P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article
https://doi.org/10.47278/journal.ijvs/2023.093

Newcastle Disease Polyclonal Antibodies as Candidate Reagents in Immunohistochemistry Diagnostic Test and Passive Immunization

Dwi Desmiyeni Putri[1,*], Ekowati Handharyani[2], Retno Damajanti Soejoedono[3], Agus Setiyono[2] and Etriwati[4]

[1] Department of Animal Husbandry, Politeknik Negeri Lampung, Indonesia
[2] Division of Pathology, School of Veterinary Medicine and Biomedical Sciences, IPB University, IPB University, Indonesia
[3] Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia
[4] Department of Laboratory of Pathology, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Indonesia
***Corresponding author:** desmiyenidwi@gmail.com

Article History: 23-251 Received: 15-Jul-23 Revised: 02-Sep-23 Accepted: 13-Sep-23

ABSTRACT

This study aimed to determine the utilization of characterized-polyclonal ND antibodies as primary antibodies in Immunohistochemistry and to determine the ND antibodies' ability to neutralize ND viruses. The ND antibodies used in this study were genotype VII ND antibodies, Sato ND antibodies, and mixed ND antibodies. Genotype VII ND antibodies, Sato ND antibodies, and mixed ND antibodies were used for VNT, but the IHC test only used genotype VII antibodies. In the IHC examination, genotype VII ND antibodies were used as primary antibodies, which were diluted in stages (1:100, 1:500, and 1:1000) to obtain the dilution that could give the best IHC examination results. Rabbit polyclonal anti-NDV HN protein antibody at 1:500 dilution was used as a positive control. Based on the results of the IHC stain, 1:1000 dilution of genotype VII ND antibodies gave the best results, with the same results as using a rabbit polyclonal anti-NDV HN protein antibodies at an antibody dilution of 1:500. Based on the VNT results, the neutralization index of Sato ND antibodies was 1.6; genotype VII ND antibodies were 1.95; and mixed ND antibodies were 1.68. This result showed that genotype VII ND antibodies have a higher ability than other antibodies to neutralize 10^4 ELD_{50}/mL NDV/Ck/BGR/11 virus. Genotype VII ND antibodies can be used as reagent candidates in the IHC test in 1:1000 dilution and as a reagent in passive immunization with a neutralization index 1.95.

Key words: Antibody, Immunohistochemistry, Immunotherapy, Viral Neutralization Test

INTRODUCTION

ND outbreaks in livestock cannot be diagnosed by clinical symptoms, pathological anatomical and histopathological changes only. Newcastle Disease (ND) does not produce a pathognomonic sign, so often confused with other poultry diseases, such as Avian Influenza and Infectious Bronchitis. In determining the diagnosis of ND diagnosis, an immunodiagnostic test is needed, such as Immunohistochemistry (IHC), which is used as an essential tool in laboratory diagnosis (Nakamura et al. 2008; Etriwati et al. 2017; Angeliya et al. 2022). The IHC method has been applied as a qualitative test that is effective, accurate, easy to implement, and inexpensive to detect the presence of ND antigens in tissues (Oldoni et al. 2005; Wakamatsu et al. 2006).

The IHC is a technique for identifying antigens based on antigen-antibody interactions (Etriwati et al. 2017; Angeliya et al. 2022). Antibodies in the IHC technique play a role in binding to homologous antigens present in infected tissue (Ramos-Vara and Miller 2014). The IHC result is strongly influenced by the antibodies used (Lipman et al. 2005). The decision about whether to use polyclonal antibodies or monoclonal antibodies depends on several factors. Commercial antibodies are generally monoclonal antibodies that have high specificity but are relatively more expensive. Polyclonal antibodies can generally be produced in rabbits, mice, and guinea pigs and can be obtained quickly (4–8 weeks) at low cost (Leenaars and Hendriksen 2005; Samiullah et al. 2006).

Antibodies have been used to prevent and treat infectious diseases in humans. Antibodies are commonly used to prevent diseases such as measles, hepatitis A,

Cite This Article as: Putri DD, Handharyani E, Soejoedono RD, Setiyono A and Etriwati, 2023. Newcastle disease polyclonal antibodies as candidate reagents in immunohistochemistry diagnostic test and passive immunization. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2023.093

hepatitis B, tetanus, varicella, rabies and vaccinia (Keller and Steihm 2000; Razonable and Chen 2022; Shrikant and Nitika 2023). The use of polyclonal antibodies containing specific immunoglobulins for certain viruses, bacteria, and toxins is a method of treatment known as passive immunization (Tizard 2021). Passive immunization has not been widely developed in animal disease control, so it is possible to develop passive immunization as an alternative strategy for ND treatment. The potential of ND antibodies as biological agents in the development of passive immunization started by analyzing the ND antibodies' activity in neutralizing the ND virus with the Viral Neutralization Test (VNT). This study aimed to determine the utilization of characterized ND antibodies as primary antibodies in the IHC test and to determine the ND antibodies' ability to neutralize ND viruses so that they could be used as reagent candidates in passive immunization.

