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Administration of Live-Attenuated Newcastle Disease (ND) Vaccines Derived from B1 and LaSota Strain and Their Effect on Broiler Antibody Titers

M. Danawir Alwi, A. Magfira Satya Apada*, Fedri Rell , Trini Purnamasari Syahid

Study Program of Veterinary Medicine, Faculty of Medicine, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Makassar, 90245, Indonesia

*Corresponding authors: A. Magfira Satya Apada (andimagfirahsatyaapada@gmai.com)

Abstract

Newcastle Disease is a contagious disease caused by Avian Paramyxovirus and infects different types of poultries including broiler. Vaccination as a preventive effort against the ND virus could employ both attenuated and inactivated vaccines. This study aims to identify the effect of administering live-attenuated LaSota and B1 ND vaccines against broiler antibody titers. This experiment employed 54 DOCs and was divided into 3 groups of treatments. Vaccination was performed at the age of 3 days old through eye drop administration. Blood specimens were taken from vena brachialis at the age of 7, 14, 21 days old. Hemagglutination Inhibition (HI) assay was analyzed using One-Way Analysis of Variance (ANOVA) followed by Least Significant Different (LSD) test if the probability is significant (P<0.05). The data of Immune Percentage is presented using descriptive quantitative analysis. The research results demonstrated that live-attenuated LaSota ND vaccination at the age of 3 days could sustain and induce immunity until the age of 21 days while B1 ND vaccination at the age of 3 days could only sustain immune protection until the age of 14 days. Live-attenuated LaSota and B1 ND vaccines did not have any significant effect on the broiler antibody titers.

Keywords: *Broiler, Newcastle Disease (ND), LaSota vaccine, B1 vaccine, Hemagglutination test, Hemagglutination Inhibition Test*

Introduction

Newcastle disease (ND) is a systemic disease infecting poultry with a high level of mortality. Newcastle disease is categorized as 4 dominant infectious diseases besides *highly pathogenic avian influenza* (HPAI), *infectious bronchitis* (IB), and *low pathogenic avian influenza* (LPAI) that causes a huge loss in poultry farming. The disease was caused by *Avian Paramyxovirus* that belongs to the family of *Paramyxoviridae* (Tabbu, 2000). Newcastle Disease is spread accross the Asian, African, American, Australian, and European Continent. Indonesia is one of the Newcastle disease (ND) endemic countries in the world and the cases of ND outbreaks were reported in almost all Indonesian regions. ND outbreak was reported in South Sulawesi from 2012 in January to 2014 in May (OIE, 2014).

Therefore, vaccination as a preventive effort to induce immunity in poultry against viral infection is necessary. ND vaccination could employ two types of vaccines. These include live attenuated (live) and inactivated (killed) vaccines. The live-attenuated vaccines could induce immunity more rapidly after the vaccination compared to the inactivated vaccines (Tabbu, 2000). The quality control on ND vaccines had been performed by the Quality Control and Veterinary Drug Administration Center (BBPMSOH) from 2009 to 2013. The centre also found that there were 96 ND single-shot vaccines, 118 ND attenuated-life vaccines, and 131 ND inactivated vaccines publicly available in Indonesia. Life-attenuated vaccine, as the most frequently used vaccine, during this period, included LaSota, B1, and Clone types (Hidayanto *et al.,* 2015).

Vaccination may also fail due to a number of factors such as low quality, inappropriate distribution mechanism, improper administration, and environmental circumstances. Vaccination failure from different countries was also reported in Pakistan (Ahmad *et al.*, 2007); Nigeria (Olugasa *et al.*, 2012); and other endemic countries. Such phenomena encourage more research on the vaccine effectiveness in stimulating antibodies. Banu (2009) confirmed that a high number of research was conducted because the vaccine manufacturers rarely performed periodical quality control on the distributed vaccines to the public.

From such facts, the authors consider the importance of identifying the effect of the live-attenuated ND vaccine derived from frequently used strains by the public on the broiler antibody titer. As a limit for the scope of the study, the authors employed live-attenuated vaccines with 2 different and most publicly available strains of LaSota and B1.

