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Effect of Toxoplasma Infection Dosage on IgG, IgM, Fetus Weight and Body Length, and Necrosis of Placenta and Fetal Heart

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ABSTRACT

One of the most prevalent zoonotic illnesses in the world, toxoplasmosis, affects both humans and animals and is caused by the parasite *Toxoplasma gondii*. The infection will trigger the immune system to increase antibody production. This study aims to determine the dose of toxoplasma that causes necrosis in rats placental and fetal hearts. This study was laboratory experimental research with a Randomized Control Trial (RCT). The study design used a post-test only with a control group design. The Ig G variable obtained from the control group (CG) and treatment group 3 (TG3) is the most significant because the mean value difference was the largest (176.56). The Ig M variable obtained from the control group with treatment group 3 (TG3) is the most significant because the mean value difference is the largest (33.47). The fetus weight variable obtained from the control group with treatment group 3 (TG3) is the most significant because the mean value difference is the largest (2.6). The body length variable obtained from the control group between treatment group 3 (TG3) is the most significant because the mean value difference is the largest (1.26). There was a significant difference in placental tissue necrosis (p = 0.034) and heart (p = 0.025) between the control group (CG) and treatment group 3 (TG3). Therefore, there was a significant difference in Ig G, Ig M, fetus weight, body length, placental tissue necrosis, and fetal heart at the 10^3 dose compared to the 10^2, 10^1, and normal doses.

1. Introduction

The parasite that causes toxoplasmosis, one of the most prevalent zoonotic illnesses that can affect both people and animals, is *Toxoplasma gondii.* (Abbas *et al.* 2019). There are large regional and national differences in the prevalence of toxoplasma infection. Certain regions—North America, Southeast Asia, Northern Europe, and the African Sahel countries-have low seroprevalence (10–30%).

Latin America and Africa's tropical nations have significant incidence, but Central and Southern European nations have moderate prevalence. (Robert-Gangneux and Dardé 2012). Infection with Toxoplasma is present in over 60% of certain populations in different parts of the world (Atthias *et al.* 2020). Because the oocyst survives better in hot, humid climates and lower altitudes, toxoplasmosis infection is frequently highest in certain regions of the world (Khan *et al.* 2017).

The toxoplasmosis parasite typically infects humans when they consume tissue cysts found in raw or undercooked meat or water tainted with oocysts. (Thai *et al.* 2019). There are two ways in which toxoplasmosis can spread: horizontally through the intake of raw vegetables, tainted milk, and undercooked meat, and vertically through the placenta from mother to fetus (Retmanasari *et al.* 2017).

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Toxoplasma gondii can infect in several ways; if infection happens in early pregnancy, it can cause congenital abnormalities, fetal death, abortion, stillbirth, or premature birth (McAuley 2014; Chen *et al.* 2017). With increasing gestational age, the rate of transmission from mother to child rises, beginning at 15% at 13 weeks gestation, 44% at 26 weeks gestation, and 71% at 36 weeks gestation (Thiébaut *et al.* 2007; Kieffer and Wallon 2013; Curcio *et al.* 2020). *T. gondii* infection will induce increased antibody production. Examination of antibodies to immunoglobulin G and immunoglobulin M (IgG, IgM) can be used to establish the diagnosis. Finding toxoplasma-specific IgG and IgM antibodies is necessary for the diagnosis of acute toxoplasmosis infection (Simanjuntak *et al.* 2019). An infection with *T. gondii* can cause oxidative stress, which can limit trophoblast invasion, decrease angiogenesis, cause endothelial dysfunction, and result in acute atherosclerosis (Jarvie *et al.* 2010; Dincel and Atmaca 2016).

