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Immunity Evaluation of Inactivated Newcastle Disease Virus Vaccine Inoculated at Different Doses in Day-old Specific-Pathogen-free Chicks

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ABSTRACT

This research aims to evaluate the immunogenicity of different doses of HIPRAVIAR® BPL2 inactivated Newcastle disease virus (NDV) LaSota vaccine. Specific-pathogen-free day-old chicks were divided into 3 different groups, and each group was vaccinated subcutaneously with the vaccine dose of 0.1, 0.2, and 0.5 ml, respectively. Blood samples were collected to measure NDV-specific antibody titers using a hemagglutination inhibition (HI) test and enzyme-linked immunosorbent assay (ELISA). The HI result showed that birds vaccinated with 0.5 ml HIPRAVIAR® BPL2 vaccine showed an increased statistically significant antibody titer compared to the other doses. Similarly, the ELISA result corroborated the HI finding. No significant difference between the results was detected when the antibody titers were measured using two ELISA kits, Biocheck CK116, and CIVTEST® AVI NDV. The percentage antibody-positive test based on HI amongst the different days post-vaccination showed that all the birds were positive from 28 to 42 days following vaccination with HIPRAVIAR® BPL2 0.5 ml (group D), whereas the highest percentage of antibody positivity were 80% and 70% at 42 days post-vaccination with HIPRAVIAR® BPL2 0.1 ml (group

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B) and HIPRAVIAR® BPL2 0.2 ml (group C), respectively. In conclusion, besides the difference in seroconversion, all the vaccine doses used had important levels of seroconversion and positivity.

Keywords: Antibody titer, ELISA, HI, inactivated vaccine, LaSota, seroconversion

INTRODUCTION

For decades, the poultry production in Malaysia has expanded steadily, in line with the establishment of feed mills, genetic improvement and veterinary services to meet the local demand and export trades (Abdurofi et al., 2017). However, the poultry industry in this region is constantly under threat by devastating diseases such as Newcastle disease (ND) and highly pathogenic avian influenza virus (HPAI) infection. ND is one of the highly contagious viral diseases causing economic losses in the poultry industry worldwide, causing a reduction in egg production, poor weight gain, morbidity, and mortality in infected chicken flocks (Alexander, 2000; Miller & Koch 2013). Other than poultry species, the disease also affects several species of wild birds such as owls, black swans, peacocks, and egrets (Shohaimi et al., 2015; Suarez et al., 2020; Mahamud et al., 2021). In addition, a study has shown wild migratory birds can act as reservoirs of virulent NDV, introducing the disease to commercial poultry farms (Naguib et al., 2022). Among the avian species, chickens are most susceptible to ND; ducks showed no clinical symptoms, while other waterfowls serve as natural reservoirs for the virus (Hines & Miller, 2012; Snoeck et al., 2013).

A single-stranded, negative-sense RNA virus causes Newcastle disease called Newcastle disease virus (NDV) or *Avian Orthoavulavirus 1* (AOAV-1) (formally called *Avian Avulavirus-1*) that belongs to the genus *Avulavirus* (ICTV, 2019; Dimitrov et al., 2019). The virus can be classified based on genotypes and pathotypes. The genotype classification is based on molecular characterization of the fusion (F) gene, where the virus can be different into 18 genotypes (genotypes I to XVIII) (Diel et al., 2012). Recently, Dimitrov et al. (2019) further refined the classification of NDV genotypes with the identification of several new genotypes (genotype I to XXIII). Presently, NDV genotype VII is causing the fifth panzootic that has spread rapidly across Asia and the Middle East (Miller et al., 2015; Dimitrov et al., 2019). The pathotype classification of the virus is based on biological characterization, where at least there pathotypes have been identified: the highly fatal velogenic strain, characterized by signs and lesions severely affecting the respiratory, gastrointestinal, and nervous systems; the moderately severe mesogenic strain; and the less severe lentogenic strain (Alexander & Jones, 2008, Jindal et al., 2010). The velogenic form of NDV caused the most significant disease in poultry species (Suarez et al., 2020). The virus is introduced into susceptible flocks through aerosols and fecal-oral routes (Samal, 2008).

