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Original Article

Evaluation of Toxoplasmosis in Female College Students

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ABSTRACT

Female students are a group of women of childbearing age who can become pregnant and have risk factors for toxoplasmosis which can cause pregnancy disorders, birth defects, and death. Seroepidemiological studies of toxoplasmosis in women are essential to determine whether infection occurs in the early stages of fertilization or earlier. The research aimed to determine the incidence of toxoplasmosis in female college students. This research was carried out in June 2023 and has received ethical recommendations from KEPK Poltekkes Kemenkes Semarang. The type of research used was analytical descriptive. Sampling used purposive sampling. The inclusion criteria were 87 students who were willing to have their blood taken. Blood is taken using the venipuncture method, then the whole blood is separated from the plasma. Plasma samples were collected and analyzed for the presence of anti-Toxoplasma gondii using the ELISA method. Then the data was analyzed using SPSS software, univariate analysis to determine the frequency distribution and percentage of each variable. The results showed that 31 students were positive for IgG anti-Toxoplasma gondii. Of these, the majority aged 20 years (n = 20, 16.1%) were positive for anti-Toxoplasma gondii IgG. Toxoplasmosis infection of 9.2% occurred in female college students who owned cats, 3.4% occurred in female college students who consumed undercooked meat, and 29.8% of female college students who consumed raw vegetables. Female college students have risk factors for Toxoplasma gondii infection so it is important to carry out screening so that Toxoplasma gondii can be detected and treated immediately.

Keywords: *ELISA, Female Student, IgG, Toxoplasma gondii.*

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INTRODUCTION

Toxoplasmosis is one of the zoonotic diseases caused by *Toxoplasma gondii*[1]. Toxoplasmosis cases are also common in humans. Humans can be infected with this disease by acquired or congenital. Human infection is acquired through several means including consumption of undercooked meat from infected animals, consumption of unpasteurized milk, direct or indirect contact with oocysts from the environment, vertical transmission during pregnancy, blood transfusions, and organ transplantation[2].

Toxoplasma gondii infection is generally asymptomatic in immunologically healthy adults. Only 10-20% of toxoplasmosis cases in children and adults show symptoms. Mild symptoms of primary infection include localized, painless cervical or occipital lymphadenopathy, usually lasting for 4-6 weeks, or nonspecific symptoms including myalgia, headache, rash, or sore throat that persist for a month or more However, it can cause a variety of clinical complications in immunocompromised patients. In immunocompromised patients it develops eye problems, mental retardation and congenital infections in children born to infected mothers, and abortions in pregnant women. If this

infection occurs during the first and second trimesters of pregnancy, it can manifest severe symptoms, such as low birth weight, hydrocephalus, intracranial calcification, and ectinocoroiditis that can be recognized at birth. Infection in the third trimester of pregnancy can result in hearing loss, visual impairment, and developmental delays[2,3,4].

The incidence of toxoplasmosis varies greatly by country and age. The prevalence rate of this parasite in the world ranges from 10% - 90% depending on social habits, climatic conditions, geographical region, socioeconomic level of society, eating habits, prevalence of contact with cats, environment, hygiene and sanitary conditions, age, sex, ethnicity, medical history, and immunogenetic characteristics affect the seropositive rate. In Indonesia, cases of toxoplasmosis in animals range from 6–70%, while in humans between 43–88% [5,6]. Based on research by Darmawan (2018) and Humaryanto (2019) on women of childbearing age in Jambi, 34% and 60% of positive IgG were found[7,8]. Another study in Central Java on male and female populations (covering Purworejo, Kebumen, Cilacap, Banyumas, Purbalingga, Banjarnegara and Wonosobo) showed a positive IgG of 90% [9].

The diagnosis of toxoplasmosis is still based on the detection of different types of antibodies, each of which is interpreted differently. Toxoplasmosis is usually diagnosed by serologic tests of *Toxoplasma gondii*-specific IgG and IgM antibodies. These tests are generally enzyme-based immunoassay or immunofluorescence tests. *Toxoplasma gondii* immunoglobulin M (IgM) antibodies are used to determine the presence of a new infection. *Toxoplasma gondii* immunoglobulin G (IgG) antibodies to see whether there is a chronic infection caused by the *Toxoplasma gondii* parasite[10,11].