2. Materials and Methods

A Randomized Control Trial is employed in this experimental laboratory investigation (RCT). The sole study design that was used was a post-test with a control group. Eight-week-old female Rattus novergicus rats weighing 200–250 grams were used in this investigation. First, for a week, all of the chosen rats were housed in the University Center Food and Nutrition's animal laboratory at Gadjah Mada University in Yogyakarta after being carefully adapted from a reliable breeder. Rats with bright eyes, fur, active movement, and a voracious appetite were provided with standard BR II food through water freely (at libitum), all in a uniform cage environment. Rats that were ill, pregnant, or that passed away throughout the course of treatment were excluded. The Center for Veterinary Research Bogor provided the *T. gondii* isolates that were used with the RH strain. The Faculty of Medicine, Universitas Sebelas Maret, has concluded in letter No. 56/UN27.06.6.1/KEP/EC/2021 that this study is ethically suitable and viable.

In this study, female rats that had gone through the pregnancy process were divided into four groups: one control group which was not given toxoplasma isolate, and the other three groups were given different doses of toxoplasma isolate, which are 10^1, 10^2, and 10^3 tachyzoites. Doses of toxoplasma with 10^4 had high levels of inflammation. Each group consisted of four pregnant rats. Toxoplasma isolate was administered intraperitoneally with a volume of 2 ml on the 12th day of pregnancy (Dubey *et al.* 1997). Then, the IgG and IgM levels were measured through rat blood samples on the 16th day of gestation. On the same day, pregnancy termination was carried out, followed by measurement of fetal body weight and length, as well as sampling of placental tissue and heart tissue.

Placental and cardiac tissue samples were then processed for histopathological preparation. Hematoxylin-Eosin staining was used to make histopathological preparations at Sebelas Maret University's Laboratory of Anatomical Pathology. The percentage of tissue necrosis in placental and cardiac tissue was assessed based on microscopic observations of histopathological preparations using four times magnification in five randomized visual fields.

The One-way ANOVA test was used for statistical analysis on data with homogeneous variance and a normal distribution. Data that were not normally distributed or not homogeneously varied were analyzed by the Kruskal-Wallis test. Data with significant differences for each treatment were then further tested with the Tukey test with a 95% confidence degree ($\alpha = 0.05$). Data that did not differ significantly were not tested further with multiple comparison tests. Data analysis was done using SPSS 18 software for Windows.

3. Results

The subjects in this study were sixteen female rats that went through pregnancy and induction with toxoplasma isolates on the 12th day of gestation. In this study, the subjects were split up into four groups, each of which received the same number of samples. Different from the control group, each group was given toxoplasma induction with different doses. Random selection was used to assign the rats to each group.

The variables IgG, IgM, fetal body weight, and length in each group had a P value >0.05 based on the normality test, indicating that the study's data was normally distributed.

Based on Table 1 ANOVA test on the IgG control group (CG) variable, the mean value of 16.08±0.71, treatment group 1 (TG1) was 104.91±2.72, treatment

group 2 (TG2) was 182.42±1.44 and Treatment 3 (TG3) was 192.64±3.99 with p-value = 0.000 <0.05, which means that there was a significant difference between the four Ig G treatments.

In the control group (CG) IgM variable, the mean value was 1.99±10.00, treatment group 1 (TG1) was 17.91±0.33, treatment group 2 (TG2) was 29.16±1.48 and treatment group 3 (TG3) was 35.46±0.37 with p-value = 0.000 <0.05 which means there is a significant difference between the four Ig M treatments.

Based on Table 2 of the control group (CG) fetal body weight variables, the mean value of 4.78±0.43 treatment group 1 (TG1) was 2.34±0.70, treatment group 2 (TG2) was 2.20±0.09 and treatment group 3 (TG3) of 2.18±0.08 with a value of p = 0.000 <0.05 which means there is a significant difference between the four fetal body weights treatments.

In the control group (CG) fetal body lengths variable, the mean value was 3.70±0.32, treatment group 1 (TG1) was 2.55±0.20, treatment group 2 (TG2) was 2.45±0.04 and treatment group 3 (TG3) of 2.43±0.05 with p value = 0.000 <0.05 which means that there is a significant difference between the four fetal body lengths treatments.