MATERIALS AND METHODS

Ethical Approval

This research has been approved by the Animal Care and Use Committee of Research and Community Services Institution, IPB University with approval number: 213-2021 IPB.

Newcastle Disease Polyclonal Antibodies

The ND antibodies used in this study were genotype VII ND antibodies, Sato ND antibodies, and mixed (characterized) ND antibodies produced in New Zealand White (NZW) rabbits (Putri et al. 2018a). Mixed ND antibodies are antibodies produced by injecting genotype VII ND antigen and Sato ND antigen into NZW rabbits. Genotype VII ND antibodies, Sato ND antibodies, and mixed ND antibodies were used for VNT, but the IHC test only used genotype VII ND antibodies.

Virus

The NDV/Ck/BGR/11 viruses were used in this study. Based on pathotype analysis, this virus is a virulent ND virus (Putri et al. 2017), and after phylogenetic analysis, the NDV/Ck/BGR/11 virus is included in genotype VII (i) ND virus (Putri et al. 2018b). ND/Ck/BGR/11 virus was used as an antigen in VNT with 10^4 ELD_{50}/mL.

Immunohistochemical Test

The tissue preparation used for IHC was native chicken tissue infected with genotype VII ND virus. In this study, IHC staining was carried out according to the procedure recommended in the Dako catalog, North America. Inc. (Dako 2013) with several modifications (Etriwati et al. 2017). The stained preparations were observed under a light microscope (Nikon, Japan). The positive result is if the antigen is brownish and negative if all sections of the preparation appear bluish and no brown antigen is found. Observations made with objective magnifications of 10, 20 and 40x.

Viral Neutralization Test

The ND antibodies used in VNT were genotype VII ND antibodies, Sato ND antibodies, and mixed ND antibodies with HI titers of 2^8, 2^9, and 2^9. The VNT was performed at 9 days old Embryonated Chicken Eggs (ECEs). Briefly, ND antibody was inactivated at 56°C for 30 min and serially diluted by 2-fold dilution. Diluted ND antibody was mixed with 10^4 ELD_{50}/ND/Ck/BGR/11 virus in an equal volume and incubated at 37°C for 1 hour. Next, the viral antibody mixture (200µL) was inoculated into the allantois cavity of 9-day-old ECEs, which was incubated at 37°C and observed for 5 days. Phosphate Buffer Saline was used as a negative control, and ND virus NDV/Ck/BGR/11 was used as a positive control. Neutralized serum ND antibody titers were calculated five days after incubation using the Reed-Muench method (Reed and Muench 1938).

Hemagglutination Inhibition Test

The hemagglutination inhibition (HI) test was performed according to the OIE terrestrial manual (OIE 2012). Antibody titer is determined from the highest serum dilution that can inhibit Hemagglutination.

RESULTS

Immunohistochemistry Test

Antibodies were generated by injecting the whole NDV/Ck/BGR/11 antigen (genotype VII) into New Zealand White rabbits used as primary antibodies in this study (Putri et al. 2017). Rabbit polyclonal anti-NDV HN protein antibody (1:500 in antibody diluent, Dako, S3022) was used as a positive control for the IHC test. Antibody dilution is performed to obtain the optimal concentration with the best IHC visualization results. Based on the results of the IHC test, a 1:1000 dilution of ND genotype VII antibody gave the best IHC results. In contrast, polyclonal rabbit anti-NDV HN protein antibodies had the best IHC results at an antibody dilution of 1:500. Immunopositive results on the IHC test can be seen in Fig. 1.

In the IHC result, the selected preparations were proventriculus, duodenum, pancreas, and heart from chickens infected with genotype VII ND virus. Genotype VII ND antibodies can detect the presence of ND antigens in several samples with a more vigorous immunopositive intensity when compared with commercial ND antibodies. From the samples examined, 100% of the ND antigens detected by commercial antibodies can also be detected by genotype VII ND antibodies. These results indicate that genotype VII ND antibody has 100% sensitivity in detecting ND virus on the IHC test (Table 1).

