

PROCEEDING



International Seminar A Role of The Veterinarian on The Global Health Challenges



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Universitas Gadjah Mada
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INFECTIOUS DOSE AND GESTATION AGE INFLUENCE ABORTION INCIDENCE IN MICE INFECTED WITH *TOXOPLASMA GONDII*

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ABSTRACT

Purpose of this study was to determine the effect of infection dose and gestation age on the incidence of abortion in mice infected with *Toxoplasma gondii* RH strain. The research was conducted in three steps. The first step, the treatment of infection was performed at 9.5 days gestations with doses of infection 10 tachyzoites of *T. gondii*. The second step, treatment of infection was performed at 16 days gestation with doses of infection 10 tachyzoites of *T. gondii*. The third step, the treatment of infection was performed at 16 days gestation with doses of infection 5 tachyzoites of *T. gondii*. Results showed that infection gestation at 9.5 days with a dose of 10 tachyzoites of *T. gondii* causes 50 percent of the parent having abortion on day 17 to day 18 followed by death. Treatment of infection was performed at 16 days gestation with doses of infection 10 tachyzoites of *T. gondii*, parent child-bearing mice were still with the placenta and treatment of infection at 16 days gestation with doses of infection 5 tachyzoites of *T. gondii* obtained all parent child-bearing. Thus on the first step, the second and third, all the parent dies 8 days after infection.

Key words: *Toxoplasma gondii*, abortion, TNF- α .

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*, invade the human and warm-blooded mammals, including birds. One-third of the human population is infected with *T. gondii* (1). The economic loss due to infectious diseases including toxoplasmosis was reported at 1.5 billion dollars USA. Socioeconomic disadvantage due to toxoplasmosis include substantial costs for the treatment of patients, mental disorders and blindness in children (2). In addition, toxoplasmosis causes reproductive disorders (infertility) and abortion especially in sheep causing great losses to the sheep industry in the world (3), the economic loss is in the form of lost lambs including abortion (4.5) and as a food contaminant (6). In human, toxoplasmosis can cause harm such as muscle disorder accompanied by symptoms of weight loss (7) and low birth weight (8). Effect of infection *T. gondii* infection in particular dose and

gestation of the abortion has not been widely reported.

Incidence of abortion in the parent with toxoplasmosis involves the immune system and fetal. One of the major proinflammatory cytokines that play a role in the abortion is tumor necrosis factor- α (TNF- α). TNF- α will affect the process of fetal development through impaired fetal growth and placental trophoblast constituent fetal development resulting in failure (9).

Infection of *T. gondii* in host immune responses evokes both humoral and cellular immune response (10). The ability of *T. gondii* arouse cellular immune response characterized by the release of Th1 response to interferon- γ (IFN- γ) produced by natural killer cells NK (11,12). The first infection, IFN- γ in this phase involves the innate immune system, NK cells and macrophages. NK cell will activate macrophages to produce TNF- α as microbicide (13). The chronic phase, T lymphocytes produce IFN- γ in large quantities. In the trophoblast, TNF- α binds to tumor necrosis factor receptor-1 (TNFR-1)

(14), and induces apoptosis that placental function as a channel disrupted the food giver. Changes in the microenvironment of the placenta due to infection *T. gondii* in decidua causes affected trophoblast apoptosis increased expression of IFN- γ (15). This study tried to find the effect of infection dose *T. gondii* and gestation against abortion in mice. We hope this research can provide information to the public so that control can be prevented toxoplasmosis especially in pregnant women.

MATERIALS AND METHODS

The samples were female mice Balb/C strain, 12 weeks, 20-30 gram obtained from the Faculty of Pharmacy, Universitas Airlangga. *T. gondii* RH strain isolate obtained from the Inter-University Centre, Universitas Gadjah Mada. PMSG hormone (Folligon, Batch No.. 2563304, Intervet International BV Boxmeer Holland) and HCG hormone (Chorulon, Batch. No. 2563304, Intervet International BV Boxmeer Holland). The study was conducted in Protozoology Laboratory, Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga. The procedure of the study include animal screening for toxoplasmosis by ELISA test, multiplication isolates *T. gondii*, mating mice, treatment involves determining gestational age and dose of infection and data analysis.

Thirty two female mice were administered with a combination of 5 IU PMSG and 5 IU HCG. Female mice were injected intraperitoneally with PMSG in 100 μ l physiological saline and after 48 hours, injected with HCG in 100 μ l of physiological saline in the same way. The mice were mated with males with a ratio of females to one male tail. The next day, the mice were examined for the presence of vaginal plug and if found positive mean 0.5 days pregnant mice (15). Mice were maintained until the expected gestational age. The study was conducted in three phases. Each phase of the study used 32 pregnant mice was divided into treatment group and control group. The first stage, the treatment of infection was performed at 9.5 days gestation with doses of infection 10

tachyzoites of *T. gondii* (16). The second phase, treatment of infection was performed at 16 days gestation with doses of infection 10 tachyzoites of *T. gondii*. The third phase, the treatment of infection was performed at 16 days gestation with doses of infection 5 tachyzoites of *T. gondii*. The control group was injected with physiological saline. Intraperitoneally infection conducted in 100 μ l physiological NaCl. Mice were maintained through gestation 21. The number of mice which had calculated, fetal aborted and contained a dead parent observed. The data are presented descriptively.