Similar to many other poultry viral diseases, the key components in the control and prevention of ND at the farm level is to enhance biosecurity practices in combination with good flock health programs, which include vaccination program as a prophylactic measure (Miller & Koch, 2013; Dimitrov et al., 2017). Vaccines against ND have been developed since the early 1950's. Presently, poultry industries depend on live and inactivated ND vaccines to prevent economic losses due to morbidity and mortality resulting from virulent NDV infection (Gallili & Ben-Nathan, 1998). Since then, continuous research and development have been done to produce various forms of ND vaccines that have high potency against circulating strains in the farm. The commonly available and ND vaccines that have been used are inactivated or killed vaccines, live attenuated ND vaccines and recombinant ND vaccines using the fowlpox virus and herpesvirus of turkey vector vaccine (Boursnell, et al., 1990; Gergen et al., 2019), and genotype-match vaccine using reverse genetic technology (Dimitrov et al., 2017; Bello et al., 2020). Regardless type of ND vaccines used, the aim of vaccination is to immunize the vaccinated chickens to offer full protection against disease challenged. However, currently available ND vaccines are unable to elicit sterilizing immunity that is able to prevent the infection of NDV (Miller et al., 2007; Miller et al., 2009; Miller et al., 2013). Hence, effective control and prevention of ND require prudent farm management, biosecurity, and effective vaccines.

Presently, ND is endemic in many countries in Asia, including Malaysia, with velogenic NDV genotype VII continuously isolated from improperly vaccinated poultry flocks (Nooruzaman et al., 2022). Thus, there is a need to evaluate the post-vaccinal induced immune response in the vaccinated chickens. The flock health against ND is monitored based on the antibody profile tested with a rapid diagnostic assay such as HI or ELISA to determine the protective antibody level for each flock (Aldous et al., 2003; Czifra et al., 1998, Miller et al., 2007).

The objective of this study was to evaluate antibody response by vaccinated chickens following vaccination with different doses of HIPRAVIAR® BPL2, an inactivated vaccine using hemagglutination inhibition (HI) test and enzyme-linked immunosorbent assay (ELISA) kits (Biocheck CK116 and CIVTEST® AVI NDV). Specifically, the effect of administering an off-label lower dose in younger animals, as the recommended full dose of 0.5 ml is difficult to apply in day-old chicks. The efficacy of vaccinations can best be evaluated with challenging experiments; however, these are expensive and time-consuming (Czifra et al., 1998). Consequently, serological tests such as the HI and ELISA are often used to assess protective response (Czifra et al., 1998).

MATERIALS AND METHODS

Vaccine

HIPRAVIAR® BPL2 (HIPRA, Spain) is an inactivated vaccine indicated to prevent

Table 1

Enzyme-linked immunosorbent assay reading and interpretations

CIVTEST® AVI NDV			BioCheck CK116 NDV		
SP value	NDV titer	Antibody status	SP value	NDV titer	Antibody status
≤0.185	0-219	-ve	<0.349	<1,158	-ve
0.185-0.234	219-317	Suspect	>0.350	>1,159	+ve
≥0.234	>317	+ve			
Cut-off titer: ≤ 317			Cut-off titer: ≤ 1,158		

NDV = Newcastle disease virus; SP = Sample to positive ratio value

ND by active immunization of birds. The vaccine comprises the inactivated LaSota strain virus (HI ≥ 1/16) formulated with liquid paraffin, administered by injecting subcutaneous 0.5 ml per bird.

Chickens

A total of 50 specific-pathogen-free (SPF) White Leghorn day-old chicks (DOCs) were used for the study (Malaysian Vaccine Pharmaceutical, Puchong, Selangor). They were raised at the Biologics Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The presence of maternally derived antibodies against NDV was rule-out by evaluating the blood of 10 randomly selected chicks. The chickens used for the study were negative against NDV. The birds were raised in a BSL-2 experimental animal facility, fed commercial feeds, and allowed to drink water *ad libitum*. The Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine approved the study protocol, UPM (reference no.: UPM/IACUC/AUP-R096/2015).

Experimental Design

The DOCs were randomly divided into four groups of 10 birds each. Birds in group A have injected subcutaneously with 0.2 ml of a sterile phosphate buffer saline (PBS) solution (1st BASE, Singapore) and thus used as control; birds in groups B and C were vaccinated subcutaneously with a dose of HIPRAVIAR ® BPL2 (HIPRA, Spain) of 0.1 and 0.2 ml, respectively. Birds in group D were vaccinated with the dose recommended by the manufacturer, 0.5 ml. The vaccinated groups were monitored for 42 days post-vaccination with weekly blood sampling to measure the antibody titer.

