

## ORIGINAL ARTICLE

# Analysis of the Cellular and Humoral Immune Response (IgG, CD4) in Rabbits Immunized with the Antigenic Protein of *Leucocytozoon caulleryi*

Dony Chrismanto<sup>1</sup>, Miyayu Soneta Sofyan<sup>1</sup>, Ira Sari Yudaniayanti<sup>2</sup>

<sup>1</sup> Department of Health, Faculty of Vocational, Universitas Airlangga, Jl. Dharmawangsa Dalam Selatan No.30, Surabaya 60286, Indonesia

<sup>2</sup> Department of Clinical Veteriner, Faculty of Veterinary Medicine, Universitas Airlangga, 60115 Surabaya, Indonesia

## ABSTRACT

**Introduction:** Leucocytozoonosis is a protozoan disease in chickens with high mortality and is an endemic in Indonesia. The aims of this research to analyze the immune response of humoral (IgG) and cellular (CD4) in rabbits immunized by the antigenic proteins of *L. caulleryi*. **Methods:** The research were done: separation and extraction of antigenic proteins of *L. caulleryi* from liver chicken, immunize rabbits with antigenic proteins, detection of IgG titers with indirect ELISA, and detection of CD4 expression with Immunocytochemistry, and visualized by fluorescein isothiocyanate with fluorescence microscope. **Results:** The immunization results indicate that the protein from the liver contains *L. caulleryi* which is antigenic and has ability to produce and increase the antibody titers (IgG) of rabbits at a dose of 500 µg per booster. Activation cellular response (CD4) was shown a greenish color by fluorescence microscopy on the fifth booster. **Conclusion:** The antigenic proteins of *L. caulleryi* dose of 500 µg with 5 times booster can induce a humoral (IgG) and cellular immune response (CD4) in the fifth booster or 10 weeks after the rabbit is immunized.

**Keywords:** Leucocytozoon caulleryi, IgG, CD4, Rabbits

## Corresponding Author:

Dony Chrismanto, M.Si

Email: donychrismanto@gmail.com

Tel: (+62) 5033869

## INTRODUCTION

Leucocytozoonosis is a blood protozoan disease in poultry caused by the genus *Leucocytozoon*, the family Plasmodiidae, and transmission via blood-sucking fly vector such as *Culicoides* sp. (1-3). The life cycle of *L. caulleryi* is complex, produce merogony in the liver or other tissue, gametocyte in blood cells, and sporogony in the intestine of flies (4-6). This disease is also called a malaria-like disease because it clinically resembles malaria in poultry caused by *Plasmodium* sp. The occurrence of leucocytozoonosis in poultry caused by *L. caulleryi* species is a type of protozoa included in phylum apicomplexa which is pathogenic and causes death in chicken and cause economic losses in poultry farm. Losses could be caused of growth retardation, decreased body weight, and decreased egg production (7).

The results of immunogenic protein characterization from Schizont *L. caulleryi* are preliminary studies for the development of subunit vaccines in preventing Leucocytozoonosis in purebred chicken (8). The development of a subunit vaccine for Leucocytozoonosis, through phylogenetic analysis of the Cytochrome B *Leucocytozoon* spp gene in purebred chickens showing that there was a high homology (> 95%) among *L. caulleryi* from various endemic areas. Thus, forming one group of relatives who can be used as a reference if vaccination is done to prevent Leucocytozoonosis in Indonesia (9).

Based on these problems, preliminary research on rabbits that are immunized using liver proteins containing *L. caulleryi* schizont has been identified and contain antigenic proteins with molecular weights of 109.6 kDa and 65.0 kDa (9). Based on the results of the characterization of antigenic proteins from *L. caulleryi*, the researchers conducted further research whether the proteins are also immunogenic and whether the schizont protein can induce cells that play a role in the immune response to produce IgG

antibodies and activate co-receptors such as CD4, inducing the Th cells to activate the immune response.

## MATERIALS AND METHODS

### Separation and extraction of Leucocytozoon from liver chicken

Leucocytozoon sp. isolated from chicken liver and undergone microscopic examination to assess the pathological changes.