Viral Neutralization Test

ND antibody titers were determined before use in VNT. The results of this study indicated that ND Sato antibodies at HI 2^6 and 2^5 titers could neutralize 100% NDV/Ck/BGR/011 because there were no ECEs infected with the ND virus in these dilutions. At titer 2^4, only one in 3 ECEs was infected with the ND virus, and at titer 2^2, all ECEs were infected with the ND virus (Table 2). ND Gen VII antibodies at HI titers more than 2^4 have 100% protection against ND virus, and at lower antibody dilutions, their ability decreases and protects 75% of ECEs only. The protective titers of ND Sato and genotype VII antibodies differ in this VNT test, and ND antibody genotype VII has a higher ability to inhibit infection. It still protects 25% of ECEs in titer 2^2 (Table 3). Mixed ND antibodies can neutralize 100% of the NDV/Ck/BGR/011 virus at a titer of 2^5 (Table 4). Based on the VNT results,

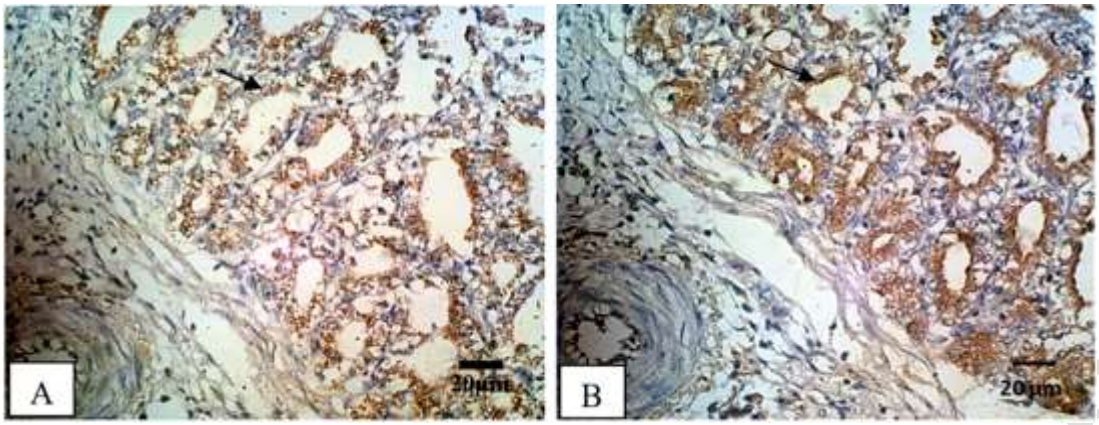

Fig. 1: The IHC results used commercial ND antibodies (A) and genotype VII antibodies (B). The immunopositive reaction was indicated by brownish color (arrows) in the cytoplasm of the epithelial cells of the proventriculus glands, which were observed with a 20x objective lens magnification.

Table 1: Sensitivity test for Genotype VII ND antibodies on IHC test

Number and type of samples	Antibody sensitivity		Percentage
	Commercial	Genotype VII	
Proventriculus (2)	2	2	100
Duodenum (2)	2	2	100
Pancreas (2)	2	2	100
Heart (2)	2	2	100

Table 2: The viral neutralization test results of Sato Newcastle disease antibodies

Antibody dilution	HI titer	Log 10 HI Titer	Infected	No infected	Accumulation			Percentage (%)	
					Infected	No Infected	Ratio	Infected	Protective
1: 4	2^6	$10^{-0.6}$	0	3	0	11	0/11	0	100
1: 8	2^5	$10^{-0.9}$	0	3	0	8	0/8	0	100
1: 16	2^4	$10^{-1.2}$	1	2	1	5	1/6	16.7	83.3
1: 32	2^3	$10^{-1.5}$	0	3	1	3	1/4	25	75
1: 64	2^2	$10^{-1.8}$	3	0	4	0	4/4	100	0

Table 3: The viral neutralization test results of genotype VII Newcastle disease antibodies

Antibody dilution	HI titer	Log 10 HI Titer	Infected	No infected	Accumulation			Percentage (%)	
					Infected	No Infected	Ratio	Infected	Protective
1: 8	2^6	$10^{-0.9}$	0	3	0	12	0/12	0	100
1: 16	2^5	$10^{-1.2}$	0	3	0	9	0/9	0	100
1: 32	2^4	$10^{-1.5}$	0	3	0	6	0/6	0	100
1: 64	2^3	$10^{-1.8}$	1	2	1	3	1/4	25	75
1: 128	2^2	$10^{-2.1}$	2	1	3	1	3/4	75	25