People who may be at high risk of contracting this parasite include pregnant women and people with weakened immune systems. Women of childbearing age are a group that has the potential to become pregnant. This population group has risk factors for toxoplasma infection that result in abnormalities during pregnancy, disability and fetal death[12]. Seroepidemiological studies of toxoplasmosis in women of childbearing age are essential to determine whether the infection occurs in the early stages of conception or earlier. Female students of childbearing age group will potentially have risk factors to get adverse effects on the occurrence of toxoplasma infection. Therefore, it is very necessary to screen for toxoplasma in the childbearing age group, to detect toxoplasma so that it can be treated immediately. The purpose of the study was to evaluate the incidence of toxoplasmosis in college students.

METHOD

This study is a type of analytical descriptive research, where research was conducted to evaluate toxoplasmosis in female students. The research was conducted at the Laboratory of the Department of Health Analysts, Poltekkes Kemenkes Semarang on May 25 - June 7, 2023. The research population was 101 female students aged 18-23 years in semester 2 and semester 4. Samples were taken using purposive sampling. The inclusion criteria are that students are willing to have their blood taken and then tested for anti-toxoplasma IgG. Based on informed consent, 87 students were willing to have their blood taken. This research does not use controls.

The material used in the study was a whole blood sample with EDTA anticoagulant. The tools used are ELISA washer, ELISA reader, incubator, centrifuge, micropipette, yellow-tip, beker glas, vacutainer, syringe, tourniquet, cotton alcohol 70%. This research received ethical recommendations from the Health Research Ethics Committee of the Health Polytechnic, Ministry of Health, Semarang Number 0480/EA/KEPK/2023.

After consent was obtained, study participants were interviewed to obtain information regarding sociodemographic status as well as epidemiological risk factors. Habits associated with *Toxoplasma gondii* infection in this study include age, place of residence, cat ownership, consumption of undercooked meat, and consumption of raw vegetables (lalapan). A 3 cc blood sample was collected from the participant by venipuncture method. Blood samples are stored overnight in the fridge at 400C. The plasma was separated from the whole blood sample by centrifugation for 10 minutes at a rate of 3000 rpm at room temperature. The plasma is separated from the red blood cells and transferred to an eppendorf tube to measure IgG. Plasma is stored in a

freezer with a temperature of -200C until use. Plasma samples were tested for *Toxoplasma gondii* using indirect ELISA.

All samples were tested in the laboratory of the Department of Health Analyst Poltekkes Kemenkes Semarang. After collecting samples, frozen plasma was thawed at room temperature and evaluated for *Toxoplasma gondii* IgG antibodies using the BIOENZYME Enzyme-Linked Associated Commercial Assay Immunosorbent Test Kit (ELISA). Based on the manufacturer's instructions, a positive or negative control is added 50 µl to the plate. Add 10 µl of sample and 40 µl of sample diluent, mix, incubate for 30 minutes at 37^0 C. Next wash 5 times and add solution of substrate A and substrate B, incubating in the dark for 10 minutes at a temperature of 37^0 C. Add 50 µl stop solution. Read OD values within 15 minutes at 450 nm using an ELISA reader. Samples with OD samples < cut off values are considered negative for anti-IgG *Toxoplasma gondii* and OD samples ≥ positive cut off values.

Data analysis was performed with the SPSS 25 software program. Data analysis uses univariate analysis, to find out the frequency and percentage distribution of each variable. The collected research data is presented in tabular form and narrated briefly.

RESULTS

The results of the study are presented in Table 1 and Table 2. Plasma samples were taken from 87 female students from semesters 2 and 4 of the D3 Medical Laboratory Technology Study Program. Of these, 12 (13.8%) participants were 18 years old, 38 (43.7%) were 19 years old, 35 (40.2%) were 20 years old, 1 (1.1%) was 22 years old and 1 (1.1%) was 23 years old. The majority of students live in boarding houses (n=75, 84.3%) and only (n=12, 13.5%) live in parental homes as shown in Table 1.

