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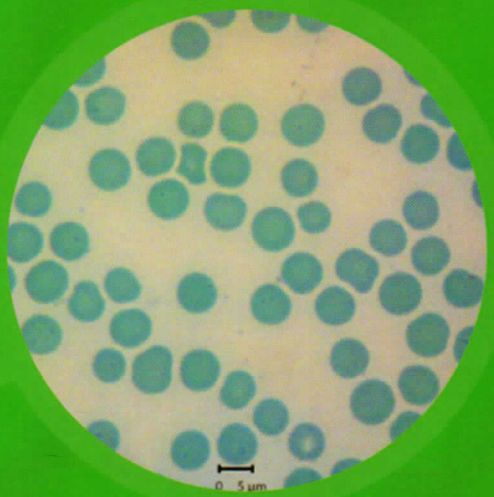

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Redaksi, penulis dan pembaca Journal of Parasite Science memberikan penghargaan dan terimakasih yang setinggi-tingginya kepada para pakar di bawah ini, selaku mitra bestari yang telah menelaah semua tulisan baik yang dimuat maupun yang ditolak sesuai rekomendasi yang disampaikan pada redaksi dalam Volume 3 No. 1, edisi Maret 2019

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DAFTAR ISI

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The Effect of Folic Acid as Supportive Therapy of Spiramycine on Weight of Foetus to *Toxoplasma gondii* – Infected Pregnant Mice (Mus Musculus)

Pengaruh Asam Folat Sebagai Terapi Pendukung Spiramycine pada Berat Janin terhadap *Toxoplasma gondii* - Tikus Hamil yang Terinfeksi (Mus Musculus)

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Abstract

This research aimed to investigate the effect of folic acid as supportive therapy of spiramycine on weight of foetus to *Toxoplasma gondii*-infected pregnant mice (*Mus musculus*). Twenty pregnant female mice were divided into four groups as C -, C +, T1 and T2. C +, T1 and T2 were infected by *Toxoplasma gondii*. C – and C + administered orally 0.5 ml aquadest, T1 administered orally 130 mg/kg BW spiramycine and 0.052 µg/g BW folic acid and T2 administered orally 0.052 µg/g BW folic acid. Experimental groups received the treatments for 5 days, then animals of each groups were sacrified. Foetuses were dissected out for observation. The weight of fetuses were measured using an analytical balance. The data weight of foetuses was presented descriptively and analyzed by ANOVA test and continued by Tukey HSD. From this study, the weight of foetuses from the pregnant mice of T1 and T2 have difference compared with the controls. The result of this research is folic acid affects the weight of foetuses to *Toxoplasma gondii*-infected pregnant mice.

Keyword : folic acid, *Toxoplasma gondii*, *Mus musculus*, foetus

Introduction

Toxoplasma gondii is an obligate intracellular protozoan which could live in all cell's host. *Toxoplasma gondii* has definitive host is family Felidae, while human and warm-blooded animal are intermediate host (Suwanti *et al*., 2012). Toxoplasmosis as zoonotic diseases that could cause problems for human and animal, especially at pregnancy. This infection could cause disruption of female reproduction process and result in fetal birth defects, blindness, premature birth, stillbirth, and abortion. Primary infections that occur in the first trimester of pregnancy causes the foetus to become infected transplacentally and suffer congenital abnormalities, this could lead premature birth to abortion (Bayat *et al*., 2013). The primary infected of pregnant women, parasitemia phase will occur first, then the maternal blood entering the placenta will infect the placenta (placentitis). Parasitic infections could be transmitted to the foetus vertically. Tachyzoite will proliferate and produce necrotic foci that cause placental necrosis and surrounding tissues, thus endangering the foetus where pregnancy expulsion or abortion may occur (Suparman, 2012). *Toxoplasma gondii* which reaches the foetus could inhibit nutrient intake, especially folic acid and amino acids from parent to child so that could be a factor causing low birth weight mice foetus.

Spiramycine is a macrolide antibiotic which is not readily cross the placenta, and therefore is not reliable for treatment of fetal infection. Spiramycine is aimed at preventing vertical transmission of the parasite to the foetus, and it is indicated only before fetal infection (Janvier, 2013).

Toxoplasma gondii, as an intracellular parasite, will infect cells, multiply, exit the host cell, then infect new cells. This process could cause cell death and tissue damage. This is due to the speed of replication of tachyzoite faster

than the ability of mitotic cells (Lavine and Arrizabalaga, 2008). Mitotic cells is one phase of the cell cycle. In the cell cycle, before entering the mitotic phase, DNA replication is needed. DNA is formed from 4 nucleotides namely adenine, thymidine, guanine, and cytosine (Alberts *et al*., 2002).

According to Talaulikar and Arulkumaran (2013), body cells use folic acid to synthesize thymidine, adenine, and guanine which needed to assemble DNA in a cell. Folic acid is a nutrition which is needed for mother in fertile period, pregnancy, prevent pregnant disruption, and abnormalities on foetus (Martinussen *et al*., 2015).

Research Materials and Method

Materials and Equipments

The materials that used in this study were male and female mice, BALB/c strain, which is 3-4 months old, healthy, puberty, and not in pregnant condition, isolate *T. gondii* RH strain tachyzoite stage obtained from the Department of Veterinary Parasitology, Faculty of Veterinary, Universitas Airlangga, folic acid, spiramycine, sterile aquadest, physiological NaCl, 70% alcohol, mice feed number 594, and ad libitum drinking water.