Table 4: The viral neutralization test results of a mixture Newcastle disease antibodies

Antibody dilution	HI titer	Log 10 HI Titer	Infected	No infected	Accumulation			Percentage (%)	
					Infected	No Infected	Ratio	Infected	Protective
1: 8	2^6	$10^{-0.9}$	0	3	0	10	0/10	0	100
1: 16	2^5	$10^{-1.2}$	0	3	0	7	0/7	0	100
1: 32	2^4	$10^{-1.5}$	1	2	1	4	1/4	25	75
1: 64	2^3	$10^{-1.8}$	1	2	2	2	2/4	50	50
1: 128	2^2	$10^{-2.1}$	3	0	5	0	5/5	100	0

the ND Sato antibody neutralization index was 1.6; genotype VII ND antibody 1.95; and mixed ND antibody 1.68. This result showed that genotype VII ND antibody has a higher ability than Sato and mixed ND antibodies to neutralize 10^4 ELD_{50}/ml NDV/Ck/BGR/11 virus.

The ability to neutralize antibodies against the ND virus can also be seen in macroscopic observations of embryos. Based on macroscopic observations, hemorrhage was seen in embryos infected with ND virus, both those injected with ND virus and a mixture of antigen and antibody with low antibody titers. Embryonated Chicken Eggs injected with ND virus only (positive control) showed generalized hyperemia with smaller embryo size compared to ECEs injected with antigen and antibody with low antibody titers (2^2) (Fig. 2). This result showed that the ECEs that received antigen and antibody with low antibody titers had a more prolonged embryo death, as seen by the growth of embryo hair. The longer death time compared to ECEs, which received ND virus only, was due to the different number of viruses that could infect the embryos. In ECEs, which received antigen and antibody with high antibody titer (more than 2^5), no embryo death was found because antibodies could completely neutralize all antigens.

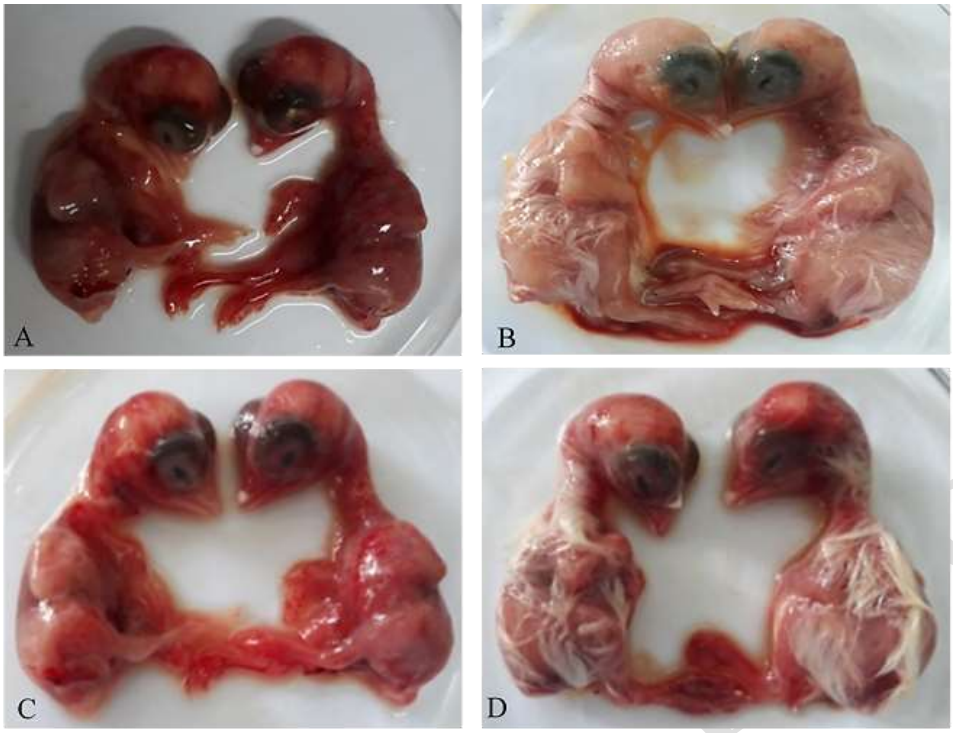

Fig. 2: Visualization of embryos on VNT; (A) positive control ECEs, found overall hemorrhages; (B) ECEs negative control, no hemorrhages, and embryos growth in line with age; (C) ECEs injected with antigen–antibody with an antibody titer of 2^2, found incomplete hemorrhages with a smaller embryo size (D) ECEs injected with antigen–antibody with an antibody titer of 2^3. Slight hemorrhages were found with a larger embryo size than the positive control embryo virus.

