Tracking Antigen Expression of Toxoplasma gondii in Mice by Immuno (Cyto) Histochemistry

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Abstract. Mouse has been an animal model that is very sensitive towards the Toxoplasma gondii infection. This research infects 30 mice with free-range chicken heart inoculate and the detection of T. gondiiby immunohistochemistry on the infected mice. The purpose of this study is to track T.gondii antigen expression by immune(cyto)histochemistry in mice. Observation over mice was conducted over 10 weeks. After 10 weeks, the mice are examined for its peritoneal fluid by microscopic and immunohistochemistry examination. Continued by the examination of internal organs to track the expression of T.gondii antigens by immunohistochemistry. The study result showed microscopic examinations on peritoneal fluid did not found any cyst or tachyzoites form of T.gondii. Examination of peritoneal fluid by immunohistochemistry was found to detect expressions of T.gondii antigen. Examinations of the mice's internal organs detected expressions of T.gondii antigen only in the brain by immunohistochemistry examinations.

Keywords: **mice, peritoneal fluid, antigen expression, immune-(cyto)-histochemistry, toxoplasma gondii**

I. INTRODUCTION

Toxoplasma gondii disease causing toxoplasmosis which had spread around the world [1]. Around 30%-65% of the world populations are infected by chronic toxoplasmosis, ranging from the lowest, moderate, and highest such as South Americans and Tropical Africa [2]. The animal prevalence is around 5-80% [3]. Various reports of toxoplasmosis prevalence and various surveys from many province in Indonesia have found that the case occurrences of toxoplasmosis are still relatively high [4] with high toxoplasmosis cases, early diagnosis is highly needed.

Mice are used as animal models to be infected by T. gondii through intra peritoneal inoculation. The inoculate given to the mice can be in the tachyzoites or bradyzoites stadium [5]. Mice are selected as the animal model in laboratories because it is highly sensitive towards T. gondii [6][7]. Field cases shows that rats have higher rates of toxoplasmosis infections compared to mice [8]. Bioassays of T. gondii are conducted in mice by inoculate infection through intra peritoneal of which it is predicted to contain the tachyzoites or bradyzoites stages [6][7]. T. gondii antigens that infects the liver, kidney, and lymph mesenteric of mice are successfully tracked by ELISA and immunohistochemistry assays [9][10]. Mice that are infected with pig-brain inoculate through bioassays are tracked with 25% positive of T. gondii cyst, while through immunohistochemistry assay it was tracked 30% positive of T. gondii antigen [11].

Immunohistochemistry assay is a method of tissue staining by using immunologic reaction and chemical. The antigen found in the tissue can be defined by monoclonal or polyclonal antigen binding towards the specific antigen and defining the location of bonding by a tracking system that recognizes the immunoglobulin from the species of the antibody origin [12]. Reporting target molecules are then made from the tracking system to react with the substrate. The purpose if this study is to track the expression of T.gondii antigen by immuno-(cyto)-histochemistry in mice. As a problem to be solved is to ask whetherwith the immuno-(cyto)-histochemistry can track the T.gondii antigen expression in mice that have been inoculated with free-range chicken heart inoculate.

II. RESEARCH METHOD

The tools used are 100 free-range chicken hearts which have been inoculated by digestion method using pepsin HCL [13]. Inoculation of free-range chicken heart by intra peritoneal inoculation was conducted in mice. Bioassay method on mice is conducted for the cultivation of T.gondii in mice. Observations over mice are conducted for 10 weeks. After 10 weeks, mice are killed by ether euthanasia. Before euthanasia, peritoneal fluid is taken to track the T.gondii by cytological examination and immunocytochemistry assay method. Cytological tracking is conducted on visceral organ (heart and liver using immunohistochemistry method).

Immunocytochemistry Method

Peritoneal fluid of mice are swabbed evenly and thinly on an object glass applied with an adhesive layer. The preparation is air dried. Immunochemistry process is then conducted. T. gondii antigen expression is observed on the preparation cells using light microscope with a 1000 times optical zoom. The appearance of brown-reddish color on the cell preparation shows a positive result of the T. gondii antigen expression.

