PRODUCTION AND CHARACTERIZATION OF IMMUNOGLOBULINE YOLK AS ANTI ANTIGEN MEMBRANE *TOXOPLASMA GONDII*

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**ABSTRACT**

Toxoplasma gondii is an obligate intracellular parasite which can infect human and other mammalian. Immunoglobulin Y technology offers several advantages better than antibody production in mammals. This research is aimed to get immunoglobulin Y from egg yolk, and to find the characterization of immunoglobuline Y according to molecular weight by SDS PAGE and targeted protein with antibodies using Western Blot. This research divided from many step: culture tachyzoites of T. gondii from intraperitoneal fluid, preparation of membrane antigen tachyzoite of T. gondii, then immunization laying hens with membrane antigen, extraction and purification immunoglobuline Y from egg yolk and then protein analyzed by SDS PAGE and Western Blot.  The result of this research showed that immunoglobulin Y from egg yolk can produced antibody against protein membrane of T. gondii and profile protein immunoglobuline Y according SDS PAGE has molecular weight 179,8 kDa. Immunoglobuline Y was analyze by Western Blot can recognize antigen epitope of T. gondii on molecular weight 35,7kDa and 78,8 kDa.  

Keywords: Toxoplasma gondii, anti membrane T. gondii, immunoglobulin Y anti membrane

**INTRODUCTION**

Toxoplasma gondii is an obligate intracellular parasite that can infect humans. Definitive host of this parasite is the cat, while the intermediary hosts include mammals, birds and reptiles nation even fish. In the life cycle of this parasite can infect a host by 3 ways: through ingestion of tissue cysts by bradizoit, by ingestion of oocysts and congenital infection with tachizoit.1 Cats infected with *Toxoplasma gondii* in all excretions will spend millions of oocysts. When the oocyst is ingested by an intermediate host such as humans, cows, goats on the various tissues will be established intermediate host groups tropozoit actively dividing to form the rest of the stadium in the form of cysts.
(bradizoit) on the network. At the intermediate host is not formed sexual stage but only just asexual stage. When cats eat mice containing cysts are formed in the sexual stage in the cat’s intestine.²

Humans infected with the T. gondii occurs not only on those who keep cats or dogs but can also occur in other people who like to eat the food of undercooked meat containing tissue cysts, drinking fresh milk undercooked, water contaminated with raw vegetables and raw contaminated by disease-causing agents toxoplasmosis.² Incidence of toxoplasmosis has not significant changed in recent years, caution and attention to these diseases has increased dramatically. About 30-50% of the world population is estimated have been infected by Toxoplasma gondii. According to Chandra, Gandhahusada research that conducted in 1995 showed that prevalence toxoplasmosis in humans ranges between 2–63%, 35–70% on cat, 75% in dogs, 11–61% in goats, 11–36% in pigs, and less than 10% on the cow.³ Research results from Fitria, showed that 46.66% pork intersection in RPH Surabaya positive toxoplasmosis.⁴

The negative impact on the human is very detrimental to the failure of pregnancy and abortion. In human and animal therapy for this disease is very expensive, the impact of livestock on the economic loss due to a decline in production. The administration of drugs such as pyrimethamine and a sulfonamide can kill tachizoit of stadium T. gondii, but these treatments are not effective on stage bradizoite. In addition, these drugs are toxic, so is not recommended for use in the long term. Prevention by vaccination not fully provided protection. Using antibodies for controlling is start assess, one of them is making vaccination not fully provided protection. Using antibodies to toxoplasmosis.

The research included in laboratory explorative research. Research design was used descriptive analysis. The animals that were used for collecting IgY were layer hens strain with 20 weeks of age, 5 hens were adapted during 2 weeks. Toxoplasma gondii passage and cultivation in Mus musculus strain Balb/C with 3 months of age.

**MATERIAL & METHOD**

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Tachizoit T. gondii cultivating and harvesting were done by infecting mice with T. gondii RH strain isolation by 10⁴ for doses in 50 mice Balb/C through intraperitoneally. Protein membrane tachizoit of T. gondii was isolated by sonication and centrifugation. Protein concentration was interpreted using spectrophotometry with 595 nm.

In Vitro cultivating and harvesting of tachizoit T. gondii were done by using infected mice with isolate T. gondii strain RH through 1 x 10⁴ on 50 Balb/C mice by intraperitoneal. Isolation of protein membrane tachizoit T. gondii used sonication and centrifugation. Protein concentration reader used spectrophotometry with 595 nm. Protein was aliquot and saved on -20° C until it was used.