Table 1. Characteristics of Respondents

	Positive IgG	**Negative IgG**	**Total**
Age (years)			
18	4 (4.6%)	8 (9.2%)	12 (13.8%)
19	12 (13,9%)	26 (29.8%)	38 (43.7%)
20	14 (16.1%)	21 (24.1%)	35 (40,2%)
22	1 (1.1%)	0	1 (1.1%)
23	0	1 (1.1%)	1 (1.1%)
Semester			
2	12 (13.7%)	27 (31%)	39 (44.7%)
4	19 (21.8%)	29 (32.5%)	48 (54.3%)
Status of residence			
Kos	27 (31%)	48 (55.2%)	75 (86.2%)
Parents' house	4 (4.6%)	8 (9.2%)	12 (13.8%)

Plasma samples were examined for the presence of anti-*Toxoplasma gondii* IgG antibodies using the ELISA method. Among the 87 samples collected, 31 (35.6%) samples were positive for anti-*Toxoplasma gondii* IgG which means they had anti-*Toxoplasma gondii* IgG and 56 (64.4%) negative anti-*Toxoplasma gondii* IgG, as shown in Table 2. Table 2 shows Habits associated with *Toxoplasma gondii* infection. The habit associated with the incidence of *Toxoplasma gondii* infection was seen in cat ownership (9.2%), while the consumption of cooked stega meat was the least (3.2%). Twenty-six people consume raw vegetables (29.8%).

Table 2. Habits associated with *Toxoplasma gondii* infection.

Variable	**IgG positive (n=31; 35.6%)**	**IgG negative (n=56; 64.4%)**
Cat ownership		
Yes	8 (9.2%)	9 (10.3%)
No	23 (26.4%)	47 (54%)
Consumption of undercooked meat		
Yes	3 (3.4%)	38 (43.6%)
No	28 (32.2%)	18 (20.6%)
Consumption of raw vegetables		
Yes	26 (29,8%)	37 (42.5%)
No	5 (5,7%)	19 (21.8%)

DISCUSSION

Toxoplasmosis is an infection caused by an intracellular protozoan called *Toxoplasma gondii*. Transmission of this disease is more prevalent in warm and humid areas and is caused by intracellular obligate protozoan parasites. High humidity and warm

climate, which can affect the survival of *Toxoplasma gondii* oocysts in the environment, can provide favorable conditions for the development of *Toxoplasma gondii*. Individuals can become infected by eating raw or undercooked meat, or by ingesting eggs released by cats in contaminated soil, water or food, or transplacental transmission of tachzoites. Although most cases of this infection in immunocompetent individuals are asymptomatic but if this infection occurs in pregnant women it can lead to congenital toxoplasmosis with severe pathological effects on the fetus such as retinochoroiditis, mental disability and abortion, stillbirth, and fetal death[13].

The immune system plays an important role in the prevention of toxoplasmosis through innate immune mechanisms and adaptive immune responses. Toxoplasmosis stimulates two types of immune responses; humoral and cellular immune responses, the first of which is associated with the formation of extracellular and invasive active tachyzoites in the circulation. Antibodies are produced by stimulating B lymphocytes (B cells); known as IgM, IgG, IgA and IgE. These antibodies functionally work on eradicating infection and eliminating parasites found free in body fluids by activating supplement pathways and the catalytic activity of the immune system[14].

One way to prevent congenital toxoplasmosis is to detect IgG in women of childbearing age before marriage. Women of childbearing age are the best group to evaluate immunity to toxoplasmosis[12,15]. Early diagnosis of toxoplasmosis, especially in women of childbearing age, is recommended because the disease can cause miscarriage, stillbirth or congenital toxoplasmosis in their babies while they are pregnant[16].

In this study we found that 35.6% of college students aged 18-23 years had seropositive for the IgG antibody *Toxoplasma gondii*. These results are in line with research findings from Al Zaheb (2017) and Al-Kadassy et al (2018) using the ELISA method in women of childbearing age who found toxoplasma IgG antibodies were also found at the age of 18-23 years[12,17]. The seropositive value in this study was significantly higher than the prevalence reported in women of childbearing age from Yogyakarta, seropositive IgG Toxoplasma in premarital women aged 20-25 years by 32.2%, 20% seropositive IgG Toxoplasma aged 26-30 years and 3.3% seropositive IgG Toxoplasma aged 31-35 years[18].

Measurement of anti-*Toxoplasma gondii* IgG in serum/plasma/other body caian using ELISA method. The parasite antigen is superimposed on the hole of the ELISA plate. After incubation with serum/plasma assay, species-specific anti-immunoglobulin antibodies labeled with enzymes or antibody-binding proteins conjugated to the enzyme (e.g., to peroxidase or alkaline phosphatase) are applied to report antigen-specific reactions, i.e. the extent to which antibodies bind to parasitic antigens. In the last step, a substrate is added that is converted by the enzyme into colored reaction products. Absorbance or optical density (OD) is measured with a spectrophotometer[19].

