Production of IgY Against Infectious Bursal Disease Virus and Purification of IgY from Egg by Using Biocompatible Technique

I Wayan Teguh Wibawan[1]

Nunki Dyah Kristanti[2]

Arviana Zulfa[2]

Kris Damar Sasi[2]

Dian Ayu Permatasari[2]

Moh. Indro Cahyono[2]

Julianto[2]

Gowinda Sibit[2]

Wyanda Arnafia[2]

[1]Department of Animal Infectious Diseases and Veterinary Public Health, Bogor Agricultural University, Bogor, Indonesia

[2]Research and Development Division, Tekad Mandiri Citra Inc., Bandung, Indonesia

Corresponding Author: Wyanda Arnafia, e-mail: wyandaa@gmail.com

KEY WORDS: IgY, IBD virus, purification, biocompatible technique

ABSTRACT

Infectious bursal disease (IBD) virus is a highly contagious pathogen that causes damage to lymphoid organs and remains a threat to the poultry industry worldwide. IBD virus infection causes immunosuppression that leads to increased susceptibility to secondary infection and failed of vaccination programs. Specific IgY against IBDV isolated from egg yolk could be applied as passive immunization to susceptible young bird in the susceptible period (19- 26 days). The aim of this study was to produce immunoglobulin Y against IBD virus in layer chicken and to purify IgY from egg yolk by using biocompatible reagent. Ten female, Isa Brown layer chickens were vaccinated with IBD vaccine. The biocompatible technique to purify of IgY from egg yolks in this study consists of two main procedures, namely lipid removal from eggs yolk and IgY precipitation from the supernatant of the first step. Separation of lipids from egg yolk was done by a combination of water dilution, freezing, and centrifugation method. Isolation of IgY was done by salting out using 8.8% NaCl at pH 4.0. This technique was able to produce high purify IgY without decreased in immunoactivity of IgY. This technique is

environmentally friendly and simple for IgY purification.

INTRODUCTION

Infectious bursal disease (IBD) virus is a highly contagious pathogen that causes damage to lymphoid organs and remains a threat to the poultry industry worldwide (Wang *et al*. 2017). IBD virus infection causes huge economic loss to the poultry industry annually (Farooq *et al*. 2012). IBD virus is a non-enveloped, double-stranded RNA (dsRNA) virus belonging to the Birnaviridae family (Mundt *et al*. 1995; Lasher *et al*. 1994; Berg 2000). IBD virus infection causes massive destruction of the B lymphocyte precursors in bursa that leads to the lymphoid depletion of B cells and marked atrophy of the bursa, thereby leading to the severe immunosuppressive disease (Käufer and Weiss 1980; Sharma *et al*. 2000; Yao and Vakharia 2001; Liu and Vakhaira 2004). This condition leads to an increased susceptibility to secondary infection and does not respond adequately to vaccinations (Malik *et al*. 2006).

Specific IgY against IBDV isolated from egg yolk could be applied as passive immunization to susceptible young bird in the susceptible period (19- 26 days). Egg yolk IgY antibodies offer a practical alternative because of their feasibility for large-scale commercial production and the relative non-invasive methods used for their preparation (Kumaran and Citarasu 2016). Immunoglobulin concentration in the yolk was equal to or greater than in serum of chicken (Malik *et al*. 2006). In addition, the other advantages of IgY production from egg yolks is rapid production process and IgY can be stored in eggs at 4 °C for at least 1 year (Amro *et al*. 2017).

The antibodies derived from chicken egg yolks are used in many applications, including immunotherapy and immunodiagnostics (Nasiri *et al*. 2016; Tabll *et al*. 2015; Dias *et al*. 2010). The production and purification technique of IgY from egg yolk is environmentally friendly, cheap, easy, and simple The aim of this study was to produce immunoglobulin Y against IBD virus in layer chicken and to purify IgY from egg yolk by using a biocompatible reagent.