The chicken that immunization using antigen from protein membrane tachizoit of T. gondii by 50 μg that was diluted on PBS and emuluted using Freund’s Complete Adjuvant through 1:1 of ratio until homogenous. Emulsion injected through intra-muscular (on femur). Immunization was repeated twice with 14-days interval. On Repetition immunization, 50 μg of antigen was emuluted using Freund’s Complete Adjuvant then 14 days after second repetition.

Isolation of anti-toxoplasma antibody on yolk used combination of chloroform and ammonium sulfate precipitate method was the chosen method that produce antibody with high purity level. Purification was done by using precipitation of ammonium sulfate 40% and using ratio between IgY supernatant: ammonium sulfate 40% was 1:1. Solution was precipitated one night on 4° C and was centrifuged with 10,000 rpm for an hour. Precipitate was taken for re-suspension using PBS then it was purified and analyzed. Immunoglobulin Y precipitation was covered with specific plastic for sonication, then added 0.5M PBS and stirred using magnetic stirrer for 24 hours in 4° C. Characterization was done by reacted antibody from purification with antigen membrane using ELISA method and Western Blot. IgY antibody titter measurement using ELISA.
Immunoglobulin Y protein analyzed using SDS PAGE. Antigenic membrane protein of *T. gondii* was identified using Western Blot method. IgY antibody titter measurement using ELISA.

**RESULT AND DISCUSSION**

The result of chicken immunization was read using Optical Density level on indirect ELISA. IgY measurement was done after the third immunization booster. ELISA was done on two samples, blood serum and immunized yolk by membrane antigen. Sample consist of yolk that taken before immunization and on the 7th, 14th and 28th day after immunization. The result of ELISA on yolk between before and after immunization of *T. gondii* membrane antigen shows that OD level increased and it significant difference between before and after immunization (p < 0.005). The 7th and 14th day after immunization, there were no differences. Result of ELISA OD level can be seen on Table 1.

Immunoglobulin Y was gotten from egg yolk that was extracted with chloroform and precipitated with ammonium sulfate 40% then the protein was analyzed using SDS PAGE.

Marker used for SDS PAGE of immunoglobulin Y could detect protein with molecular weight between 10 kDa-260kDa. Protein molecular weight assessment had done using regression analysis between Rf and BM log. On this research, protein band on marker had line equation y = \(-2.501x^5 + 2.920x^4 - 2.202x^3 + 3.467x^2 - 3.253x + 2.597\). On the 2nd column, immunoglobulin without dilution, showed 6 protein bands with molecular weight 179.8kDa, 130.4kDa, 70.6kDa, 59.1kDa, 38.6kDa and 25 kDa. On the 3rd column, immunoglobulin Y with dilution ration 1:5 showed 4 protein bands, 179.8 kDa, 67.4 kDa, 61.6 kDa and 38.6 kDa. On the 4th column, immunoglobulin Y with dilution ration 1:10 showed 4 protein bands, 179.8 kDa, 67.4 kDa, 61.6 kDa and 38.6 kDa. Column 5th, immunoglobulin Y with dilution ration 1:20, showed protein bands with molecular weight were 179.8 kDa dan 67.4kDa. Concentration of immunoglobulin Y protein from preparation was 0.16 μg/μl.

The result of Western Blott, using antigen that were *T. gondii*, was reacted with polyclonal antibody from egg yolk which were Ig Y. Then, it compared with antibody from rabbit that was already immunized using *Toxoplasma gondii* proteins, IgG. Used marker on SDS PAGE could detect protein with 20 kDa–120 kDa of molecular weight. Molecular weight assessment of immunoglobulin Y had been done using regression analysis between Rf and BM log. On this research, protein band on marker had equation as \(y = -3.103x^4 + 5.506x^3 - 2.515x^2 - 0.936x + 2.246\). The 1st column was antigen of *T. gondii* membrane which was reacted with rabbit Ig G. This column showed protein band with molecular weight 35.7 kDa. The 2nd and 3rd column were antigen of *T. gondii* membrane that was reacted with chicken IgY and showed protein band with molecular weight 35.7 kDa and 78.8 kDa.

