

ADDITION OF ANTI- *Toxoplasma gondii* MEMBRANE IMMUNOGLOBULIN Y TO REDUCE NECROTIC INDEX IN MICE'S LIVER

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**Abstract**

**Background** The study aims to determine the effect of administering anti-*T. gondii* membrane IgY against liver damage (Necrotic index) and the effectiveness of the antibody's delivery time.

**Materials and Methods** This research was a laboratory experiment with five treatments and five replications. Each treatment used female mice (*Mus musculus*) as animal models. The treatment groups consisted of a P0 group (not infected), P1 group (infected), P2 group (anti- *T. gondii* membrane IgY given one day before infection), P3 group (anti-*T. gondii* membrane IgY given together with infection) and P4 group (anti-*T. gondii* membrane IgY given two days after the infection). A dose of anti- *T. gondii* membrane IgY as many as 75 ug/head and infectious dose of 10 tachyzoites/head were given. Four days after infection mice were sacrificed and examined. Finally, necrotic index in histopathological liver using Hematoxylin Eosin.

**Results** The percentage of necrotic index liver showed that result treatment of P2 and P3 treatment that lower than another treatment.

**Conclusion** Thus, it can be concluded that administration of anti-*T. gondii* membrane IgY can reduce liver cell necrotic index and it was greatest when given before and simultaneously with infection.

**Key words:** *Toxoplasma gondii*, immunoglobulin Y, liver damage.

**Introduction**

The negative impact of *Toxoplasma gondii* infection in human is very detrimental particularly related to failure the pregnancy. It can cause several problems to a fetus such as abortion, stillborn (stillbirth), neo-natal infant mortality (mortality), weak born, congenital abnormality of mental retardation, eyes abnormalities which range from mild to blindness and hydrocephalus (Suwanti, 2005). Acute infection of *Toxoplasma gondii* can attack tissue and artificial infection by intraperitoneal causes necrosis in liver, spleen, and pancreatic in mice (Riganti *et al.*, 2003). In an experimental infection *T. gondii* strains RH in mice (*Mus musculus*) and leads to tissue damage which mostly liver damage (Mordue *et al.*, 2001). The liver damage is related to the apoptosis and necrosis liver cells (Mordue *et al.*, 2001). Liver damage caused by *Toxoplasma gondii* infection leads to mice mortality.

The control of Toxoplasmosis which includes prevention and treatment has been considered ineffective so far (Hokelek, 2003). Treatment with pyrimethamine and sulfadiazine could inhibit the synthesis of folic acid which is necessary for parasite replication. The immunization with protein ESA antigenic can generate an immune response but still unable to provide protection since dead mice is being still observed at the 8<sup>th</sup> day (Mufasirin, 2013). Thus treatment and prevention still need to be evaluated.

The use of immunoglobulins Y (IgY) as a passive immunization in some diseases have been investigated. The antibodies produce anti- protein membrane *T. gondii* (Praptiwi, 2012). Immunoglobulin Y can bind membrane proteins with molecular weight at approximately 30-35 kDa. The IgY can reduce placenta damage at mice infected by *T.gondii* (Suwanti *et al.*, 2011). Immunoglobulin Y anti-ESA also can reduce the apoptosis index of trophoblast in mice infected by tachyzoite stadium of *T.gondii* (Fajarwati, 2013). Therefore based on those findings, it is imperative to conduct a research focusing on the use of Immunoglobulin anti-membrane to know if it can reduce liver damage caused by *Toxoplasma gondii* infection.

Given anti-membrane, IgY is bounded to P30 (SAG-1) protein of tachyzoite. Those proteins serve as binding molecules during the invasion of *T.gondii* to host cell (Praptiwi, 2012). The bond formed between antibody and P30 (SAG-1) protein will obstruct tachyzoite to be bounded to the host cells; thus it may thwart the infection. Infected cells stimulate overproduction of pro-inflammatory cytokines; however, to due the bond formed between two molecules, it does not occur.