### Immunize rabbits with schizont proteins extract of *L. caulleryi*

This research used six rabbits and the research procedure was approved by the Ethical Committee of Veterinary Medicine Faculty, Universitas Airlangga, No: 630-KE (3). The rabbits were injected with 500 µg Leucocytozoon sp. antigen, with repeated injections (booster) up to 5 times and a time span every two weeks.

### Detection of IgG antibody with indirect ELISA

Analysis of the humoral immune response was done by examining rabbit blood serum to measure the increase in antibody titer (IgG) after the immunization process every two weeks (10).

### Detection of CD4 expression with Immunocytochemistry

Analysis of cellular immune responses was done examining Peripheral Blood Mononuclear Cell (PBMC) from rabbit blood for detection of CD4 expression (3, 10). The results were examined by using a fluorescence microscope, to detection of CD4 expression.

## RESULTS

Based on examination results with an indirect ELISA test to determine IgG antibody titers based on Optical Density (OD) and analysis with Multivariate descriptive statistical tests with Tests Within Subject Contrasts on the achievement of the average optical density (OD) value and the average standard deviation of three rabbits (N) serum with 5 replications (5 x booster), the first injection with a dose of 500 µg (control) showed an OD value of 0.24633, then the first booster increased by 0.34200, or an increase of 1.5 times that of the control, thus in the second booster there was an increase of almost 4 times that of control, amounting to 0.87667. Based on descriptive statistical tests showing OD values on boosters 0, 1, and 2 with the same notation (a) that is not different between these boosters but significantly different from OD values at boosters 3,4 and 5 (notation b). These results indicate that the value of OD increased significantly starting from booster 3 of 1.59767 or 7 times the increase of control (booster 0) or 2 times of the second booster of 0.87667. The increase

in OD value increased to the fifth booster to 1.93933 such as which is presented in Table I.

**Table I : Average Optical Density (OD) Value of the Immunized Rabbit Serum with *L.caulleryi***

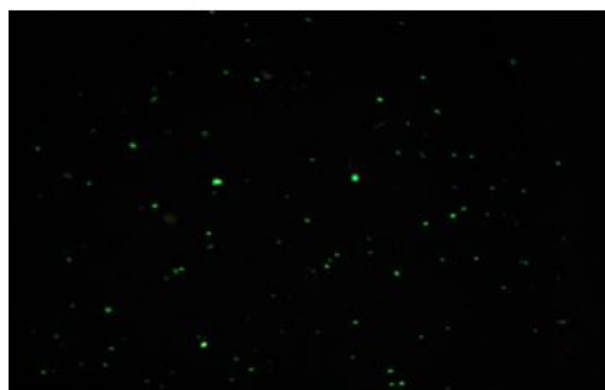
Booster	Mean	Std. Deviation	N
Booster_0	.24633 <sup>a</sup>	.021221	3
Booster_1	.34200 <sup>a</sup>	.080169	3
Booster_2	.87667 <sup>a</sup>	.331037	3
Booster_3	1.59767 <sup>b</sup>	.376990	3
Booster_4	1.75500 <sup>b</sup>	.493268	3
Booster_5	1.93933 <sup>b</sup>	.511608	3

Note: Different superscripts in the same column show significant different of OD (p <0.05)

## DISCUSSION

According to Abbas, 2005 (11) the increase in antibody titer is due to activation of memory cells (B cells) that occur after the 30th day of immunization and the adaptive immune system, as well as the nervous system, can remember (memory) especially in gaining experience on antigen exposure. The immunization results indicate that the protein from the liver contains schizont *L.caulleryi* which is antigenic and can induce antibodies (IgG) as immune response of rabbits at a dose of 500 µg per booster (12).

Based on this, in accordance with the results of research conducted that rabbits who have been immunized with protein from chicken liver containing *L.caulleryi* as much as 500 µg each injection with 5 replications, there is an increase in antibody titer (IgG) which significantly increased on the repetition (booster) to 3 and continue to increase until the last booster (5th). An increase in antibody titer with an OD value indicator indicates that B lymphocyte cells have produced immunoglobulin IgG (7,10), which means there is a role for CD4 as signal transduction that has activated Th cells to increase cells that play a role in the immune system as expressed in Fig.1.