Preparation of membrane antigen protein of *Toxoplasma gondii* on tachizoit stadium was continued with characterization using (SDS PAGE) method. This method was the common used of electrophoresis method. Electrophoresis method was used for protein characterization based on molecular weight. The result of SDS PAGE analysis of *T. gondii* membrane protein showed 35.4 kDa, 59.8 kDa; 66.8 kDa; 81.9kDa; 86.8kDa which were more than 118 kDa (Figure 2). Based on Chalghoumi, et al., (2009), 10-100 kDa protein for vaccine were needed.

**Table 1.** Averages of IgY OD level on yolk that was immunized with *T. gondii* membrane antigen

| Yolk                        | OD Level Average | OD Level Deviation |
|-----------------------------|------------------|--------------------|
| Before Immunization         | 0.9856<sup>a</sup> | 0.5424             |
| 7th day after the 3rd immunization | 1.5332<sup>b</sup> | 0.2201             |
| 14th day after the 3rd immunization | 1.8873<sup>b</sup> | 0.3788             |
| 28th day after 3rd immunization | 1.8303<sup>b</sup> | 0.3640             |

Different superscript on the same column show significant difference (p < 0.005)
IgY also had high affinity against antigen. Fab (antibody fragment) on IgY could recognize antigen epitope more than on IgG. Structure of heavy and light chain of IgG and IgY was relatively similar which was two heavy chains on IgY had molecular weight 67–70 kDa on each chain and two light chain with molecular weight 25 kDa on each. The differences of IgG and IgY only on CH4 chain on Fc. OD level on ELISA result both on yolk and on serum which was immunized with antigen of T. gondii membrane showed that there was significant different between before immunization and after the 3rd immunization. It showed that antigen of T. gondii membrane was immunogenic. Based on Abbas, forming of immunoglobulin antibody would increase on the 2nd antigen exposure with the same antigen type. B cell would produce immunoglobulin in 5 days after antigen exposure and immunoglobulin level would be kept on 23 days. If booster was done every 14 days, the increasing of antibody would occur after the 3rd immunization. On the screening of both yolk and serum on the 7th day and 14th day after the 3rd immunization did not show significantly difference. It indicated that between 7th day and 14th day after the 3rd immunization immunoglobulin Y production was relatively constant on the chicken body. On the 28th day after immunization did not show significantly difference. Antibody testing on chicken serum was done until the 14th day after the 3rd immunization immunoglobulin Y production was in accordance with Michael et al., who said that two days after the 3rd immunization immunoglobulin level would be kept on 23 days. If booster was done every 14 days, the increasing of antibody would occur after the 3rd immunization. On this research, protein band with molecular weight 25 kDa and 38.6 kDa might be fragment from Fab IgY on light chain, while protein with molecular weight 59.1 kDa, 61.6 kDa, 67.4 kDa and 70.6 kDa were fragment from Fab IgY on heavy chain. This was in accordance with Michael et al., who said that two heavy chains on IgY had molecular weight 67–70 kDa on each and two light chain with molecular weight 25 kDa on each. Protein band which had molecular weight 130.4 kDa, 179.8 kDa was probably the fragment from complex bond of Fc receptor Igy (FcR-Igy) and the whole molecule from IgY. This was in accordance with He and Bjorkman, which was said that FcR-IgY complex and whole IgY had molecular weight between 150 kDa–180 kDa. The result of Western Blot using antigen from T. gondii membrane which was reacted with polyclonal IgY antibody showed protein band reaction with molecular weight 35.7 kDa and 78.8 kDa (Figure 2). On 35.7 kDa of Western Blot showed that IgY antibody could recognize antigen epitop, that was shown by band reaction between protein from antigen which was 35.7 kDa (SAG 1) T. gondii with 38 kDa of IgY molecule (light chain Fab IgY). P30 T. gondii (SAG1) was the major protein on RH strain and had molecular weight around 30–38 kDa. Surface antigen (SAG) was protein which took role on attachements. SAG was protein on the tachizoiot surface that consist of glycospilosphatidilinositol (GPI) and helpfuly gave signal on attachment process between SAG and ligan on the cell surface that would be infected. Western Blot result which had reacted with primary antibody of rabbit (IgG) also showed protein band with molecular weight 82 kDa. It proved that the recognizing epitope of T. gondii antigen against Fab IgG of