Materials and Methods

This study uses an experimental design aiming to conclude the effect from different treatments on a range of variables while comparing it to the control variables and analyzing the results. The measurement for antibody titers was performed in Maros Veterinary Medicine Center. The research was conducted for one month from November to December 2016 with 54 One-Day Old Chicks. Vaccination was performed at the age of 3 days old through eye drop administration. Blood specimens were collected using a 1 ml syringe at the age of 7 days and a 3ml syringe at the age of 21 days. Antibody titer measurement was performed using chicken sera. The samples were divided into 3 different treatment groups: T0 (without vaccine administration), T1 (LaSota vaccine administration), P3 (B1 vaccine administration). The blood specimens taken from *vena brachialis* were left for 24 hours and the sera were removed from the blood clots. The collected blood serums proceeded Hemagglutination (HA) test to determine the 4 HAU. The measurement of antibody titer was performed by means of Microtiter Hemagglutination-Inhibition Test adopting OIE 2008 modified procedures by the Laboratory of Virology, Maros Veterinary Center.

Results and Discussion

a. Antibody titer

Means of antibody titers were measured using *Geometric Mean Titre* (GMT) The data results from the Hemagglutination Inhibition test were analyzed using One-way Analysis of Variance (ANOVA) and followed by Least Significant Difference test if the significance value equal to $P \leq 0,05$.

Table 1. Means of Antibody titers against Newcastle Disease on different treatments (T0, T1, T2)

Treatments	Test Results					
	Titer means (log 2± SE)			Accumulated Value		
	7 Days	14 Days	21 Days	7 Days	14 Days	21 Days
T0	6,71±0,09	5,09±0,09	3,16±0,06	104.88	34.22	9.00
T1	7,15±0,08	5,80±0,07	4,30±0,06	142.22	56.00	19.77
T2	7±0,06	5,67±0,07	3,97±0,05	128	51.22	15.77

Description :

- Means of antibody titers are converted into *Geometric Mean titer* (GMT) and expressed in log2 ± *standard error*

Based on the assessment standard of *Geometry Mean Titre* (GMT) in the Hemagglutination Inhibition test, the accumulated titer of ≥ 16 or ≥ HI log 2^4 is defined as protective against ND while the accumulated titer of < 16 or < HI log 2^4 is defined as nonprotective against ND (OIE, 2012). Table 1. illustrated the means of antibody titers from the three groups at the age of 7, 14, and 21 days as poultry immune status.