**Figure 1 : CD4 expression in Rabbit T cells, visualized by Fluorescein isothiocyanate (FITC) (200x magnification).**

Co-receptor of CD4 T cell activation has begun to be seen in the 4th booster but the amount of circulation is still small, while in the 5th booster there is an increase in the fading, which is in accordance with the increasing antibody titer (IgG). It is known that CD4 serves as signal transduction when the antigen is introduced with T Cell Receptor (TCR) and strengthens T cell bonding with Antigen Presenting Cell (APC) in the introduction of MHC molecules to activate T cells, and about 65% of T cells mature in the blood and lymphoid tissue express CD4 (10).

## CONCLUSION

The antigenic proteins of *L.caulleryi* dose of 500 µg with 5 times booster can induce a humoral (IgG) and cellular immune response (CD4) in the fifth booster or 10 weeks after the rabbit is immunized.

## ACKNOWLEDGEMENT

We would like to thank the Rector and Dean of Vocational Faculty, Universitas Airlangga for the research funding that had been granted in 2019.

## REFERENCES

1. You YC, Jiunn-Siow W, Ching-Chang Y. Culicoides arakawae (Diptera: Ceratopogonidae) population succession in relation to leucocytozoonosis prevalence on a chicken farm in Taiwan. Vet Parasitol. 2000;93:113-120.
2. Labuschagne K. Survey of Culicoides spp at The National Zoological Gardens of South Africa (Pretoria). Tshwane University of Technology, South Africa.; 2007.
3. Lastuti ND, Suprihati E, Chrismanto D, Ma'ruf A. Antigenic protein of Leucocytozoon caulleryi schizont inducing cellular immune response:TLR-2 and CD4 as marker. ASSEHR. 2017;98:67-70.
4. Imura T, Sato S, Sato Y, Sakamoto D, Isobe T, Murata K, et al. The apicoplast genome of Leucocytozoon caulleryi, a pathogenic apicomplexan parasite of the chicken. Parasitol Res. 2014;113:823–8.
5. Zhao W, Cai B, Qi Y, Liu S, Hong L, Lu M, et al. Multi-Strain Infections and 'Relapse' of Leucocytozoon sabraezii Gametocytes in Domestic Chickens in Southern China. PLoS One. 2014;9(4):e94877.
6. Zhao W, Liu J, Xu R, Zhang C, Pang Q, Chen X, et al. The Gametocytes of Leucocytozoon sabraezii Infect Chicken Thrombocytes, Not Other Blood Cells. PLoS One. 2015;10(7):e0133478.
7. Lee H, Koo B, Jeon E, Al. E. Pathology and molecular characterization of recent Leucocytozoon caulleryi cases in layer flocks. J Biomed Res. 2016;30(6):517–24.
8. Ito A, Gotanda T. The correlation of protective effects and antibody production in immunized chickens with recombinant R7 vaccine against Leucocytozoon caulleryi. J Vet Med Sci. 2002;64(5):405–11.
9. Suprihati E, Yuniarti W. The phylogenetics of Leucocytozoon caulleryi infecting broiler chickens in endemic areas in Indonesia. Vet World. 2017;10(11dsq):1324-1328.
10. Lastuti N, Yuniarti W, Hastutiek P, Suwanti L, Chrismanto D. Humoral and cellular immune response induced by antigenic protein of Sarcptes scabiei var. caprae. Vet World. 2018;11(6):819–23.
11. Abbas A., Litchman A. Cellular and Molecular Immunology. 5th. Ed. International Edition. Philadelphia, Pennsylvania: Elsevier Saunders Inc.; 2005. 41–105, 411–432 p.
12. Dennis VU, Akira I, Fletcher P del V, Kazutoshi S, Hiromitsu K. "Antibody Response and Protective Immunity of Chickens Vaccinated with Booster Dose of Recombinant Oil-Adjuvanted Leucocytozoon caulleryi Subunit Vaccine, Avian Dis. 2014;58(4):609–15.