DISCUSSION

Newcastle Disease (ND) is a highly contagious viral disease of poultry and devastatingly impacts poultry production (Chukwudi et al. 2012; Mao et al. 2022; Akhtar et al. 2023). Since the discovery of the ND virus on Java Island in 1926 (Mao et al. 2022), the disease has continued to spread to various regions (Ahmed et al. 2022; Lebdah et al. 2022). It has become one of the endemic poultry diseases in Indonesia. Vaccination is one method applied to commercial farms to minimize ND cases (Mahmood and Sabir 2021). However, poultry infected with ND is still found. Based on OIE (2009) showed that in 2007, 1.500–8.000 chickens were infected with the ND virus each month in Indonesia. In 2009 and 2010, clinical outbreaks of ND in vaccinated commercial birds were reported to cause mortality up to 70–80% (OIE 2009).

Immunohistochemistry is one of the most developed methods in biomedical research. The IHC method is used to identify proteins and other macromolecules in tissues and cells. The antibodies used to localize antigens in cells or tissues are widely used in many essential discoveries (Nakamura et al. 2008; Burry 2011; Etriwati et al. 2017; Angeliya et al. 2022). Polyclonal antibodies can be produced quickly and at a lower cost than monoclonal antibodies (Lipman et al. 2005; Putri et al. 2018a; Putri et al. 2022). Polyclonal antibodies have better specificity than monoclonal antibodies. This is because polyclonal antibodies are produced by large numbers of B cell clones that produce multiple antibodies against a particular epitope. Serum-containing polyclonal antibodies are a combination of antibodies with a particular specificity. An important reagent used in the IHC test is the primary antibody. The primary antibody is used as a specificity control and ensures the primary antibody binds to the proper epitope on the antigen (Etriwati et al. 2017; Angeliya et al. 2022).

The IHC method can diagnose ND accurately, quickly, and more economically than serological and molecular methods (Ramos-Vara and Miller 2014). Based on the results of the IHC test using a light microscope, ND antigens appear as reddish-brown spots clustered in an area or spread over all tissue locations. The presence of horseradish peroxidase (HRP) as an enzyme in the complex antigen-antibody bond causes a color change when a substrate is given. The chromogen used in this method is diaminobenzidine (DAB), so positive cells infected with a virus (immunopositive) appear brown in their cytoplasm. At the same time, negative results will not show any brown color (Etriwati et al. 2017; Angeliya et al. 2022).

Based on the IHC test result, genotype VII ND antibodies showed the best visualization results at a 1:1000 dilution. Genotype VII ND antibodies showed a more vigorous intensity of immunopositive results than commercial ND antibodies. This result is due to genotype VII ND antibodies having various antibodies against all the structural proteins of the ND virus. However, commercial ND antibodies only react positively with the HN protein of the ND virus. Based on the results of the IHC test in several organs, ND virus immunopositive reactions were seen in the cell membranes and cytoplasm of epithelial cells in the duodenum and proventriculus. An immunopositive reaction on the cell membrane indicates the presence of viral particles (HN protein) attached to receptors on the cell membrane at the start of infection or during the phase before the virus starts budding. In the pre-budding stage,

viral surface proteins have been assembled on the infected cell membrane (Harrison et al. 2010).

Based on the results of AGPT, ND antibody genotype VII can react with both homologous and heterologous ND antigens. Genotype VII ND antibodies are expected to be used to detect the presence of ND virus from various genotypes, so it is expected to detect other ND genotypes even though they have never been found in Indonesia. This should be noted, because the sensitivity of immunodiagnostic reagents can lead to misdiagnosis due to false negative reactions. The ND polyclonal antibody is the primary antibody in the IHC test because polyclonal antibodies can recognize many epitopes of the ND virus. They provide robust detection and are more tolerant to slight changes in antigen (Mutneja et al. 2018).