Based on Table 3 which shows a post hoc test of the IgG variable, the strongest significance difference was found between control group (CG) with treatment group 3 (TG3) as it had the largest Mean Difference value (176,563) compared to the control group (CG) with treatment group 1 (88,835) and the treatment group 2 (166.348).

Next, data from post hoc test of the IgM variable, the strongest significance difference was found between control group (CG) and group 3 (TG3) as it had the largest Mean Difference value (33.470) compared to the control group with treatment group 1 (15.920) and treatment group 2 (27.168).

Post hoc test of the fetal body weights variable, the strongest significance difference was found between the control group with treatment group 3 (TG3) as it had the largest Mean Difference value (2,600) compared to the control group with treatment group 1 (2,448) and treatment group 2 (2,580).

Post hoc test of the fetal body lengths variable, the strongest significance difference was found between the control group with treatment group 3 because it had the largest Mean Difference value (1,268) compared to the control group with treatment group 1 (1,155) and treatment group 2 (1,253).

The data in Table 4 shows a significant difference in placental tissue necrosis (p = 0.034) and heart tissue necrosis (p = 0.025) between the control group (CG) and treatment group 3 (TG3).

On histopathological observation of normal placenta and not induced by toxoplasma isolate, normal placental tissue was found with necrotic focus of less than 5% (Figure 1A). In the histopathological observation of the placenta induced by toxoplasma at a dose of 10^1, the placental tissue was found to be normal with less than 20% necrotic focus (Figure 1B). On the histopathological observation of the placenta induced by toxoplasma at a dose of 10^2, normal placental tissue was found with necrotic focus of less than 50% (Figure 1C). On the histopathological observation of the placenta induced by toxoplasma at a dose of 10^3, normal placental tissue was found with necrotic focus of less than 75% and the placenta appeared atrophic (Figure 1D).

On histopathological observation of a normal fetal heart and not induced by toxoplasma isolates, the heart with normal ventricular walls was observed (Figure 2A). While in fetal heart tissue induced by toxoplasma at a dose of 10^1, the atrial wall (a) appeared slightly widened and thinned while the ventricular wall (v) was relatively normal (Figure 2B). In the heart tissue induced by toxoplasma at a dose of 10^2, the atrial wall

Table 1. Results of one way ANOVA test on rat IgG and IgM levels after toxoplasma induction

	Mean±SD				P value
	CG	TG1	TG2	TG3	
Ig G (ng/ml)	16.08±0.71	104.91±2.72	182.42±1.44	192.64±3.99	<0.001**
IgM (ng/ml)	1.99±10.00	17.91±0.33	29.16±1.48	35.46±0.37	<0.001**

*signifikan pada α < 0.05, **signifikan pada α < 0.001

Table 2. Results of one way ANOVA test on fetal body weight and length

	Mean±SD				P value
	CG	TG1	TG2	TG3	
Fetal body weights (gram)	4.78±0.43	2.34±0.70	2.20±0.09	2.18±0.08	<0.001**
Fetal body lengths (cm)	3.70±0.32	2.55±0.20	2.45±0.04	2.43±0.05	<0.001**

Table 3. Results of tukey post-hoc analysis on rats IgG and IgM levels after toxoplasma induction and fetal body weight and length