Immunohistochemistry Method

Visceral organ sample of mice which includes the heart, lungs, liver, and brain are prepared for this examination. Those organ samples are processed to make histological preparation, making block squares using paraffin. Paraffin removal of the preparation (blocked antibody) with xylene as many as 3 times each lasting for 3 minutes. Rehydration of the preparation samples are conducted using ethanol with the concentration of 100%, 95%, and 70 % consequently for two minutes, two minutes, one minute and last with water for a minute. It is then soaked in peroxidase blocking solution at room temperature for 10 minutes. Then the preparationsamples are incubated in pre-diluted blockingserum at25°C for 10 minutes. The preparation samples are then soaked in anti-Toxoplasma gondii antibody at 25°C for 10 minutes. Then washed with Phosphate Buffer Saline (PBS) for 5 minutes. The preparation samples are then incubated with secondary antibody (conjugated to horse radish peroxidase) at 25°C for 10 minutes. It is then washed with PBS for 5 minutes. The preparation samples are incubated again with peroxides at 25°C for 10 minutes. It is then washed again with PBS for 5 minutes. The preparation samples are then incubated with chromogenic DAB (Diaminobenzinidine) at 25°C for 10 minutes. It is then incubated with Hematoxylin Eosin for three minutes. The preparation samples are then washed with flowing water. Then cleaned and given drops of mountingMedia.The preparation samples are then closed-covered by a coverslip. Observation is conducted to look at the expression of T. gondii antigen (reddish-brown in color) on the tissue using light microscope with a 1000x zoom.

III. RESULTS AND ANALYSIS

Result of microscopic examination of mice peritoneal fluid after 10 weeks of inoculation with free-range chicken liver inoculate did not show any cysts or tachyzoites of T. gondii, continued by immunocytochemistry examination showed cell structures with reddish-brown colorings. Result of immunohistochemistry of the mice brains, heart, lungs, and liver only showed structures of reddish-brown in the brain tissue.

Microscopic examinations which relies on the sensibility of the eyes on the microscope, for the swabbed sample of mice peritoneal fluid was not able to find any form of cyst or tachyzoites of T. gondii. While the Immunocytochemistry examination of the swabbed peritoneal fluid sample succeeded to detect cell forms with reddish-brown colorization. This is due to the sensibility of the eyes in reading the results; direct examination with microscope makes it hard to find T. gondii in the preparation samples. It means that immunohistochemistry detection succeeded to trace antigen expression of T. gondii in the mice peritoneal fluid. As suggested by Casartelli-alvas (2014) that histology and Immunohistochemistry are diagnostic tools that are very useful due to its specification [14]. It is stated that immunohistochemistry examination for T. gondii has a sensitivity of 7% and specificity of 9%. The occurrence of reddish-brown colorization shows antigen reaction is occurring in that area with specific T. gondii monoclonal antibody which shows positively that there is an expression of antigen in that place.

Visceral organs examinations of the mice heart, liver, and brain tissue by immunohistochemistry only succeeded to show T. gondii expressed in the mice brain tissue. Which means to detect T. gondii in tissues after reacted through immunohistochemistry with antibody labeled enzyme, it makes it easier to trace T. gondii antigen with the expression the reddish-brown colored structures. [15]. Based on the study of Esquivel et al. (2015) on post mortem autopsy patient in Durango Hospital of Mexico shows T. gondii cyst are more likely to be found in brain tissues compared to heart tissues through Immunohistochemistry assays [16]. A difference was also shown by the study of Silva et al. (2013) on sheep that immunohistochemistry assays found T. gondii to be positive in liver tissues, although serological results shows a low titer [17].

The occurrence of different results from some organs that are examined may be due to each organ's different distribution of antigen, therefore the concentration of antigen in the site is not enough to react with the monoclonal T. gondii antigen. In this study, only the infected brain tissue of mice showed positive reaction, while on the Casartelli-alvas (2014) showed positive reaction with the chicken liver, and by Silva et al. (2013) on infected sheep liver. Also the correlation with the bioassay results that was conducted towards the free-range chicken liver in this study, showed the amount of T.gondii cysts in the tissue

per gram from brain or heart tissue of the chicken could be higher than in infected pigs and lower than infected sheep.