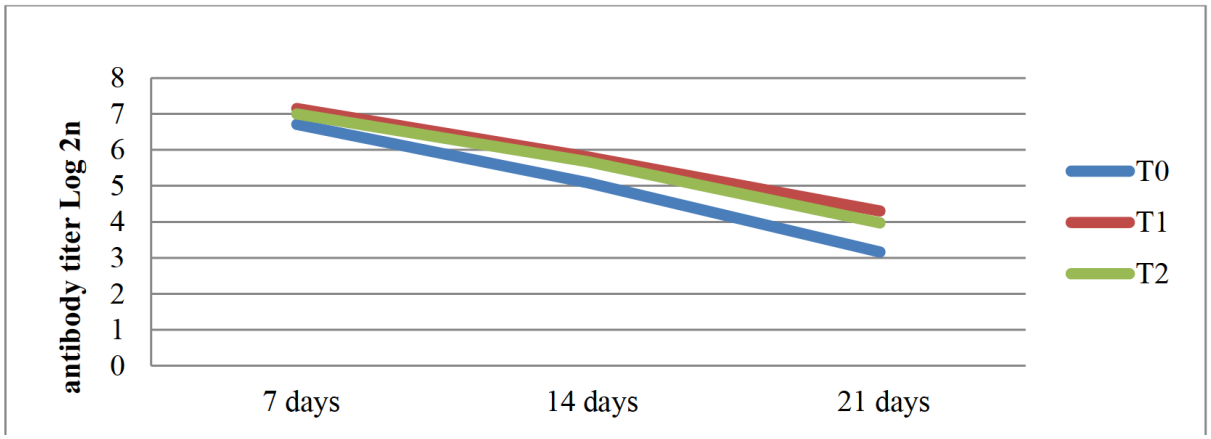

Figure 1. Line chart of antibody titer means in each treatment group

As can be seen from Figure 1, the line chart of antibody titer means from T0, T1, and T2 groups indicate a decrease over 3 times of sample observation. The antibody titer means from the T0 group (without treatment) showed lower antibody titers compared to the other treatment groups administered with LaSota vaccines (T1) and B1 vaccines (T2) while the treatment groups administered with LaSota vaccines (T1) showed higher antibody titers than those administered with the B1 vaccine (T2). Such results indicated the presence of immune response within 3 days of post-LaSota (T1) and B1 (T2) vaccination. The initial immune response after the administration of the ND live-attenuated vaccine was *cell-mediated immunity* (CMI) observed after 2-3 days of post-vaccination (Tabbu, 2000). Similar findings was also found by Al-Shahery *et al.*, (2008) stating that the cell-mediated immune response caused by ND virus can be detected within 2-3 days after the vaccination. Reynold and Maraqa (2000) reported that CMI response may be much faster to emerge in poultry administered with live-attenuated ND vaccine.

At the age of 14 days, lower antibody titer means are observed from the T0, T1, and T2 treatment group compared to the antibody titer means at the age of 7 days. Antibody titers

may be defined as protective in a titer of ≥ 16 or ≥ HI log 2^4 (OIE, 2012). The results of the GMT conversion indicated a decreasing *maternal antibody* from the T0 treatment group at the age of 14 days although the mean was still considered as protective (34,22>16). The declining antibody titer in the T0 group correlates with the decline in maternal antibodies. A study performed by Jalil *et al.,* (2009) employing broilers as the samples found that maternal antibodies in DOC will be reduced in half every 7 days. Rahman *et al.,* (2002) further stated that such decline may occur in 5 days until all of the antibodies are diminished. The declining antibody titer means observed in T1 and T2 groups were stimulated by the neutralization by maternal antibody and therefore, the response of the administered vaccine was less effective. Antigen neutralization from live-attenuated vaccination occurred because of the high concentration of maternal antibodies during the vaccination at the age of 3 days. Annisaa (2013) confirmed that in addition to neutralization, the decreasing antibody titers observed from the vaccinated sample groups are strongly related to the declining maternal antibody in the chickens.

Antibody titers observation at the age of 21 days illustrates a decline from the all (T0, T1, and T2) groups. The decline in the antibody titer from the T0 group indicated declining maternal antibody and the chickens' lowered protection against ND virus (GMT = 9.00 < 16). Declining maternal antibodies more frequently occurred as the chickens grow older until they reached an unprotected state against infection (Tuscany, 2016). The difference in antibody titers between groups (T1 and T2) with the unvaccinated group (T0) was caused by the decreasing maternal antibody until it reached below the ND protective standard.

Table 2. The Results of One-Way Analysis of Variance (ANOVA)

No.	Collection of Sera	One-Way ANOVA test results *(p)*
1	7 Days	0.370
2	14 Days	0.048
3	21 Days	0.000

Note: Significant probability is (*P*< 0.05)

The results of one-way ANOVA (Table 2) indicate a smean ignificant difference among the three treatment groups at the age of 14 days (0,048 < 0,05) and 21 days (0,000< 0,05) and therefore, this is followed by LSD test. There is no significant difference observed from the three treatment groups at the age of 7 days (0,370 > 0,05) and therefore, an LSD test is not required.

Table 3. Least Significant Difference (LSD) Test on The Samples at The Age of 14 Days.

Treatment Groups		LSD Test Results (*P*)
T0	T1	0.024
	T2	0.047
T1	T0	0.024
	T2	0.772
T2	T0	0.047
	T1	0.772

Note: Significance Probability is (*P*< 0,05)

Based on the LSD test, a significant difference is identified in means between T0 and T1 groups (*P*: 0,024<0,05) and between T0 and T2 groups (*P*: 0,047<0,05). On the contrary, there is no significant difference identified in means between T1 and T2 groups (*P* : 0,772>0,05)

Table 4. Least Significant Difference (LSD) Test on the Samples at The Age of 21 Days

Treatment Groups		LSD Test Results (*P*)
T0	T1	0.000
	T2	0.002
T1	T0	0.000
	T2	0.439
T2	T0	0.002
	T1	0.439

Note: Significance Probability is (*P*<0,05)

LSD test results on the samples at the age of 21 days indicate a significant difference in means between the T0 and T1 group (*P*: 0,000<0,05) and between the T0 and T2 group (*P*: 0,002 <0,05). However, there is no significant difference identified in the means of antibody titers between the T1 and T2 groups (*P*: 0,439>0,05).