Dependen variable	Group		Mean difference	Sig.	95% confidence interval	
					Lower bound	Upper bound
IgG (ng/ml)	CG	TG1	88.835*	0.000	92,756	84,914
		TG2	16.348*	0.000	170,269	162,426
		TG3	176.563*	0.000	180,484	172,641
	TG1	TG2	77.513*	0.000	81,434	73,591
		TG3	87.728*	0.000	91,649	83,806
	TG2	TG3	10.215*	0.000	14,136	6,294
IgM (ng/ml)	CG	TG1	15.920*	0.000	17,121	14,719
		TG2	27.168*	0.000	28,369	25,966
		TG3	33.470*	0.000	34,671	32,269
	TG1	TG2	11.248*	0.000	12,449	10,046
		TG3	17.550*	0.000	18,751	16,349
	TG2	TG3	6.303*	0.000	7,504	5,101
Fetal body weights (gram)	CG	TG1	2.448*	0.000	2,097	2,798
		TG2	2.580*	0.000	2,230	2,930
		TG3	2.600*	0.000	2,250	2,950
	TG1	TG2	0.133	0.426	0.218	0.483
		TG3	0.153	0.362	0.198	0.503
	TG2	TG1	0.133	0.426	0.483	0.218
		TG3	0.020	0.903	0.330	0.370
Fetal body lengths (cm)	CG	TG1	1.155*	0.000	0.863	1.447
		TG2	1.253*	0.000	0.960	1.545
		TG3	1.268*	0.000	0.975	1.560
	TG1	TG2	0.098	0.481	0.195	0.390
		TG3	0.113	0.418	0.180	0.405
	TG2	TG3	0.015	0.913	0.277	0.307

Table 4. Results of kruskal-wallis analysis on rats placenta and heart necrosis

	Mean±SD		P value
	CG	TG3	
Placenta	31.25±22.5	68.75±12.5	0.034*
Heart	1.25±2.5	32.5±21.79	0.025*

(a) appeared dilated and necrotic while the ventricular wall (v) was relatively normal (Figure 2C). In the cardiac tissue induced by toxoplasma at a dose of 10^3, the atrial wall (a) appeared dilated and necrotic, while the ventricular wall (v) was thickened and appeared atrophic (Figure 2D).

4. Discussion

In determining the diagnosis of toxoplasmosis, there are several tests that can be carried out including serological tests, histological examination and isolation of *T. gondii* (Yuliawati and Nasronudin 2015). IgM can develop right away after being infected with *T. gondii* and go away in a few months, but IgG can occur in the initial one to two weeks of infection and last for years or even life. The serological tests and the methodology utilized determine the sensitivity and specificity of the tests. A study conducted and compared 6 test studies using the IgM ELISA showed that the sensitivity was between 93-100% and specificity was 99.1% 77.5 (Yuliawati and Nasronudin 2015). The combination of IgG and IgM ELISA tests is most often used (Halonen and Weiss 2013).Infectious disorders in humans and animals, including toxoplasmosis, are commonly diagnosed using the well-known serological diagnostic technique known as ELISA (Enzyme Linked Immunosorbent Assay) (Subekti and Kusumaningtyas 2011).

Rats are more resistant than mice to *T. gondii* although infection depends on the strain of the animal, individual immunity, method of infection, and the stage and strain of *T. gondii* infected (Hartati *et al.* 2007). These rodents also have short maturity and high adaptation from the biological and morphological levels to survive on land and water (Khademvatan *et al.* 2017).

Filisetti and Candolfi (2004) found that in the humoral immune response. The first component of