**Figure 2.** The result of T. gondii membrane protein characterization using Western Blot. 1st column was T. gondii antigen which was reacted with rabbit IgG antibody, 2nd and 3rd column were T. gondii antigen which were reacted with chicken IgY antibody and the 4th column was marker. The red sign was protein with 78.8 kDa of molecular weight, green sign was protein with 35.7 kDa of molecular weight.

to provoke immune response. It fulfill requirement for immunization so hopefully could provoke immune response. From this result was in accordance with the Suwanti’s research that major protein on membrane had molecular weight between 60 kDa–200 kDa. 66 kDa–70 kDa of protein also found in membrane and ropty T. gondii was proven by Bonhomme et al. (1990) which was cited by Suwanti. It was proven by protein characterization result of P22 recombinant from tachizoiot membrane that showed protein band between 35 kDa–40 kDa. Molecular weight of protein band above 118 kDa could not be determined using regression equation between log of molecular weight and Rf from marker, since those protein out of regression line.

Antibody was produced from induced egg yolk with immunization from membrane T. gondii protein. On this research, immunization was done via intra-muscular for three times using mix of Complete Freund Adjuvant at the 1st immunization and Incomplete Freund Adjuvant at the 2nd and 3rd immunization. The aim of CFA and IFA given was to induce the bigger immune response. It fulfill requirement for induced the bigger immune response. It fulfill requirement for immumogenic. Based on Abbas, forming of immunoglobulin antibody would increase on the 2nd antigen exposure with the same antigen type. B cell would produce immunoglobulin in 5 days after antigen exposure and immunoglobulin level would be kept on 23 days. If booster was done every 14 days, the increasing of antibody would occur after the 3rd immunization. On the screening of both yolk and serum on the 7th day and 14th day after the 3rd immunization did not show significantly difference. It indicated that between 7th day and 14th day after the 3rd immunization immunoglobulin Y production was relatively constant on the chicken body. On the 28th day after immunization did not show significantly difference. Antibody testing on chicken serum was done until the 14th day after the 3rd immunization. On this research, protein band with molecular weight 25 kDa and 38.6 kDa might be fragment from Fab IgY on light chain, while protein with molecular weight 59.1 kDa, 61.6 kDa, 67.4 kDa and 70.6 kDa were fragment from Fab IgY on heavy chain. This was in accordance with Michael et al., who said that two heavy chains on IgY had molecular weight 67–70 kDa on each and two light chain with molecular weight 25 kDa on each. Protein band which had molecular weight 130.4 kDa, 179.8 kDa was probably the fragment from complex bond of Fc receptor Igy (FcR-Igy) and the whole molecule from IgY. This was in accordance with He and Bjorkman, which was said that FcR-IgY complex and whole IgY had molecular weight between 150 kDa–180 kDa. The result of Western Blot using antigen from T. gondii membrane which was reacted with polyclonal IgY antibody showed protein band reaction with molecular weight 35.7 kDa and 78.8 kDa (Figure 2). On 35.7 kDa of Western Blot showed that IgY antibody could recognize antigen epitop, that was shown by band reaction between protein from antigen which was 35.7 kDa (SAG 1) T. gondii with 38 kDa of IgY molecule (light chain Fab IgY). P30 T. gondii (SAG1) was the major protein on RH strain and had molecular weight around 30–38 kDa. Surface antigen (SAG) was protein which took role on attachements. SAG was protein on the tachizoiot surface that consist of glycospilosphatidilinositol (GPI) and helpfuly gave signal on attachment process between SAG and ligan on the cell surface that would be infected. Western Blot result which had reacted with primary antibody of rabbit (IgG) also showed protein band with molecular weight 82 kDa. It proved that the recognizing epitope of T. gondii antigen against Fab IgG of
rabbit was occur. Fab IgG of mammalian on heavy chain had molecular weight 67-70 kDa and light chain 25 kDa.\textsuperscript{8} On the result of Western Blott using rabbit IgG antibody showed 35.7 kDa of molecular weight. It meant that Fab IgG could recognize protein of tachizoit membrane and proved there was similarity of Fab structure from IgG and IgY. Fragment from molecular antibody was antigen binding fragment and Fc was crystallizable fragment (constant) as biology effector. On aves, IgY Fc receptor was known as FcRY. In fact, aves FcRY had similarity with FcRn IgG on mammals, whereas FcRn also act as MHC1 which could bind with antigen peptide for T cell. The similarity of FcRY and FcRn was could bind with immunoglobulin molecule on pH ≤ 6 and did not bind on pH ≥ 7. FcRY which bind with the whole IgY molecule had dimer structure with N terminal chain and cyctin receptor for binding peptide from antigen. FcRY bonded on IgY CH4 chain. The differences between FcRY and FcRn were on recognizing ligand receptor of CH3-CH4 IgY and CH2-CH3 IgG whereas IgY ligand had double ability than IgG ligand. Antigen epitope could be recognized by more IgY molecule than mammalian immunoglobulin.\textsuperscript{10}