Hemagglutination (HA) and Hemagglutination Inhibition (HI) Tests

HI tested the harvested sera to determine the NDV-specific antibody titer. Hemagglutination (HA) and Hemagglutination Inhibition (HI) tests were performed using the NDV strain LaSota and a chicken erythrocytes suspension (Beard et al., 1975) following standard procedures (World Organisation for Animal Health

Table 2
NDV HI antibody titer following vaccination with different doses of HIPRAVIAR® BPL2 vaccine

Vaccine dose	Antibody titer, $\log_2$ (Geometric mean ± SD) at different days post-vaccination							Grand mean
	0	7	14	21	28	35	42	
A (PBS)	ND	1.0±0.0[a]	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1[abc]
B (0.1 ml)	ND	1.0±0.0	1.89±1.23	3.76±1.95	4.03±1.78[+]	4.53±1.70[+]	4.23±1.93[+]	3.24[*c]
C (0.2 ml)	ND	1.0±0.0	1.71±1.15	2.95±2.16	3.20±2.38	3.52±2.67	4.10±2.47[+]	2.75[*c]
D (0.5 ml)	ND	1.0±0.0[a]	2.46±1.83	5.55±1.34[+]	6.46±1.35[+]	7.37±0.70[+]	7.46±0.85[+]	5.05[*ab]

ND = Not detected; SD = Standard deviation; PBS = Phosphate buffer saline
[+]Chicks with a geometric mean antibody titer of $\geq 2^4 (\geq 16)$ were considered positive (seroconverted) (WOAH, 2012);
Values with different superscripts differ significantly at $p < 0.05$;
Values with * are significantly different at $p < 0.05$ when compared to the control;
Values with "a" are significantly different at $p < 0.05$ when compared to the HIPRAVIAR® BPL2 0.1 ml group at the same time point;
Values with "b" are significantly different at $p < 0.05$ when compared to the HIPRAVIAR® BPL2 0.2 ml group at the same time point;
Values with "c" are significantly different at $p < 0.05$ when compared to the HIPRAVIAR® BPL2 0.5 ml group at the same time point

[WOAH], 2012). Serum titers of 1:8 (2^3) or lower were considered negative for antibodies against NDV (WOAH, 2012).

ELISA

The antibody response against ND was evaluated using two commercially available indirect ELISA test kits, CIVTEST® AVI NDV kit (HIPRA, Spain) and BioCheck CK116 NDV (BioCheck, The Netherlands). The ELISA was conducted based on the respective manufacturer's protocol. The test was validated using the mean absorbance values (OD) of the positive and negative controls, and the results were interpreted based on the sample-to-positive ratio (SP) values (Table 1). Meanwhile, ELISA results were considered positive based on the recommended SP values of the kits (Table 1).

Statistical Analyses

The comparison between groups at different time points was conducted by the one-way ANOVA (analysis of variance) test, followed by the Tukey HSD (honestly significant different) post hoc test. The differences between groups showing *p*-values below 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The induction of antibody response correlates with the level of protection against ND (Bello et al., 2020). In a previous study, at least 95% of the SPF birds with detectable antibody titers in serum were protected against a challenge with the Herts strain of NDV (Maas et al., 1999). Therefore, this research was conducted to

Table 3

Number of chickens seroconverted based on HI test following vaccination with different doses of HIPRAVIAR® BPL2 vaccine

Vaccine dose	Number of HI NDV antibody positive chicks / Total number of chicks at different days post inoculation						
	0	7	14	21	28	35	42
A (PBS)	ND	0/10	0/10	0/10	0/10	0/10	0/10
B (0.1 ml)	ND	0/10	0/10	2/10	6/10	7/10	8/10
C (0.2 ml)	ND	0/10	0/10	1/10	6/10	6/10	7/10
D (0.5 ml)	ND	0/10	0/10	3/10	10/10	10/10	10/10

ND = Not detected; HI = Haemagglunation inhibition; NDV = Newcastle disease virus; PBS = Phosphate buffer saline

Table 4

Number of chickens seroconverted based on ELISA following vaccination with different doses of HIPRAVIAR® BPL2 vaccine

Vaccine Dose	Number of ELISA NDV antibody-positive chicks / Total number of chicks on different days post-vaccination											
	CIVEST® AVI NDV						BioCheck CK116 NDV					
	7	14	21	28	35	42	7	14	21	28	35	42
A (PBS)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
B (0.1 ml)	0/10	1/10	8/10	8/10	9/10	8/10	0/10	1/10	9/10	9/10	9/10	9/10
C (0.2 ml)	0/10	0/10	7/10	7/10	7/10	7/10	0/10	1/10	7/10	7/10	7/10	7/10
D (0.5 ml)	0/10	1/10	10/10	10/10	10/10	10/10	0/10	2/10	10/10	10/10	10/10	10/10