Antibodies have been used for centuries to treat several infectious diseases in humans, such as diphtheria, tetanus, hepatitis A, hepatitis B, and rabies (Keller and Steihm 2000). Antibodies as therapeutic agents work by opsonizing and neutralizing antigens, which can stimulate the immune system to process and eliminate antigens (Ascoli and Aggeler 2018). Several diseases in humans, such as COVID-19, and in animals, such as IB and AI, have developed passive immunization using immunoglobulin Y (IgY) (Lardinois et al. 2014; Constantin et al. 2020). The virus neutralization test is one of the selected test methods to determine the potential of antibodies as immunotherapy reagents.

In this study, the viruses used in VNT were ND/Ck/BGR/11, which belonged to the ND virus genotype VII (Putri et al. 2018a). Sato is an ND isolate generally used as a standard ND virus and belongs to genotype III. Sato antibody is a heterologous antibody against ND/Ck/BGR/11 viruses. The antibody mixture consisting of Sato and genotype VII was declared heterologous. Heterologous antibodies have a lower neutralizing ability than homologous antibodies (Samiullah et al. 2006) since there are fewer neutralized epitopes of genotype VII ND virus. The VNT results showed that all the ND antibodies produced in this study could neutralize the genotype VII ND virus with different indices. Neutralizing activity of antibodies against antigens is needed to protect against viral infections, although this humoral immune system usually works together with cellular immunity (Tizard 2004). The VNT results indicated that genotype VII ND antibodies could be used as potential passive immunization reagent candidates to neutralize ND antigens circulating in the field.

Research on the application of antibodies as passive immunization reagents has been carried out by Lardinois et al. (2014), using Ig Y antibodies for ND and AI. ND and AI antibodies (Ig Y) can be applied *in ovo* to 14-day-old ECEs through the yolk sac (Lardinois et al. 2014). The use of antibodies as immunotherapy is limited by several factors, namely the need for a specific diagnosis before use and the fact that passive immunization is more effective as prophylaxis than therapy for some diseases (Berry 2018; Pelletier and Mukhtar 2020). Differences in donor species and antibody recipients also require further study before these antibodies are used as immunotherapy. Antibodies for human therapy must go through the humanized stage before being applied to humans (Chames et al. 2009). Some functional limitations of antibodies in immunotherapy, such as pharmacokinetics and impaired interaction with the immune system, require further research to use antibodies as immunotherapy (Chames et al. 2009).

Antibodies continue to be explored for their use as reagents in disease control. In animal disease control, Hassanzadeh et al (2006) developed antibodies as immune complex (antigen-antibody) vaccines for Infectious Bursal Disease. Administration of the IBD immune complex vaccine *in ovo* or subcutaneously can induce humoral immunity in chickens (Hassanzadeh et al. 2006). It is possible to develop vaccines from genotype VII ND immune complexes, and it is hoped that using ND antigens that are homologous to field isolates can induce ND protective antibodies in vaccinated chickens.

The reagent in the form of genotype VII ND antibodies is expected to replace commercial antibodies used for immunodiagnostic tests and as an alternative for passive ND immunization in valuable birds (pet birds) and grandparent stock or parent stock poultry farms and can also be used in the development of ND immune complex vaccines, which can be applied to commercial chicken farms such as broiler and layer commercial farms.

Conclusion

ND antibodies that have been characterized can be used as candidate reagents in the IHC test or the development of passive immunization. Genotype VII ND antibody with 1:1000 dilution gave the best results on IHC staining. Genotype VII ND antibody was best able to neutralize ND virus from field isolates.

Acknowledgments

This research was funded by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia in Hibah Bersaing Research Grant No 018.04/Pl 15.8/2016.

Authors' Contributions

DDP, EH, RDS, and AS conceived and planned the experiments. DDP and EE conducted experiments in the laboratory. DDP, EH, RDS, and AS contributed to the interpretation of the results. DDP is the lead in writing the manuscript. All authors provided critical feedback and helped to shape the research, in analysis and in manuscript writing.

REFERENCES

Ahmed HM, Amer SA, Abdel-Alim GA, Elbayoumi KM, Kutkat MA and Amer MM, 2022. Molecular characterization of recently classified Newcastle disease virus genotype VII.1.1 isolated from Egypt. International Journal of Veterinary Science 11(3): 295-301. https://doi.org/10.47278/journal.ijvs/2021.097

Angeliya L, Kristianingrum YP, Asmara W and Wibowo MH, 2022. Genetic characterization and distribution of the virus in chicken embryo tissues infected with Newcastle disease virus isolated from commercial and native chickens in Indonesia. Veterinary World 15(6): 1467-1480. https://doi.org/10.14202/vetworld.2022.1467-1480

Akhtar T, Shahid S, Asghar A, Naeem MI, Aziz S and 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