*Toxoplasma gondii* infection can stimulate immunological reaction reaction, such as excessive release of cytokines like including IFN  $\gamma$ , IL-18 and TNF  $\alpha$  (Mordue et al., 2001). Over induced cytokines cause liver cell damage, including necrosis. Mice infected by *T.gondii* oocyst suffer from liver necrosis (Sasmita, 2006).

This condition is caused by the overproduction of pro-inflammatory cytokines (Mordue et al, 2001). Liver damage during *T.gondii* infection is caused by over-expression of cytokines, n-amely IFN  $\gamma$ , IL-12, and TNF  $\alpha$ . High level of IFN  $\gamma$  induced by *T.gondii* happen at the initial stage of infection (Denkers and Gazzinelli, 1998). This interferon is produced by NK, CTL, and Th1. The presence of IFN- $\gamma$  gives signal to macrophage to produce TNF- $\alpha$ , and NO (Denkers and Gazzinelli, 1998; Warea, 2008). Accumulated-NO becomes toxic for those kinds of cells (Liesenfeld *et al.*, 1999) and can lead to cells necrosis. IL-2 expressed by Th1 activates CTL and NK subsequently produces Fas-Ligan (Malhi et al., 2006). The decrease of TNF  $\alpha$  will lower necrosis.

## Materials and Methods

Female mice (gestation age of 9.5 days) were infected with a *Toxoplasma gondii* strained in RH stage with a dose of 10 tachyzoite per mouse in 200  $\mu$ l of physiological NaCl. Infection is done intraperitoneally. Parent mice are otherwise infected with *Toxoplasma gondii* when the tachyzoite stage is present in intraperitoneal fluid within 4 days after infection. Antibodies of Ig Y are in the yolk. Combination of chloroform and precipitation with ammonium sulfate is a preferred method to produce purest of antibodies. Comparison between egg yolks with PBS at 7.2 pH and its suspension are incubated for 30 minutes at room temperature and occasionally shaken. Then 3000 rpm centrifugation for 15-minutes supernatant is taken. Animal models in this research were 25 female mice who were 2-3 months old BALB/C-strained, weight 20-25 grams, and mated with 25 male mice of 4-5 months (weight 30-35 grams) in a monogamous relationship. Pregnant mice were divided into 5 treatment groups which consisted of 5 mice in each group. The treatment groups consisted of a P0 group (not infected), P1 group (infected), and P2 group (anti-membrane *T. gondii* IgY is given at one day before infection), P3 group (anti-membrane *T. gondii* IgY is given together with infection) and P4 group (anti-membrane *T. gondii* IgY is given two days after infection).

The infections doses are 10 tachyzoite (Mufasirin, 2011) for each mouse that was diluted of 200  $\mu$ l physiological NaCl and given intraperitoneal injection. The infection was performed simultaneously for all groups at 9.5 days of pregnancy except for P0. Addition IgY of anti-ESA *T.gondii* was 75  $\mu$ g/mice and given orally. Four days after infection, mice were sacrificed and tachyzoite in intraperitoneal liquid was examined.

**Table 1: Grouping of Experimental Treatment**

Treatment	Information
P0	Non-infected
P1	Infected
P2	anti-membrane <i>T. gondii</i> IgY is given at one day before infection
P3	anti-membrane <i>T. gondii</i> IgY is given together with infection
P4	anti-membrane <i>T. gondii</i> IgY is given two days after infection

Histological testing for liver cell was kept inside 10% formalin buffer which subsequently undergo hispatological step by using HE (Hematoxylin Eosin). Ethical considerations using the health and ethics committee, Animal Care and Use Committee (ACUC) of the faculty of Veterinary Medicine Airlangga University approved this study.

## Results

The results of native intraperitoneal liquid method indicated that all mice for group P1, P2, P3 and P4 which were infected with tachyzoite *T.gondii* at 9.5 days age of pregnancy shows positive infection. The picture of tachyzoite from intraperitoneal liquid is presented in (figure 1).