Note. PBS = Phosphate buffer saline; ELISA = Enzyme-linked immunosorbent assay; NDV = Newcastle disease virus

Table 5

Detection of NDV-specific antibody titer by CIVTEST® AVI NDV ELISA following vaccination with different doses of HIPRAVIAR® BPL2 vaccine

Vaccine dose	Antibody titer (arithmetic mean) using CIVTEST® AVI NDV kit on different days post-vaccination							Grand mean
	0	7	14	21	28	35	42	
A (PBS)	7.8	0.9	0.0	0.0	0.0	0.0	3.0	1.67*^{abc}
B (0.1 ml)	7.8	1.2	115.6	2406.6	2160.1	3118.4	3122.7	1561.77*^{c}
C (0.2 ml)	7.8	1.6	49.9	1670.8	1784.7	3031.9	3763.1	1472.83*^{c}
D (0.5 ml)	7.8	2.7	176.9	4947.7	6171.3	7821.8	11224.8	4336.14*^{ab}

Note.
PBS = Phosphate buffer saline; ELISA = enzyme-linked immunosorbent assay; NDV = Newcastle disease virus
Chicks were considered positive based on the recommended SP value (>317) of the CIVTEST® AVI NDV ELISA kit;
Values with different superscripts differ significantly at $p < 0.05$;
Values with * are significantly different at $p < 0.05$ when compared to the control;
Values with "a" are significantly different at $p < 0.05$ when compared to the HIPRAVIAR® BPL2 0.1 ml group at the same time point;
Values with "b" are significantly different at $p < 0.05$ when compared to the HIPRAVIAR® BPL2 0.2 ml group at the same time point;
Values with "c" are significantly different at $p < 0.05$ when compared to the HIPRAVIAR® BPL2 0.5 ml group at the same time point

Table 6

Detection of NDV-specific antibody titer by BioCheck CK116 NDV ELISA following vaccination with different doses of HIPRAVIAR® BPL2 vaccine

Vaccine dose	Antibody titer (arithmetic mean) using CIVTEST® AVI NDV kit on different days post-vaccination							Grand mean
	0	7	14	21	28	35	42	
A (PBS)	156.0	43.0	17.0	15.0	44.0	28.0	61.0	52.0^{abc}
B (0.1 ml)	156.0	24.0	434.0	5287.0	6014.0	7197.0	5328.0	3491.43*^{c}
C (0.2 ml)	156.0	30.0	257.0	4636.0	5598.0	7015.0	6882.0	3510.57*^{c}
D (0.5 ml)	156.0	24.0	1262.0	12239.0	14520.0	13140.0	15501.0	8120.29*^{ab}

Note.
PBS = Phosphate buffer saline; ELISA = Enzyme-linked immunosorbent assay; NDV = Newcastle disease virus
Chicks were considered positive based on the recommended SP value (>1159) of the BioCheck CK116 NDV ELISA test;
Values with different superscripts differ significantly at $p < 0.05$;
Values with * are significantly different at $p < 0.05$ when compared to the control;
Values with "a" are significantly different at $p < 0.05$ when compared to the HIPRAVIAR® BPL2 0.1 ml group at the same time point;
Values with "b" are significantly different at $p < 0.05$ when compared to the HIPRAVIAR® BPL2 0.2 ml group at the same time point;
Values with "c" are significantly different at $p < 0.05$ when compared to the HIPRAVIAR® BPL2 0.5 ml group at the same time point

evaluate the immune response generated following inoculation of different doses of commonly used inactivated Newcastle vaccine to chicks. It was evaluated weekly during the entire life of a typical broiler chicken (42 days of life).

A previous study has reported that positive HI test results were a good indicator of immunity (Czifra et al., 1998). Our findings agree with Igwe et al. (2019), who reported that increased doses of the LaSota vaccine in broiler chickens significantly increased the antibody response to ND.

Based on the HI results, birds vaccinated with 0.5 ml HIPRAVIAR® BPL2 vaccine showed a statistically significant higher antibody response titer than the other doses (Table 2).