MATERIALS AND METHODS

Immunization of Hens

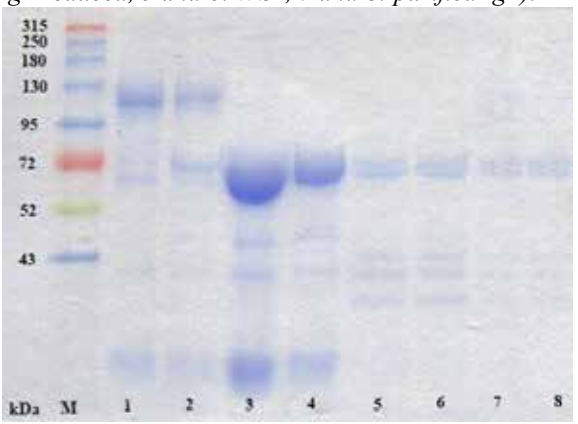
The vaccine used in this study is a commercial inactivated oil-based vaccine against IBD virus (Medivac Gumboro Emulsioan®, PT. Medion, Indonesia). Ten female, Isa Brown layer chickens (30 weeks old), were used. The chickens were reared in an individual cage with free access to water and feed. The layer chickens at 30 weeks of age were primed with oil-based IBD vaccine (0.5 ml dose/chicken with intramuscular injection). Boosting was performed two times with an interval of 14 days (same dose and route of the vaccine as described previously). Serum and eggs were collected before the first vaccination and 7 days after of each boosting.

IgY Separation and Purification

Egg yolk was manually separated from the egg white of all collected eggs manually. Procedure for isolation of IgY refers to Hodek *et al*. 2013 with minor modification using biocompatible chemicals. This procedure was divided into two stages: preparation of water-soluble fraction (WSF) of yolk and precipitation of IgY. WSF was prepared by diluting yolk 7 times (by volume) with aquadest and pH adjusted to 5.0 with 0.5 M HCl. The mixture froze at -20 °C and thawed at 4 °C. The aggregated egg yolk granules were sedimented by centrifugation at 13,500 g for 15 min at 4 °C and supernatant was collected. The supernatant was filtered with filter paper to get clear filtrate of WSF.

The second purification stage was precipitation of IgY. IgY. Precipitation was done by using sodium chloride because of its biocompatibility. Solid NaCl was added to WSF until 8.8% NaCl in the total volume of WSF. Mixtures were stirred for 2 hours at room temperature, pH was adjusted with 0.5 M HCl to 4, and then centrifuged at 3,700 g for 20 min at 4 °C. Supernatants were dis-

Figure 1*.The SDS-PAGE profile of IgY (M: marker, 1 and 2: standard IgY non-reduced,3 and 4: standard IgY reduced, 5 and 6: WSF, 7 and 8: purified IgY).*



carded and the pellets dissolved in PBS.

IgY Confirmation by ELISA and SDS-PAGE

The ELISA was used to evaluate the titer of specific antibody against IBD virus in serum and purified IgY. This assay was also used to evaluate the effect of pH in purification process on bioactivities and stability of purified IgY. The ELISA was done four-times:

- in pre-vaccination
- 1-week post first vaccination
- 1 week post first boosting, and
- 1-week post second boosting.

ELISA was done by using IDEXX Infectious Bursal Disease Virus Antibody Test Kit (USA). The test procedure was done base on the kit manual. Values were recorded at 650 nm absorbance. ELISA plate was read using an ELISA plate reader.

SDS-PAGE gel electrophoresis was used to determine the molecular weight of the purified IgY, and to assess the purified IgY after the purification process. The SDS-PAGE samples were WSF, purified IgY, and chicken IgY (as a positive control). Protein samples were analyzed on 10% SDS-PAGE gels under reduced and non-reduced conditions. Gels were run at 200 V for 1 hour, and then stained with Coomassie Blue. The gels were destained in 40% methanol with 10% acetic acid until a clear background was reached.

RESULTS

In this study, purification of IgY from egg yolk by using biocompatible technique was only used with safe chemicals. Separation of lipid from egg yolk was done by the combination of water dilution, freezing, and centrifugation method. Aquadest as the safe substance was used in this first procedure. This method was effective, efficient, and inexpensive for separating lipid from the mixture.

The second step in this study aimed to isolate of IgY from WSF. Precipitation method was done by using a salting-out procedure. NaCl 8.8% as the biocompatible substance was able to precipitate IgY from the mixture. This procedure was done at pH 4.0 at room temperature. SDS-PAGE analysis (Figure 1) and showed the optimal IgY precipitation result. This result revealed the purity of purified IgY after this final step.