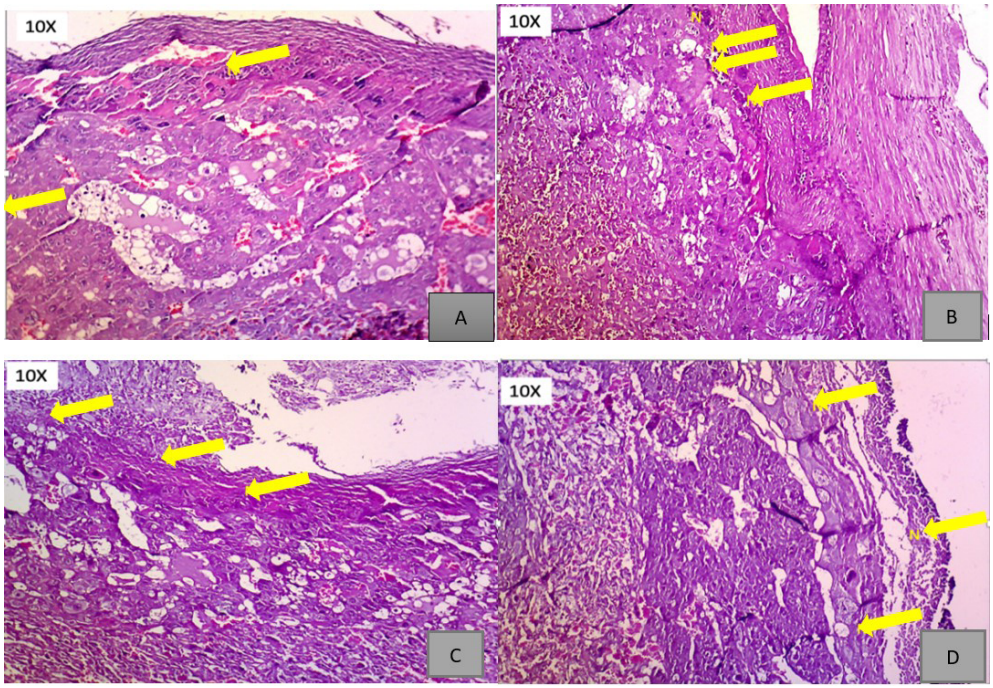

Figure 1. Histopathological preparations of placental tissue necrosis with 4x microscope magnification: (A) control group placental tissue (without toxoplasma induction), (B) placental tissue group 1 (toxoplasma induction dose of 10^1), (C) placental tissue group 2 (toxoplasma induction dose of 10^2), (D) placental tissue group 3 (toxoplasma induction dose of 10^3) (yellow arrows indicate areas of necrosis)