Ascoli CA and Aggeler B, 2018. Overlooked benefits of using polyclonal antibodies. Biotechniques 65(3): 127-136. https://doi.org/10.2144/btn-2018-0065

Berry CM, 2018. Antibody immunoprophylaxis and immunotherapy for influenza virus infection: Utilization of monoclonal or polyclonal antibodies? Human Vaccines & Immunotherapeutics 14(3): 796-799. https://doi.org/10.1080%2F21645515.2017.1363135

Burry RW, 2011. Controls for immunocytochemistry: An update. Journal Histochemistry Cytochemistry 59(1): 6-12. https://doi.org/10.1369/jhc.2010.956920

Chames P, Van Regenmortel M, Weiss E and Baty D, 2009. Therapeutic antibodies: successes, limitations and hopes for the future. Brazillian Journal of Pharmacology 157(2): 220-233. https://doi.org/10.1111%2Fj.1476-5381.2009.00190.x

Chukwudi OE, Chukwuemeka ED and Mary U, 2012. Newcastle disease virus shedding among healthy commercial chickens and its epidemiological importance. Pakistan Veterinary Journal 32(3): 354-356.

Constantin C, Neagu M, Diana Supeanu T, Chiurciu VA and Spandidos D, 2020. IgY - turning the page toward passive immunization in COVID-19 infection (Review). Experimental and Therapeutic Medicine 20(1): 151-158. https://doi.org/10.3892%2Fetm.2020.8704

Dako, 2013. Dako's General Instructions for Immunohisto-chemical Staining. California (US): Dako North America Inc.

Etriwati, Ratih D, Handharyani E and Setiyaningsih S, 2017. Pathology and immunohistochemistry study of Newcastle disease field case in chicken in Indonesia. Veterinary World 10(9): 1066-1071. https://doi.org/10.14202/vetworld.2017.1066-1071

Hassanzadeh M, Fard MHB and Tooluo A, 2006. Evaluation of the immunogenisity of immune complex Infectious Bursal Disease vaccine delivered in ovo to embryonated eggs or subcutaneously to day-olds chickens. International Journal of Poultry Science 5(1): 70-74. https://doi.org/10.3923/ijps.2006.70.74

Harrison MS, Sakaguchi T and Schmitt AP, 2010. Paramixovirus Assembly and Budding: Bulding Particles that Transmit Infection. International Journal Biochemistry Cell Biology 42(9): 1416-1429. https://doi.org/10.1016%2Fj.biocel.2010.04.005

Keller MA and Steihm ER, 2000. Passive immunity in prevention and treatment of infectious diseases. Clinical Microbiology Reviews 13(4): 602-614. https://doi.org/10.1128%2Fcmr.13.4.602-614.2000

Lardinois A, Van den Berg T, Lambrecht B and Steensels M, 2014. A model for the transfer of passive immunity against Newcastle disease and avian influenza in specific pathogen free chickens. Avian Pathology 43(2): 118-124. https://doi.org/10.1080/03079457.2014.880407

Lebdah M, Tantawy L, Elgamal AM, Mohamed M, Elsafty MM, Elhusseiny MH and Mohamed ME, 2022. Molecular detection and characterization of virulent newcastle disease viruses from different avian species in Egypt. International Journal of Veterinary Science 11(2): 189-195. https://doi.org/10.47278/journal.ijvs/2021.084

Leenaars M and Hendriksen CF, 2005. Critical steps in the production of polyclonal and monoclonal antibodies: evaluation and recommendations. ILAR Journal 46(3): 269-279. https://doi.org/10.1093/ilar.46.3.269

Lipman NS, Jackson LR, Trudel LJ and Weis-Garcia F, 2005. Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. ILAR Journal 46 (3): 256-268. https://doi.org/10.1093/ilar.46.3.258

Mahmood MS and Sabir R, 2021. Preparation and evaluation of avian influenza (H9) and Newcastle disease (thermostable i-2 strain) bivalent vaccine for commercial poultry. Agrobiological Records 3: 17-23. https://doi.org/10.47278/journal.abr/2020.017

Mao Q, Ma S, Schrickel PL, Zhao P, Wang J, Zhang Y, Li S and Wang C, 2022. Review detection of Newcastle disease virus. Frontiers in Veterinary Science 9: 936251. https://doi.org/10.3389%2Ffvets.2022.936251

Mutneja M, Mohan C, Long KD and Das C, 2018. An Introduction to Antibodies and Their Applications. 3rd Ed. Darmstadt (DE): Merck.