CONCLUSIONS

To sum up briefly, the result from profil analysys of membrane protein of tachizoit \textit{T. gondii} was protein with molecular weight 35.4 kDa, 59.8 kDa, 66 kDa, 81 kDa and 86 kDa. Immunoglobulin Y from egg yolk could produce antibody anti protein of \textit{T. gondii} membrane. Based on Western Blot result, could be concluded that protection mechanism of immunoglobulin Y was on Fab which could recognize epitop of \textit{T. gondii} antigen with molecular weight 35.7 kDa and 78.8kDa

REFERENCES

1. Hanafiah, M., Wisnu N, Mufti K., dan Fadrial K. 2009. Produksi dan Isolasi Protein Membran Stadium Bradizoit \textit{Toxoplasma gondii}: Suatu Usaha untuk Mendapatkan Material Diagnostik dalam Mendiagnosa Tokoplasmosis. Fakultas Kedokteran Hewan Universitas Syiah Kuala. Aceh. Vol. 10 No: 3: 156–164.
2. Hiswani. 2003. Tesis: Tokoplasmosis Penyakit Zoonosis Yang Perlu Diwaspadi Oleh Btu Hamil. Fakultas Kesehatan Masyarakat. Universitas Sumatera Utara. Chandra, G. 2001. \textit{Toxoplasma gondii}: Aspek Biologi, Epidemiologi, Diagnosis dan penatalaksanaannya.
3. Chandra, G. 2001. \textit{Toxoplasma gondii}: Aspek Biologi, Epidemiologi, Diagnosis dan penatalaksanaannya. http://www.emedice.com. (Juni 2011).
4. Ardhiiani, F. 2008. Insidensi Tokoplasmosis pada Babi di RPH Pengirian Surabaya dan RPH Gadang Malang. Fakultas Kedokteran Hewan Universitas Airlangga. Surabaya.
5. Arabpour, M., Mojgan B. Maryam N., Seyyed H.A. 2011. African Journal of Biotechnology Vol. 10(40): Cloning and expression of \textit{Toxoplasma gondii} tachyzoit P22 protein. Department of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran., pp. 7746–7750.
6. Suwanti, L.T. 1996. Identifikasi dan produksi Antibodi Monoklonal Protein Membran \textit{Toxoplasma gondii} Stadium takizoit. Tesis Pasca sarjana Universitas Gadjah Mada. Yogyakarta.
7. Chalghoumi, R., B. Yves, P. Daniel dan T. Andre. 2009. Hen Egg Yolk Antibodies (IgY), Production and Use for Passive Immunization Against Bacterial Enteric Infection in Chicken. Gembloux Agriculture University. Belgium. 295–308.
8. Michael, A., S. Meenatchisundaram, G. Parameswari, T. Subbraj, R. Selvakumaran dan S. Ramalingam. 2010. Chicken Egg Yolk Antibodies (IgY) as an Alternative to Mammalian Antibodies. Indian J. Science Technology. 3(4): 468–474.
9. Ko K. and Ahn D.U, 2007. Preparation of Immunoglobulin Y from Egg Yolk Using Ammonium Sulfate Precipitation and Ion Exchange Chromatography. Poultry Science. 86: 400–407.
10. He, Y and Pamela J.B. 2011. Strukture of FcRY, an avian immunoglobuli receptor related to mammalian mannose receptor, and its complexes with IgG. California Institute of Technology. USA. Page: 12431–12436.
11. Abbas, A.K., A.H. Lichtman and J.S. Pober. 2000. Cellular and Molecular Immunology. W.B. Saunders Company, Philadelphia. p. 235–338.
12. Carruthers, V.B. 2002. Host Cell Invasion by the Opportunistic Pathogen \textit{Toxoplasma gondii}. Acta Trop. 81: 111–122.