The analysis on the antibody titer indicates that the T1 (LaSota vaccine) group and T2 (B1 vaccine) group have higher antibody titer compared to the T0 treatment in 3 times antibody titer mean calculation (at the age of 7, 14, and 21 days). The results confirmed the significant effect of administering LaSota (T1) and B1 (T2) vaccines on the antibody titer of the chicken at the age of 14 and 21 days. T1 (LaSota vaccine) group has higher titer antibody means compared to the T2 (B1 vaccine) group. However, statistically, the difference is not significant at the age of 7, 14, and 21 days. Such finding indicated that ND live-attenuated vaccine derived from LaSota strain had no significant difference from ND live-attenuated vaccine derived from B1 strain on chicken antibody titers. This was because LaSota and B1 strain originated from the similar virus strain, lentogenic. According to a study performed by Tabby (2000) the ineffectiveness of ND virus is strongly related to the virus strain, contact period, virus dosages, and growth media.

b. Immune Percentage

The immune percentage indicates a percentage of samples with protective antibody titer in one treatment group. The standard for optimal immune percentage is ≥ 80% and the poor immune percentage is < 80% (Tabbu, 2000; Suryani, 2015).

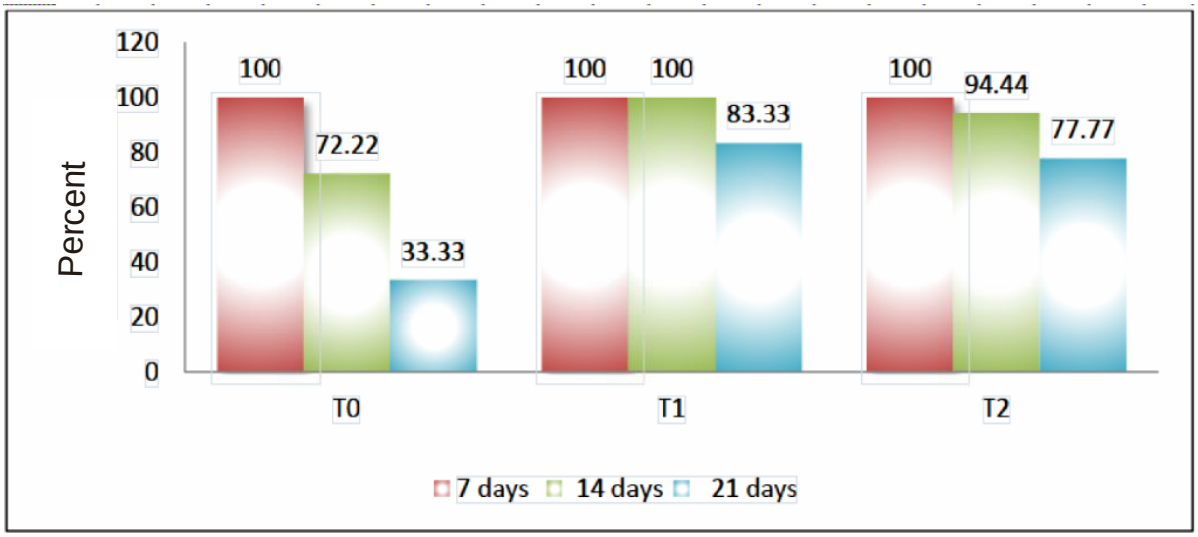

Figure 2. Bar Chart of Immune Percentage in Treatment Groups

Calculation results of immune percentage from three treatment groups at the age of 7 days account for 100%. This indicates each sample from all the treatment groups (T0, T1, and T2) at the age of 7 days has protective antibody titers. Such a condition was promoted by the high concentration of maternal antibodies at the age of 7 and the less optimal vaccine responses. Maternal antibody in DOC transferred by the hens through the yolk. According to Silva and Tambougi (2010), the presence of maternal antibodies induces immune protection to the young chicks against any foreign antigen. High maternal antibodies in chicks could neutralize vaccine antigens and reduce their effectiveness (Dimitrov, 2016).