Nakamura K, Ohtsu N, Nakamura T, Yamamoto Y, Yamada M, Mase M and Imai K, 2008. Pathologic and immunohistochemical studies of newcastle disease (ND) in broiler chickens vaccinated with ND: Severe nonpurulent encephalitis and necrotizing pancreatitis. Veterinary Pathology 45(6): 928-933. https://doi.org/10.1354/vp.45-6-928

Oldoni I, Brown CC, King DJ, Samal S and Seal BS, 2005. The use of in situ hybridization and immunohistochemistry to study the pathogenesis of various newcastle disease virus strains and recombinants in embryonated chicken eggs. Microbial Pathogenesis 39(3): 69-75. https://doi.org/10.1016/j.micpath.2005.04.002

OIE, 2012. Newcastle disease. Infection with Newcastle Disease Virus. Office International des Epizooties, Paris, pp: 555-574.

OIE, 2009. Newcastle Disease Virus in Indonesia. Office International des Epizooties, France.

Pelletier JPR and Mukhtar F, 2020. Passive monoclonal and polyclonal antibody therapies. Immunologic Concepts in Transfusion Medicine 251-348. https://doi.org/10.1016%2FB978-0-323-67509-3.00016-0

Putri DD, Handharyani E, Soejoedono RD, Setiyono A, Mayasari NI and Poetri ON 2017. Pathotypic characterization of Newcastle disease virus isolated from vaccinated chicken in West Java, Indonesia. Veterinary World 10(4): 438-444. https://doi.org/10.14202/vetworld.2017.438-444

Putri DD, Handharyani E, Soejoedono RD, Setiyono A and Poetri ON, 2018a. Production and characterization of Newcastle disease antibody as a reagent to develop a rapid immunodiagnostic test tool. Veterinary World 11(7): 895-901. https://doi.org/10.14202/vetworld.2018.895-901

Putri DD, Handharyani E, Soejoedono RD, Setiyono A, Mayasari NI and Poetri ON, 2018b. Genotype characterization of Newcastle disease virus isolated from commercial chicken flocks in West Java, Indonesia. Pakistan Veterinary Journal 38(2): 184-188. http://dx.doi.org/10.29261/pakvetj/2018.041

Putri DD, Poetri ON, Candra AA and Soejoedono RD, 2022. Production of hyperimmune serum against genotype VII Newcastle disease virus in rabbits with several applications. Journal Advance Veterinary Animal Research 9(2): 211-220. https://doi.org/10.5455%2Fjavar.2022.i586

Ramos-Vara JA and Miller MA, 2014. When tissue antigens and antibodies get along: Revisting the technical aspects of immunohistochemistry-the red, brown, and blue technique. Veterinary Pathology 51(1): 42-87. https://doi.org/10.1177/0300985813505879

Razonable RR and Chen P, 2022. Editorial: Neutralizing Antibodies in the Prevention and Treatment of COVID-19. Frontiers in Immunology 13:938069. https://doi.org/10.3389/fimmu.2022.938069

Reed LJ and Munch H, 1938. A simple method of estimating 50 percent and points. American Journal Hygiene 27(3): 493-497. https://doi.org/10.1093/oxfordjournals.aje.a118408

Samiullah M, Rizvi F, Anjun AD and Shah MFA, 2006. Rising hyperimun serum againt Avian Paramyxovirus (APMV-1) and Pigeon Paramyxovirus (PPMV-1) in rabbits and their cross reactivity. Pakistan Journal Biological Science 9(11): 2184-2186. http://dx.doi.org/10.3923/pjbs.2006.2184.2186

Shrikant N and Nitika N, 2023. Monoclonal antibody: future of malaria control and prevention. Transactions of The Royal Society of Tropical Medicine and Hygiene 117(9): 673-674. https://doi.org/10.1093/trstmh/trad027

Tizard IR, 2021. Passive immunization. Vaccines for Veterinarians (6): 141-152. https://doi.org/10.1016%2FB978-0-323-68299-2.00021-6

Tizard IR, 2004. An Introduction of Veterinary Immunology 7th ed. Philadelphia (US): Elsevier.

Wakamatsu N, King DJ, Kapczynski DR, Seal BS and Brounw CC, 2006. Experimental pathogenisis for chicken, turkeys and pigeons of exotic Newcastle disease virus from a outbreak in California during 2002–2003. Veterinary Pathology 43(6): 925-933. https://doi.org/10.1354/vp.43-6-925