Asia, presents a significant challenge (Roohani et al., 2015; Dimitrov et al., 2017; Hu et al., 2022). Genotype VII isolates are highly virulent, rapidly spreading, and have been associated with reduced vaccine efficacy, especially when conventional genotype II vaccines are used (Miller et al., 2015).

The dominance of genotype VII strains in Malaysia has made them the most economically significant NDVs, causing repeated outbreaks in both commercial and backyard poultry farms. This situation is mirrored in other countries, such as Nepal, where a genotype I strain was recently detected for the first time in backyard flocks (Napit et al., 2023). Although genotype I is generally considered lentogenic, its discovery highlights the need for enhanced surveillance and molecular characterization of local NDV strains.

Our vaccine candidate, NDV 1174/08, was classified as lentogenic based on F gene sequencing and pathogenicity testing (Suriani et al., 2016). Although its complete genotype remains undetermined, preliminary results suggest it is immunogenic in both SPF and village chickens. Matching vaccines to local field strains, such as NDV 1174/08 to Malaysian genotype VII strains, may help close the efficacy gap and improve outbreak control. Vaccination remains the most practical and cost-effective method for ND control, especially in resource-limited settings (Al-Garib et al., 2019). In Haryana, India, for instance, lentogenic vaccines are routinely used for broilers, while both lentogenic and mesogenic strains are used in breeders and layers (Joshi et al., 2021). In Iran, Sarcheshmei et al. (2016) showed that while vaccination reduced mortality after challenge with virulent NDV, vaccinated birds could still harbor and transmit the virus. This reinforces the need for integrated control strategies beyond vaccination alone.

The route of administration also plays a crucial role in vaccine uptake and efficacy. In this study, oral administration was used due to its simplicity and practicality, particularly for field conditions and smallholder farmers. Previous studies have shown comparable immune responses using oral or spray administration (Abdoshah et al., 2022; Steensels et al., 2024). Abdi et al. (2016) further demonstrated that cereal grains can serve as effective oral vaccine carriers, offering an alternative delivery method for rural or extensive poultry systems. However, the current study had limitations, including a small sample size (n=9 per group) and the lack of a challenge trial. These factors constrain the generalizability of our findings. Future research should incorporate larger cohorts, controlled challenge models, and longitudinal monitoring to validate efficacy and duration of protection. Furthermore, early vaccination strategies (e.g., in ovo or within the first week of life) could be explored to induce lifelong immunity, provided they do not adversely impact chick viability or growth (Mayers et al., 2017).

While vaccine access is relatively good in urban and peri-urban settings, rural poultry producers who represent the majority in many developing countries often face barriers to access due to logistical challenges, poor infrastructure, and limited commercial incentives for private suppliers (Enahoro et al., 2021). This highlights the importance of government-led vaccination programs and community-based distribution models

to reach these underserved populations.

Importantly, vaccination alone is insufficient to fully control ND. Biosecurity practices are essential to prevent virus introduction and transmission within flocks (FAO, 2002). In Malaysia, genotype VII NDV outbreaks continue despite vaccination programs, primarily due to poor hygiene, lack of sanitation, uncontrolled bird movement, and exposure to wild birds (Omar, 2025). Basic biosecurity measures such as clean water, disinfected feeding systems, and quarantine protocols have been shown to significantly reduce disease risk, even in village systems (Leow et al., 2015).

Numerous studies confirm that a holistic approach—combining appropriate vaccine strains, effective administration, good management, and strong biosecurity—offers the best chance of controlling Newcastle disease (Tulu, 2019; Bello et al., 2018). In backyard and village systems, where disease burden remains high, targeted education and intervention strategies will be critical to improving flock health and reducing the socioeconomic impact of ND.

This study has several limitations that should be considered when interpreting the results. The relatively small sample size may limit the statistical power of the analysis and reduce the generalizability of the findings to broader populations. This limitation was primarily due to the preliminary nature of the study and the availability of samples at the time of experimentation. Additionally, the evaluation was based on a single vaccine dose, which may not fully represent the immune response that could be achieved with multiple or booster doses. Furthermore, the study did not include a challenge trial; therefore, the protective efficacy of the vaccine against actual pathogen exposure could not be directly assessed. To address these limitations, future studies should incorporate larger sample sizes, explore varied dosing regimens, and include challenge trials to more comprehensively evaluate the vaccine's protective capacity and long-term efficacy.

4. CONCLUSION

The findings of this study highlight the significant potential of the NDVac 1174/08 vaccine, particularly when administered via the oral route, in offering protective immunity against Newcastle Disease (ND) for up to 49 days. This extended duration of immunity is especially meaningful for rural and smallholder poultry systems, where logistical and financial barriers often limit the feasibility of frequent vaccinations or booster doses. The oral administration method not only simplifies the vaccination process but also enhances accessibility, especially in remote areas where veterinary services are scarce. By eliminating the need for injections and trained personnel, this approach empowers farmers to administer vaccines themselves, increasing coverage and compliance in village settings. Moreover, a 49-day immunity window can provide sufficient protection through critical production periods, such as brooding and early growth stages, where mortality from ND tends to be highest. This has direct implications for improving poultry productivity, food security, and household incomes, particularly in low-resource environments where poultry is a primary livelihood asset. The findings also contribute valuable data to the ongoing search for sustainable, low-cost vaccination strategies in endemic regions. In conclusion, the study demonstrates that oral administration of NDVac 1174/08 is not only effective in inducing long-lasting immunity but also offers a practical and scalable solution for Newcastle Disease control in village chickens. Further field evaluations and cost-benefit analyses are recommended to facilitate its integration into national and community-level vaccination programs. Future research should focus on field evaluations of NDVac 1174/08 across diverse farming systems and its integration into comprehensive vaccination strategies to maximize its impact on disease control and economic sustainability.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare related to this article.

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