ELISA is mainly used for routine screening of *Toxoplasma gondii* infection because it is very sensitive (allows quantitative and semi-quantitative antibody measurement), can be easily used to test large populations in a short time, with the ability to detect IgG and IgM[20]. With 97% specificity and 100% sensitivity, serological tests such as ELISA are considered one of the best laboratory methods for determining serum toxoplasmosis antibody levels[15].

Toxoplasma gondii antibodies are indicative of *Toxoplasma gondii* infection, and the infection lasts a long time (generally lifelong). Significant levels of IgM antibodies to viruses, bacteria, or other infectious agents are interpreted as a sign of acute infection, whereas high specific IgG levels are consistent with persistence of immunity in the convalescent phase after prior infection[21].

IgM anti-*Toxoplasma gondii* is known as a marker of acute infection that appears earlier and declines faster than IgG antibodies. IgM antibodies are often first detected after primary infection. The diagnosis of newly acquired *Toxoplasma gondii* infection is generally based on detection of specific IgM antibodies, followed by detection of specific IgG antibodies 1-3 weeks later.11 IgG antibodies to *Toxoplasma gondii* can be detected 1-2 weeks after infection. It usually peaks in 1-2 months and decreases at different rates. Because it can last a lifetime at residual titers, these antibodies are an indicator of previous infection. These antibodies are used as

diagnostic markers for chronic infection[20].

A positive IgG result indicates past infection, but cannot provide information on when the infection occurred. Serological results may vary depending on the individual, his immune background, and the serological techniques used. In the clinic, serologic detection of IgM and IgG antibody levels is the basis for identifying infections and the most commonly used methods[22,23,24].

Several female collage students who participated in this study owned cats and tested positive for *Toxoplasma gondii* IgG antibodies. The results obtained from this study are different from Enitan's research (2020), where respondents kept cats as pets and none of these categories tested positive for Toxoplasma gondii IgG antibodies[25]. Stray cats defecating in parks and sandboxes can put some people at risk of contracting *Toxoplasma gondii*, whether they own cats or not[26]. Cats that do not receive routine toxoplasmosis vaccinations may be at higher risk of infection with *Toxoplasma gondii*. Cats are the definitive host for Toxoplasma gondii. The *Toxoplasma gondii* parasite reproduces in the cat's body until it reaches the oocyst stage which the cat excretes in its feces. To prevent your cat from contracting Toxoplasma, bathe your cat frequently, pay attention to the type of cat food you give it, and vaccinate your cat against *Toxoplasma gondii*[27]. Humans can also contract *Toxoplasma gondii* infection when they swallow raw meat containing tissue cysts[28].

Many female collage students who consume raw vegetables still test positive for *Toxoplasma gondii* anti-IgG. Transmission of *Toxoplasma gondii* by consumption can occur when oocysts containing sporozoites are orally contaminated with contaminated food. Although packaged and ready-to-eat fresh vegetables pose a low risk, these products have been shown to be contaminated with pathogenic microorganisms and could theoretically pose a risk of toxoplasmosis[28].

Our study has some limitations. First, we did not use an IgM test that could help determine if a woman-acquired Toxoplasma infection was recent. Secondly, it would be ideal to include more samples, but this is not possible due to financial constraints. This study provides information that female students are at risk of Toxoplasma infection. Hence the need to raise awareness about toxoplasmosis, especially the mode of transmission, so that women can take steps to protect themselves and avoid contracting this parasite.

CONCLUSION

Anti-IgG *Toxoplasma gondii* was found in female students. The most positive results of toxoplasmosis are found at the age of 19 years. This study provides new epidemiological data on the seroprevalence of *Toxoplasma gondii* infection in women of childbearing age from college students. The serological status of women of childbearing age provides information about immunity, which can help to prevent congenital infections by identifying women at risk. This research can be useful information for health policymakers for counseling and screening programs. Early diagnosis of toxoplasmosis, especially in women of childbearing age, is recommended as premarital preparation. For further researchers, they can increase the number of examination samples and add anti-*Toxoplasma gondii* IgM examination.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. We conducted this research with full transparency and free from any outside influence that could harm this research, including any personal or professional relationships that could be construed as a conflict of interest.

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