ELISA was done in this study to determine the concentration of IgY in serum and purified IgY. The highest titer of IgY against IBD virus in serum was observed 1 week after the first booster (Table 1). The ELISA

Table 1. *The titer of IgY against IBD virus in serum and purified IgY in several time*

Time	The titer of IgY in serum (ELISA unit)	The titer of purified IgY (ELISA unit)
Pre-vaccination	5797	5447
1-week post first vaccination	6150	4526
1-week post first booster	10347	6687
1 week after the second booster	7320	8016

result showed that the titer of purified IgY was increased from 1-week post first vaccination until 1 week after the second booster. The results of ELISA assay indicated that low pH at purification process did not damage the IgY. Specific anti- IBD virus IgY at the end of purification process can recognize IBD virus.

DISCUSSION

The results of this study show that it is possible to produce of IgY from chicken eggs with a chicken immune system boosted by vaccination with IBD virus. This method offers several advantages by producing IgY from chicken eggs. Namely, no blood sampling is needed, considerable amounts of antibodies can be obtained at a fairly low cost, and IgY can be stored in eggs at 4 °C for several times before using. Therefore, the production of polyclonal antibodies through the immunization of chicken makes IgY a good alternative. This technique can produce the antibodies in a large amounts with high quality from simple methods of production without an invasive procedure. The IgY from chicken eggs has the potential of expanding to other antigens (Amro *et al*. 2017; Sudjarwo *et al*. 2017; Grando *et al*. 2017; You *et al*. 2014; Aguilar *et al*. 2014).

The IgY titer was increased gradually from 1 week after vaccination until 1 week after the first booster. In 1 week after the second booster, the IgY titer in serum was decreased slightly. Furthermore, the IgY titer of purified IgY from egg yolks always shows a gradual increase from the beginning of vaccination process until 1 week after the second booster. The increase of IgY in serum have different times with the increase of IgY in the egg. Serum levels of IgY raised more rapidly than in the egg. This indicated the need for several time to transfers and to deposit IgY from blood vessels to egg yolks. In addition, the IgY in serum was decreased faster than in the egg.

Biocompatible technique in this study has successfully purified chicken IgY antibody in egg yolks of hyperimmunized hens. This method consists of two main steps; namely, lipid removal from egg yolk and IgY precipitation from the supernatant of the first step. In the first step of the procedure, only aquadest and HCl are added to the yolks. Those substances are environmentally friendly. Separation of lipid from egg yolk was done by a combination of water dilution, freezing, and centrifugation method. These methods are simple, cost-effective, have simple equipment requirements, and simple handling processes.

NaCl precipitation at low pH in the second step is a cheap and rapid method to precipitate IgY from the mixture of proteins. The salting-out procedure was able to precipitate of IgY with low NaCl concentration in a single step. High purify IgY was produced in this step. The SDS-PAGE result of purified IgY showed the molecular weight patterns agreed with the expected molecular mass. The reactivity of IgY was not affected by low pH during the purification. Hodek *et al*. 2013 study also explained the extraction of total IgY from egg yolk by using NaCl precipitation. That study demonstrated that this technique can purify IgY until 97% with no any decrease in immunoreactivity of purified IgY. Moreover, the most common and safe materials (tap water, NaCl) were used within the whole procedure providing the final IgY preparations fully acceptable for various human applications, such as food additives, peroral medications or cosmetics.

CONCLUSION

The IgY against IBD virus can produce from chicken eggs with chicken immune system boosted by vaccination with IBD virus. This method offers several advantages in the production of IgY from chicken eggs; namely no blood sampling is needed, considerable amounts of antibodies can be obtained in a fairly low cost, and IgY can be stored in eggs at 4 °C for several time before using. Biocompatible technique in this study has successfully purified IgY from egg yolks with high purity and immunoactivity. This technique is environmentally friendly, simple, cost-effective, a simple equipment requirement, and simple handling process.

ACKNOWLEDGEMENT

The authors highly appreciate the Tekad Mandiri Citra Inc. for financial assistance in the execution of this project.