RESULT AND DISCUSSION

Observations at 9.5 days pregnant mice infected with *T. gondii* infection by as much as 10 tachyzoites dose, showed 100% infection treated mice on day 17 to day 18 showed symptoms of illness, 50% had abortion and death. Symptoms of abortion is characterized by blood around the vagina and obtained a number of fetal aborted on a pedestal enclosure. Parent on days 17 to 18 after surgery, intraperitoneal fluid and fetal examined. The result of the intraperitoneal fluid was found tachyzoites stage. The results showed all the parent dies before the child's birth. Fetus results of surgery have multiple abnormalities including smaller than normal size, larger than the head of the agency, maceration and decay. Observations on the 16 days pregnant mice were infected with 10 tachyzoites of *T. gondii* shows all the parents gave birth on the 19-20 days, the puppies born dead with multiple abnormalities, among others, there were still wrapping fetal membranes (placenta), shrinkage / drying and maceration. Observations on the infection of 16 days gestation dose infection 5 tachyzoites of *T. gondii*, the entire stem can be delivered on days 19-20 with a live birth. The entire stem of mice either the first stage, the second and the third died 8 days after infection.

The placenta is an organ that is sensitive to infection *T. gondii* in pregnant mice. The uterus and placenta susceptible to infection *T. gondii* associated role of IFN- γ and TNF- α . Infection of *T. gondii* in host immune

responses evokes both humoral and cellular immune responses. *Toxoplasma gondii* is able to evoke an immune response characterized by cellular Th1 released response with IFN- γ at the beginning of the infection involves the innate immune system, NK cells that produce IFN- γ and macrophages produce TNF- α and will activate this phase, NK cells are the main cells producing IFN- γ as microbicide. Macrophages produce TNF- α in the large quantities. In chronic phase, T lymphocytes produce IFN- γ . In the trophoblast, TNF- α binds to TNFR-1 and induces apoptosis that placental function as a channel disrupted the food giver. Placental inflammation in mice pregnant by a marked increase in decidual lymphocytes that at 1, 2 and 3 weeks produce IFN- γ and decidual macrophages that produce TNF- α pregnancy that affect changes in the microenvironment of the placenta (15). Impaired function of the placenta due to apoptosis of placental cells causes disruption of fetal development even in severe conditions can be aborted. The huge in causing abortion are role of cytokines IFN- γ and TNF- α is abortogenic (17). Another factor in its favor is *T. gondii* strain was used in the research included virulent strain can increase proinflammatory cytokines in high quantities. Previous studies compared the expression of proinflammatory cytokines due to *Toxoplasma gondii* RH strain and Beverley strain that are avirulent strain. *T. gondii* RH strain was able to induce an increase in IFN- γ and IL-12, TNF- α compared to the strain on limpha Beverley (18) so that in this study the levels high of abortions occur due to IFN- γ and TNF- α .

Infection *T. gondii* in a pregnant mother has some manifestations good at holding itself and the fetus was conceived. As soon as the infected host, *T. gondii* will develop and spread throughout the body. Distribution and transmission of *T. gondii* from mother to child in touch with the infected macrophages or leukocytes. Tachyzoites transmission to the brain can be happened by the leukocytes (19). The parasite develops in the trophoblast and later to spread to the fetus include chorionic tissue and fetal blood flow involved resulting in the spread of global to the fetus. Due to infection of the fetus causes immunological

responses that affect fetal development. Effect of an infection caused by *T. gondii* is directly fetal death aborted or decay or drying depends on factors including microorganism uterine environment in the uterus. Preliminary results obtained some fetal abnormalities such as shrinkage, decay and drying. Infection in pregnancy causes an increase proinflammatory cytokines IFN- γ and TNF- α that can cause death and increased fetal resorption (20).

CONCLUSIONS

Incidence of abortion in pregnant mice was infected with *T. gondii* was influenced by gestational age and dose of infection. Infection of gestation 9.5 days with a dose of 10 tachyzoites of *T. gondii* caused 50 percent of the parent having abortion on day 17 to day 18 followed by death. Infection at 16 days gestation with doses of infection 10 tachyzoites of *T. gondii*, parent child-bearing mice were mostly dead with multiple abnormalities, among others, there were still wrapping fetal membranes (placenta), shrinkage / drying and maceration and infection at 16 days gestation with doses of infection 5 tachyzoites of *T. gondii* obtained all normal childbirth.

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