The equipments used in this research were cages made of plastic tub covered by wires, water container, syringe sonde spuit, hemositometer, 1 ml spuit, 5 ml spuit, gloves, surgical masks, cover glasses, object glasses, microscope. For dissection preparation, the equipments used were sterile scalpel, sterile pincet, forceps, section set, petri dish, analytical balance, and sterile containers. For chemical preparation, the equiments used were erlenmeyer, mortar, pestle, watch glass, and analytical balance.

Trial Preparation

The 20 female mice used in this study are divided into 4 treatment groups and put into the cage, each treatment group is consisted of 5 mice with details for weight mice foetuses weighing. Prior to treatment, the mice were adapted for one week in order to adapt to the new environment. After one week, one male mice was added to one female mice in each group to facilitate mating. Mice usually mate at night. The next day, for pregnancy examination was looked at the vaginal plug in the vagina of mice, which means there was a copulation between female and male mice. If vaginal plug were found that means mice was 0.5 day pregnant (Suwanti, 2005). After one week, female mice were checked for pregnant condition. Mice were infected with *Toxoplasma gondii* at 12th day of pregnancy. Then, began treatments on 13th day of pregnancy.

Isolate Preparation

Preparation of *Toxoplasma gondii* isolates required 3 male mice. Isolates reproduced by injection to healthy mice through intraperitoneal with tachyzoites infections per mice. Mice that had been infected *Toxoplasma gondii* awaited for 24 - 48 hours or until the mice show symptoms of parasitemia with weak mice, lethargy, apathy, standing feathers, and quick breath. Then tachyzoites were harvested by mice sacrificed. Mice were section on the abdomen and then give 5 ml of physiological NaCl in the peritoneum cavity, as a medium of tachyzoites. Taken intraperitoneal fluid used a syringe until the peritoneal fluid was run out then calculated tachyzoites with the hemositometer improve Neubauer and used in the treatment (Mufasirin, 2011).

Treatments

In this study the research was used 20 female mice and divided into 4 groups and each treatment has 5 female mice. The treatments were began at 13th day of pregnancy. The four treatments were :

a. Control Negative Group

C - group : 5 mice treated with aquadest 0.5 ml / head / day to seen normal weight of mice foetuses

b. Control Positive Group

C + group : 5 mice infected tachyzoites of T. gondii with a dose of 5 through intraperitoneal and treated with aquadest 0.5 ml / head / day to seen changes weight of mice foetuses

c. First Treatment Group

T1 group : 5 mice infected tachyzoites of T. gondii with a dose of 5 through intraperitoneal and were treated with Spiramycine 130 mg / kg body weight / day and Folic Acid 0.052 µg / g body weight / day are given for 5 days to seen changes in weight of mice foetuses

d. Second Treatment Group

T2 group : 5 mice infected tachyzoites of T. gondii with a dose of 5 through intraperitoneal and were treated with Folic Acid 0.052 µg / g body weight / day are given for 5 days to seen changes in weight of mice fetuses.

Data Collection

Data collection was obtained during the treatment period up to a maximum of 5 days after treatment. Mice were sacrificed in the third trimester or on the eighteenth day of pregnancy in each group, then takes mice foetuses for the preparation of weighing. Made preparations were carried out at Protozoology Laboratory, Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga.

Observations of weight mice foetus were performed to measure the changes weight mice foetus of the T1 and T2 groups that compared with the preparations in the C - and C + groups used as controls. Observations of the preparations were used an analytical balance.

Data Analysis

The data were processed by statistic used One-Way Analysis of Variance test (ANOVA) and Tukey Honestly Significance Difference (HSD). The analysis was used the Statistic Package for the Social Science (SPSS) 23.0 program.

Result and Discussion

The examination result obtained from each treatment groups of C – (without infection of *T. gondii* and administration of 0.5 ml aquadest), C + (with infection of *T. gondii* and administration of 0.5 ml aquadest), T1 (with infection of *T. gondii*, administration of 130 mg/kg BW spiramycine and 0.052 µg/g BW folic acid) and T2 (with infection of *T. gondii* and administration of 0.052 µg/g BW folic acid). Experimental groups received the treatments for 5 days, then animals of each groups were sacrificed with dislocation os cervicalis. Foetuses were dissected out on abdomen for observation. The data of weight foetus were obtained from mice at 18th day of pregnancy. The weight of foetuses were measured using an analytical balance (Appendix 1).

The data obtained from each treatment group. After the foetuses were removed from the placenta and weighed, the foetuses of the five mice of each group were added up. The data were proceeded with Statistical Product and Service Solutions (SPSS) program using ANOVA, obtained significant less than 0.5% ($p<0.05$). Therefore, it continued to use Tukey HSD. The data used for SPSS were weight of foetuses from pregnant mice which has total same or similar foetuses. The results of data processing showed that T2 were significantly difference with C +, while not significantly difference with C -. Then, T1 not significantly difference with C +, while significantly difference with C -. The result of weight of foetuses from pregnant mice, shown in Table 1. below:

Table 1. Mean and Standard Deviation of Weight of Foetuses from Pregnant Mice

Treatments	Number of Pregnant Mice (head)	Number of Foetuses From Pregnant Mice (head)	Mean ± Standard Deviation of Weight Foetuses (g)
C –	5	49	$1.174^{c} \pm 0.107$
C +	3	29	$0.890^{a} \pm 0.258$
T1	5	46	$0.948^{ab} \pm 0.378$
T2	5	42	$1.115^{bc} \pm 0.196$

Superscript : a, b, c : different notations in same columns showed significantly difference 5% ($p<0.05$).

Conclusion

Based on the results of the research, it could be concluded that the addition of folic acid as supportive therapy of spiramycine affected in the weight of foetus to *Toxoplasma gondii*-infected pregnant mice. Folic acid could be maintain the weight of foetuses which were infected by *Toxoplasma gondii*.

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