Antibody titers from the T1 and T2 groups at the age of 14 days represent a protective immune percentage (≥80%) accounted for 100% and 94.44% respectively. High immune percentages of T1 and T2 groups have stimulated the vaccination that was capable of sustaining antibody titer above the ND protective standards. Meanwhile, T0 group can be categorized as slightly protective (72,22% < 80%). This indicated the declining maternal antibody in line with the declining protection against ND in chicken. A study performed by Marttiana (2011) also found a similar trend of declining maternal antibodies with age in the control group.

T1 and T2 groups at the age of 21 days still had a high immune percentage although T2 groups showed a slight decrease in the immune percentage (77,77% < 80 %). On the other hand, the immune percentage of the T0 group drops too far from the protective category (33,33%<80%). From the 3 times of observation on immune percentage, a declining immune percentage with age was identified from all the groups (T1, T2, P3). The existing significant difference between the vaccinated (T1 and T2) and the unvaccinated group (T0) was caused by the dropping maternal antibody to below the standardized protective level against ND. The dropping rate of immune percentage was affected by both challenges in the pathogens and the poultry condition.

c. *Coefficient of Variation*

The coefficient of variation (CV) of the observations from the three groups indicates the group administered with the live-attenuated vaccine (T1 and T2) has higher homogeneity compared to the control group (T0) This indicates the positive effect of vaccination through eye drop administration on the homogeneity of antibody titer distribution. Homogenous antibody titers may occur in the vaccinated group (T1 and T2) due to controllable vaccine dosages through eye drop administration. Methods of vaccination are one important factor that affects antibody

titer homogeneity in a group. In a study performed by Aryopurtanto (2011), a treatment group was identified as heterogeneous due to vaccination via drinking water. Homogeneity in the antibody titers from the control group (T0) was caused by the inherited maternal antibody reared by a similar DOC supplier company and therefore, the hens had homogenous antibody titers.

Table 4. *Coeffcient of Variation (CV)*

Treatments	*Coefficient of Variation* (CV)		
	7 Days	14 Days	21 Days
T0	17.12	28.39	33.43
T1	15.26	19.13	21.51
T2	12.85	20.33	23.44

Conclusion and Recommendation

Vaccination is one preventive act against Newcastle Disease by administering biological products containing attenuated pathogens and therefore, it could stimulate antibodies and the immune system. Live-Attenuated Vaccines derived from LaSota strain and B1 awakened the formation of antibody titers. Live-attenuated ND vaccine derived from LaSota and B1 strain had no significant effect on the broiler antibody titers.

Further field surveillance on screening or detecting the mutated virus that successfully adapted to the current habitats is necessary. In addition to that, further research on the maternal antibody may neutralize live-attenuated vaccines administered to the young chicks.

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References

Ahmad, M.D., Mamoona C., Muhammad, F.R., dan Hamad, B.R. 2007.*Evaluation of Two Vaccination Schemes Using Live Vaccines against Newcastle Disease in Chickens.*University of Veterinary and Animal Sciences. Lahore: Pakistan.

Al-Shahery, M.N, Al-Zubedy A.Z, Al-Baroodi, S.Y. 2008. Evaluation Of Cell Mediated Immune Response in Chickens Vaccinated With New Castle Disease Virus. *Iraqi J Vet Sci*. 22:21-24.

Annisaa, F.L.N. 2013. *Studi Kemampuan Vaksin Aktif ND-IB: Pembentuk Kekebalan dan Perlindungan terhadap Paparan Virus IB pada Ayam Pedaging [The Study of Live-attenuated ND-IB vaccine effectiveness: Immune Formation and Protection against IB virus infection in Broiler] . [skripsi]*. Institut Pertanian Bogor: Bogor

Aryoputranto, R.R., 2011. *Gambaran Respon Kebal Newcastle Disease Pada Ayam Pedaging Yang Divaksinasi Newcastle Disease Dan Avian Influenza Pada Berbagai Tingkat*

*Umur [Illustration of Immune Response against Newcastle Disease in Broilers Vaccinated with ND and AI Vaccines at Different Age].[skripsi].*Institut Pertanian Bogor: Bogor.