REFERENCES

1. Aguilar I, Sánchez EE, Girón ME, Estrella A, Guerrero B, Rodriguez-Acosta FA. Coral snake antivenom produced in chickens (Gallus domesticus). *Rev Inst Med Trop Sao Paulo* 2014;56(1):61-66.
2. Amro WA, Al-Qaisi W, Al-Razem F. Production and purification of IgY antibodies from chicken egg yolk. *J Genet Eng Biotechnol* 2017; in press.
3. Berg TP. Acute infectious bursal disease in poultry: A review. *Avian Pathol* 2000;29:175–194.
4. Dias da Silva W, Tambourgi DV. IgY: a promising antibody for use in immunodiagnostic and in immunotherapy. *Vet Immunol Immunopathol* 2010;135(3-4):173-180.
5. Farooq A, Rabbani M, Muhammad K, Akram Z, Ahad A, Fatima Z, Kamal T, Anwar Z. Passive immunization in infectious bursal disease virus infected birds using chemically purified immune yolk immunoglobulins (IgY). *Afr J Microbiol Res* 2012; 6(12):2993-2998.
6. Grando TH, Baldissera MD, de Sá MF, do Carmo GM, Porto BCZ, Aguirre GSV, Azevedo MI, de Jesus FPK, Santurio JM, Sagrillo MR, Stefani LM, Monteiro SG. Avian antibodies (IgY) against Trypanosoma cruzi: Purification and characterization studies. *J Immunol Methods* 2017;449:56-61.
7. Hodek P, Trefil P, Simunek J, Hudecek J, Stiborova M. Optimized protocol of chicken antibody (IgY) purification providing electrophoretically homogenous preparations. *Int J Electrochem Sci* 2013;8:113–124.
8. Käufer I, Weiss E. Significance of bursa of fabricius as target organ in infectious bursal disease of chickens. *Infect Immun* 1980;27:364–367.
9. Kumaran T, Citarasu T. IgY-technology: Production of antibodies in chickens and use in therapy of infectious diseases. *Int J Sci Res Mod Educ* 2016;1:29–35.
10. Lasher H, Shane S. Infectious bursal disease. *Worlds Poult Sci J* 1994;50:133–166.
11. Liu M, Vakharia VN. VP1 protein of infectious bursal disease virus modulates the virulence in vivo. *Virology* 2004;330(1):62-73.
12. Malik MW, Ayub N, Qureshi IZ. Passive immunization using purified IgYs against infectious bursal disease of chickens in Pakistan. *J Vet Sci* 2006;7(1):43–46.
13. Mundt E, Beyer J, Müller H. Identification of a novel viral protein in infectious bursal disease virus-infected cells. *J Gen Virol* 1995;76:437-443.
14. Nasiri K, Nassiri MR, Tahmoorespur M, Haghparast A, Zibaee S. Production and characterization of egg yolk antibody (IgY) against recombinant VP8-S2 antigen. *Pol J Vet Sci* 2016;19(2):271-279.
15. Sharma JM, Kim IJ, Rautenschlein S, Yeh HY. Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. *Dev Comp Immunol* 2000;24(2-3):223-235.
16. Sudjarwo SA, Eraiko K, Sudjarwo GW, Koerniasari. The potency of chicken egg yolk immunoglobulin (IgY) specific as immunotherapy to Mycobacterium tuberculosis infection. *J Adv Pharm Technol Res* 2017;8(3):91-96.
17. Tabll A, Abbas AT, El-Kafrawy S, Wahid A. Monoclonal antibodies: Principles and applications of immunodiagnosis and immunotherapy for hepatitis C virus. *World J Hepatol* 2015;7(22):2369-2383.
18. Wang H, Shan S, Wang S, Zhang H, Ma L, Hu L, Huang H, Wei K, Zhu R. Fused IgY Fc and Polysaccharide Adjuvant Enhanced the Immune Effect of the Recombinant VP2 and VP5 Subunits-A Prospect for Improvement of Infectious Bursal Disease Virus Subunit Vaccine. *Front Microbiol* 2017;14(8):2258.
19. Yao K, Vakharia VN. Induction of apoptosis in vitro by the 17-kDa nonstructural protein of infectious bursal disease virus: possible role in viral pathogenesis. *Virology* 2001;285(1):50-58.
20. You Z, Yang H, Xin W, Kang L, Gao S, Wang J, Zhang T, Wang J. Preparation of egg yolk antibodies against BoNT/B and their passive protection in mouse models. *Hum Vaccin Immunother* 2014;10(8):2321-2327.