Necrotic index is defined as the average number of liver cells which undergo necrosis among total cells. The numbers of necrotic cells in 6 field of observation were calculated with 400x magnification. The result showed that addition of IgY anti-membrane *T.gondii* can reduce necrotic index liver cell, since the percentage of administered IgY anti-membrane *T. gondii* was lower than positive control. Negative control group (P0) was different from P1, P2, P3 and P4. While P2 was different from P4 and control (P1), yet but not different with P3. P3 was different from P4, P0 and P1; yet, no different from P2. P4 was different from P0, P1, P2, and P3.

## Discussion

Necrotic index was the highest at P1 and the lowest at P0. It implies that *T.gondii* infection can cause necrosis in liver. This result is in line with previous researches which showed that tachyzoite infection strain RH can cause necrosis in liver cells (Mordue et al., 2001; Sukthana et al., 2003). Mice infected with oocyst *T.gondii* also caused necrosis in liver (Sasmita, 2006). The necrosis in liver by tachyzoite *T.gondii* infection was caused by the overproduction of pro-inflammatory cytokines (Mordue et al., 2001).

The production of pro-inflammatory cytokine can cause necrosis by stimulating macrophages to produce TNF- $\alpha$ . Necrosis index of a group IgY anti-membrane (P2, P3, and P4) index showed the decrease in necrosis compared to P1 group. Thus, it indicates that IgY can suppress necrosis of liver cell. The decline of necrotic index may be caused by the ability of IgY anti-membrane to bind SAG-1 (P30) membrane protein of tachyzoite which is known to influence the attachment process during an invasion into host cells. The protein is SAG-1 (P30) membrane which participates in the binding step during tachyzoite invasion to host cells (Praptiwi, 2012). Therefore tachyzoite which cannot be bound to the host cells prevent the immunological reaction cause necrosis.

The obtained result illustrated that IgY can lower liver necrosis (Takano et al., 2010; Zhen et al., 2011). IgY anti-*Escherichia coli* O111 is able to suppress the necrosis in liver by inhibiting the production of TNF- $\alpha$  by IgY (Zhen et al., 2011). TNF- $\alpha$  is the inflammatory cytokines serving as stimulant of necrosis (Mordue et al., 2001). The decline of TNF- $\alpha$  production leads a decrease cells necrosis.

Among the treatment groups, the lowest necrotic index is P2. It implies that the addition of IgY anti-membrane *T.gondii* before infection is the most effective way. This could be caused IgY bond of anti-membrane to protein SAG-1 (P30) tachyzoite; thus, it cannot be bounded to the host before tachyzoite reached its target. In addition, presenting IgY anti-membrane before infection will help opsonization, so it can increase phagocytosis process resulting in an inhibition of infection.

There is no significant difference between P2 and P3. However, a distinct result was shown by P4. This may be due to the administered period of IgY anti-membrane was not far from the fourth treatment-4. Necrotic index of P4 was higher compared to the rest. It may be caused by tachyzoite which reached its target before forming bound with IgY anti membrane. Tachyzoite could reach its target cells four days post-infection (Suebeki, 2006). The provision of IgY anti-membrane along with and after infection is less effective, because time needed for invasion is faster than time it takes for phagocytosis by macrophages. Tachyzoite's entry into its target cells requires 15-30 seconds, while phagocytosis by phagocytic cells only takes 2-4 minutes (Subekti, 2006).

## Conclusion

Based on the obtained results, it can be concluded that the addition of IgY anti-membrane *T.gondii* can reduce necrotic index in mice liver cells. Regarding administration period, the most effective result is achieved before infection. Therefore, it can be said that IgY anti-membrane is a promising candidate to be developed as a molecule to prevent Toxoplasmosis.

**Conflicts of Interest:** The authors declare that there are no conflicts of interest regarding the publication of this paper.

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