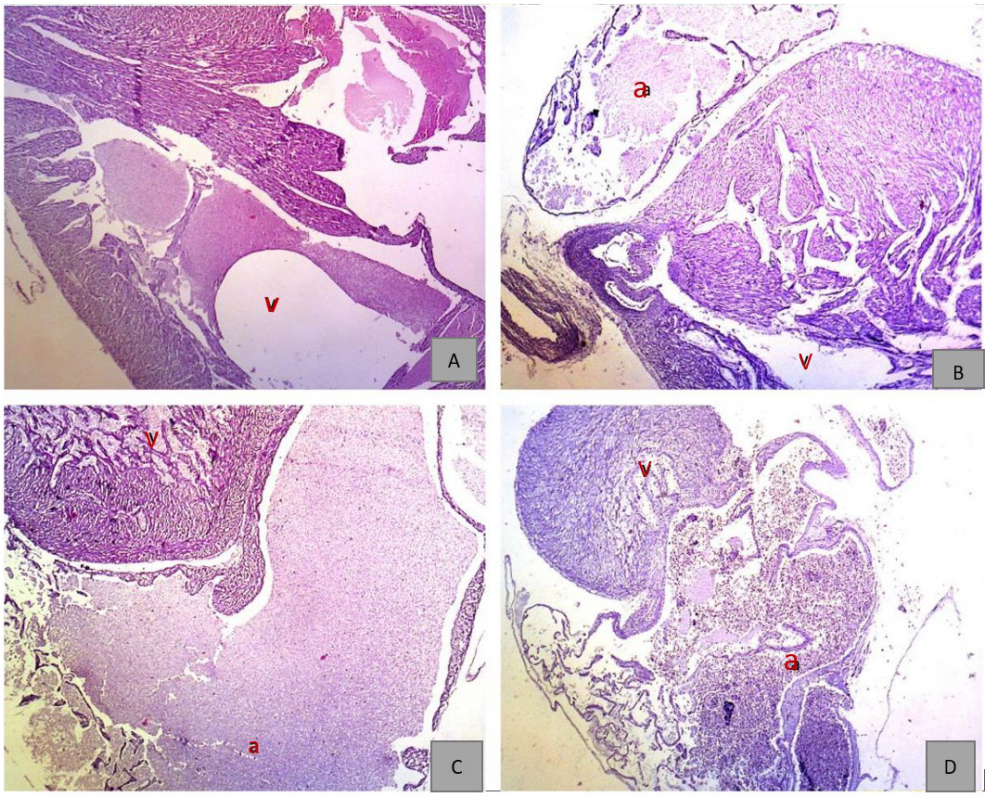

Figure 2. Histopathological observations of fetal heart tissue necrosis with 4x microscope magnification: (A) control group heart tissue (without toxoplasma induction), (B) heart tissue group 1 (toxoplasma induction dose of 10^1 tachyzoites), (C) cardiac tissue group 2 (Toxoplasma induction dose of 10^2 tachyzoites), (D) group 3 heart tissue (Toxoplasma induction dose of 10^3 tachyzoites) (a: atrium, v: ventricle)

an antibody to develop in a toxoplasma infection is IgM, which is seen in the peritoneal fluid two days after infection. While in serum, When the first week of infection is over, IgM can be detected. This goes accordingly with the findings in this study, which on the examination of rat serum on the 7th day after injection of toxoplasma isolate in rats, it was found that there was a significant increase in IgM levels ($p<0.001$). The highest rate of congenital infection in rat fetuses was found in rats inoculated with 10,000 strains of toxoplasma oocysts on the 12th day of gestation (Dubey *et al.* 1997).

This study revealed that treatment group 3 (TG3) had significantly greater body weight and length than the control group (CG). According to Hurt *et al.* 2022, When compared to toxoplasma-negative women, toxoplasmosis exhibits a statistically significant correlation with a lower birth weight and premature birth rate. The odds of preterm delivery and low birth weight were found to be 1.7 and 1.9 times higher, respectively.

Histopathological analysis revealed macrophage infiltration in necrosis areas in the Central Nervous System (CNS), lungs, liver, heart, and muscles. Toxoplasmosis can cause pathological changes in a variety of organs, including the brain, neurons, microglia, liver parenchyma, heart, skeletal muscle, fetal membranes, and leukocytes (Rina *et al.* 2021). The multinucleated placental syncytiotrophoblast, which makes up the placenta's outer layer and comes into direct contact with maternal blood during two stages of attachment and intracellular replication, is infected by *T. gondii* infection (Ander *et al.* 2018).

When *T. gondii* infection affects the heart, the main complication is myocarditis, which is characterized by an influx of inflammatory cells and either myocyte necrosis or not. Cardiac tissue damage depends on the intensity of the inflammatory response and *T. gondii* tachyzoites in myocytes (Zhou *et al.* 2021).

Placental tissue's necrosis was found in several previous studies associated with *T. gondii* infection. In a sheep model that underwent abortion in the acute phase (abortion on days 7 to 14) after toxoplasma infection, thrombosis and infarct necrosis of the placental tissue was found. This was caused by vascular damage to the placental tissue. However, the cause of vascular damage in sheep placental tissue that causes thrombosis and necrosis of infarction is not known with certainty (Castano *et al.* 2014). Several hypotheses concerning the pathophysiology have been proposed, such as the idea that a *T. gondii* infection causes the mother's body temperature to rise (Owen *et al.* 1998) and an increase in IFN-γ secretion, which then induces trophoblasts to secrete fibrinogen-like protein 2 (fgl2), which is a prothrombotic factor (Castano *et al.* 2014).

Increased IFN-γ due to *T. gondii* infection was found in sheep (D Verhelst *et al.* 2015) and rats (Ikeda Rina *et al.* 2021). This is consistent with this study's results, which showed a significant difference ($p = 0.034$) in the percentage of necrosis area in the placental tissue of rats induced by toxoplasma isolates compared to the control group.

In conclusion, there was a significant difference in Ig G, Ig M, fetus weight, body length, placental tissue necrosis, and fetal heart at the 10^3 dose compared to the 10^2, 10^1, and normal doses.

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