The sera were collected at 0, 7, 14, 21, 28, 35, and 42 days post-vaccination (dpv) to determine the HI NDV antibody titer. Pre-vaccination screening confirmed that the 1-day-old chicks were free of NDV HI antibodies. As shown in Table 2, the chicks from group A inoculated with PBS remained negative throughout the study. At the same time, treatment group D (0.5 ml) developed the highest HI antibody titer, followed by group B (0.1 ml) and group C (0.2 ml). Generally, the mean $\log_2$ HI NDV antibody titer for groups B, C, and D gradually increased from 7 to 42 dpv (Table 2). Group B HI NDV antibody titer increased from 1.89±1.23 to 4.23±1.93, group C increased from 1.71±1.15 to 4.10±2.47, and group D increased from 2.46±1.83 to 7.46±0.85. The percentage distribution of HI positivity amongst the different days post-vaccination showed that all the birds were positive at 28 days following vaccination with HIPRAVIAR® BPL2 0.5 ml, while 80 and 70% of the birds were positive at 42 days post-vaccination with HIPRAVIAR® BPL2 0.1 and 0.2 ml, respectively (Table 3).

HI NDV antibody titer in group B reached 80% seropositive birds with 4 $\log_2$ antibody titer. Group C showed similar results, reaching 70% seropositive birds with 4 $\log_2$ antibody titer. Group D showed excellent results with 100% seropositive birds with 7 $\log_2$ antibody titer. According to Mahamud et al. (2022), the mean HI antibody titer of 7 $\log_2$ following vaccination of SPF chickens with live ND vaccine can confer 100% protection against challenges with virulent NDV genotype VII. Further study involving challenge trials is required to confirm the protective immunity of the studied inactivated vaccine. However, in many Southeast Asia countries where ND is endemic, commercial broiler chickens were vaccinated with a combination of live and inactivated to induce vaccine-induced protective immunity (Dimitrov et al., 2017).

Besides HI titers, ELISA is another routine serological assay to determine ND vaccination profiles (Aldous et al., 2003; Miller et al., 2007; Mahamud et al., 2022). Previous studies have also evaluated the correlation of antibody titers detected using both assays in the detection of vaccine-induced immunity (Brown et al., 1990). In this study, the ELISA was performed using the CIVEST® AVI NDV and BioCheck CK116 NDV kit (Tables 4, 5, and 6). Both ELISA kits could detect 100% seropositive

birds in group D starting from 21–42 days post-vaccination. Furthermore, 90% of the birds were seropositive at 21–42 dpv with HIPRAVIAR® BPL2 0.1 ml using a BioCheck CK116 NDV kit (Tables 4 and 5). Although the antibody titer-positive birds were 80% at 21, 28, and 42 dpv with HIPRAVIAR® BPL2 0.1 ml after being tested with CIVTEST® AVI NDV kit, 90% of the birds, however, were positive at 35 days (Tables 4 and 6). When tested using both kits, there was a steady 70% positivity at 21–42 dpv of HIPRAVIAR® BPL2 0.2 ml (Table 4). This steady 70% positivity in this group is probably associated with poor vaccine administration, as the 30% negative birds did not seroconvert. Thus, there are no significant differences between the antibody titers measured using Biocheck CK116 and CIVTEST® AVI NDV kits, even though there is some variation in the percentage coefficient of these ELISA kits. The variations are probably associated with the inherent differences in the assay on the quality and quantity of the NDV antigen and other reagents used (Mao et al., 2022).

The serological method used in this study showed Group B detected 80% (HI), 80% (CIVEST® AVI NDV), and 90% (BioCheck CK116 NDV) seropositive birds following vaccination with 0.1 ml of HIPRAVIAR® BPL2. On the other hand, group C could only reach 70% seropositivity detected using all methods. Meanwhile, group D shows the highest seropositivity at 100% using all serological methods.

CONCLUSION

The study showed birds vaccinated with the HIPRAVIAR® BPL2 vaccine at different doses, thus significantly seroconverted compared to the control group. The variation of antibody titer detection using serological methods such as HI and ELISA of two different kits does not affect the seropositivity of the birds. Group B reached 80% (HI), 80% (CIVEST® AVI NDV), and 90% (BioCheck CK116 NDV) seropositivity. Group C reached 70% seropositivity detected using all methods. Meanwhile, using all serological methods, group D reached the highest seropositivity at 100%. However, groups B and C were only able to achieve 4 $\log_2$ antibody titer compared to group D 7 $\log_2$, which is usually associated with protective immunity. Further study involving challenge trials is required to confirm the protective immunity of the studied inactivated vaccine. The titers detected in the groups indicate the ability of the vaccine to generate a dose-dependent seroconversion as group D, 0.5 ml, had the higher seroconversion compared the groups B, 0.1 ml, and C, 0.2 ml. The negative seroconversion on HI in some of the samples from Groups B and C indicates a challenge to vaccine application in a lower dose administration as some birds did not seroconvert later.

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