Banu, N.A., Islam, M.S., Chowdhury, M.M., dan Islam M.A. 2009. *Determination of immune response of Newcastle disease Virus Vaccines in Layer Chickens.* Bangladesh Agricultural University, Mymensingh: Bangladesh.

Dimitrov, K.M., Afonso, C.R., Yu, Q., Miller. P.J. 2016. Newcastle disease vaccines, A solved problem or a continuous challenge?.*Journal Veterinary Microbiology.*

Hidayanto, N.K., Ramlah, Ardiawan, F., dan Suryati, Y*. Pengujian vaksin Newcastle disease (ND) di BBPMSOH Tahun 2009-2013 [Quality Control on Newcastle Disease Vaccine in Quality Control and Veterinary Drug Administration Center in 2009-2013].* Balai Besar Pengujian Mutu dan Sertifikasi Obat Hewan, Gunungsindur. Bogor

Jalil, M.A., Samad, M.A., dan Islam, M.T., 2009. *Evaluation of maternally derived antibodies against Newcastle disease virus and its effect on vaccination in broiler chicks.* Bangladesh J. Vet. Med., 7: 296-302.

Marttiana, E. 2011. *Gambaran respon kebal terhadap virus Avian Influenza (AI) h5 pada ayam petelur yang divaksinasi dengan vaksinAI-ND inaktif [Laying Hens' Immune Response against h5 Avian Influenza (AI) Vaccinated with AI-ND Inactivated Vaccines].* [skripsi]. Institut Pertanian Bogor. Bogor

OIE. 2012. *Newcastle disease, in manual of standars for diagnostic tests and vaccines.* Paris ;OIE.

OIE. 2014. *NDV in Indonesia. Detailed country(ies) disease incidence*. http// www. oie.int / wahis/public.php. Retrived online o June, 5th 2016 at 22. 00 WITA.

Rahman, M.M., Bari, A.S.N., Girasuddin, M., Islam, M.R., Alam, J., Sil, G.G. 2002. Evaluation of Maternal and Humoral Immunity against Newcastle Disease Virus in Chicken. *International Journal of Poultry* Sci 1(5):161-163.

Reynolds, D.L., dan Maraqa, A.D. 2000. Protective immunity against Newcastle disease: the role of antibodies specific to Newcastle disease virus polypeptides. *Avian Disc*; 44(1):138-44

Sianita, N., Ziaul, H., dan Kusriningrum, R. 2011. Respon Antibodi dan Protektivitas pada Ayam Pasca Vaksinasi Menggunakan Vaksin ND aktif lv12 [Antibody Responses and Protection in Chickens during The Post-Vaccination of lv12 Live-Attenuated ND vaccines]. *Veterinaria medika*. Vol. 4 : 2

Silva, W.D., Tombourgi, D.V. 2010.IgY: A promising antibody for use in immunodiagnostic and in immunotherapy. *Veterinary Immunology and Immunopathology* 135 : 173–180

Suryani, L. 2015. *Deteksi Titer Antibodi dan Identifikasi Faktor Penyebab Kegagalan Vaksinasi Terhadap Newcastle Disease pada Ayam Petelur Di Desa Bulo Kabupaten Sidenreng Rappang [Antibody Titer Detection and Identification of ND Vaccination Failure Factors in Laying Hens at Bulo Village, Sidenreng Rappang] .* [skripsi]. Universitas Hasanuddin: Makassar

Tabbu, C.R. 2000. *Penyakit Ayam dan Penanggulangannya [Poultry Diseases and Their Treatment].* Penerbit Kanisius. Yogyakarta

Tuscany, N., Soejoedono, R.D., Poetri, O.N. 2016. *Evaluasi keberadaan antibodi asal induk terhadap virus avian influenza dan infectious bursal disease pada ayam broiler [Evaluation of maternal antibody against avian influenza virus and infectious bursal disease in broiler]. [Skripsi].* Institut Pertanian Bogor: Bogor