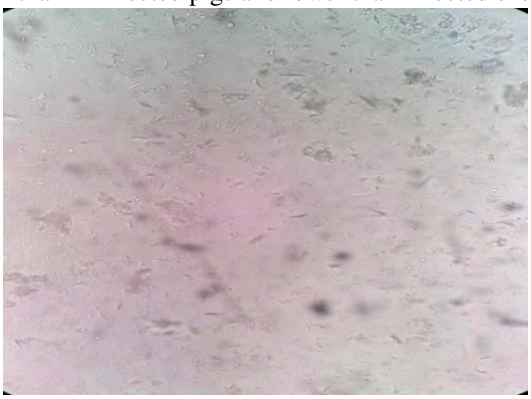

Fig 1. Microscopic view of peritoneal fluid. Neither forms of cysts nor tachyzoites of T.gondii was found.

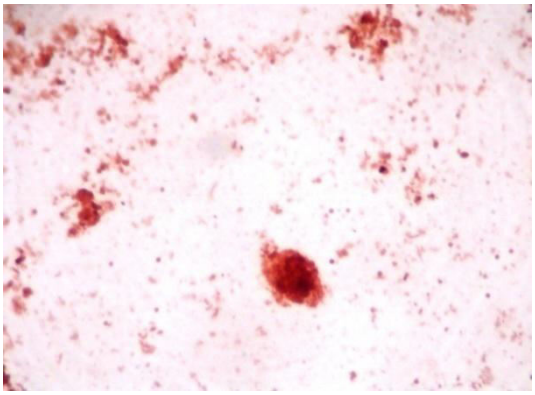

Fig 2. Peritoneal fluid by immuno histochemistry assay. Shown a reddish-brown coloured structure.

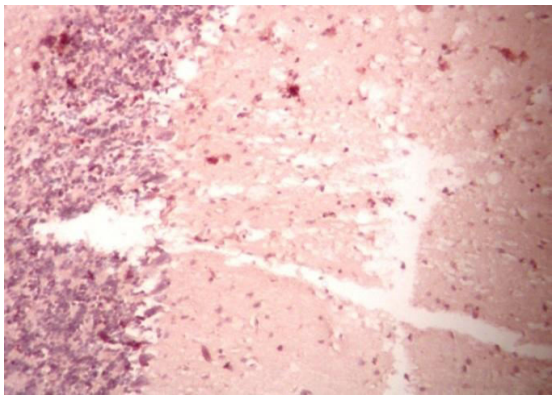

Fig 3. Mice brain tissues by immuno histochemistry. Shown a reddish-brown coloured structure as a positive result of T.gondii expression in the brain.

From various serological diagnostic methods for T.gondii such as T.gondiiModified Aglutination Test (MAT), Indirect Haemaglutination Test (IHAT), the antibody was detected 7 days after the infection. Using the ELISA and IFAT (Indirect Fluorescence Antibody Technique) the antibody was detected 14 days after the infection [18]. Which means the serological diagnosis is very determined by the occurrence of antibody after the infection, due to the arising false-negative state. Also from the false-negative state of the bioassay can be; due to have no parasite from the tissues in the bioassay, has a parasite but not detected, or due to a low concentration of parasitic agent from the infectious inoculate due to a procedural error in the digestion method [19].

Immunohistochemistry assay is to trace local antigen in the tissue. This reaction becomes specific due to its use of specific monoclonal antibody (AbMo T.gondii). A false-negative serological examination can be fatal to the animals infected with T.gondii, because an examination with Immunochistochemistry can positively trace T.gondii antigen when the serologic testing on the animals was low The immunohistochemistry on the sheep's liver was able to found traces of T.gondii antigen although the examination using MAT showed a low titer [17]. This condition shows the importantly of immunohistochemistry assays to control the spread of zoonosis diseases.

IV. CONCLUSION

Immunohistochemistry was successful in tracing the expression of T.gondii antigen in mice peritoneal after 10 weeks of inoculation with free-range chicken liver inoculate. The immunohistochemistry succeeded in tracing the expression of T.gondii in the mice brain tissue and as bioassay result of T.gondii from the free-range chicken liver.

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