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Survival of *Toxoplasma gondii* in Goat Milk after Pasteurization with Low Temperature and Long Time

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Abstract: The purpose of this study was to determine survival of *Toxoplasma gondii* tachyzoite RH strain in goat milk after being pasteurized in low temperature for along time. In this study vivo trial was done, i.e., using mice that were infected with a *Toxoplasma gondii* tachyzoite intraperitoneally in a dose of $2.76x10^6$. The infected mice were divided into 3 groups; (1) pasteurized milk and tachyzoite heated at a temperature of 63°C for 30 minutes (T), (2) pasteurized milk and tachyzoite without being heated as a positive control (PC) and (3) pasteurized milk without tachyzoite as a negative control (NC). The results showed that tachyzoites did not detect in the peritoneal fluid in group T and NC. However, *T.gondii* tachyzoites were detected in group PC whose amount was nearly equal to the amount before and after the mice infection.

Key words: Pasteurization · Goat milk · *Toxoplasma gondii*

INTRODUCTION

Toxoplasma gondii, an obligate intracellular apicomplexan parasite protozoan, is widely distributed and can infect many species of warm-blooded animals; thus, it is considered as significant zoonotic pathogen [1]. Toxoplasmosis in sheep is a parasitic disease of great importance for veterinary medicine, husbandry and public health since it causes productive and economic losses, as well as damage to human health due to consumption of contaminated meat and milk, which can facilitate zoonotic transmission [2]. This disease is of major economic importance in raising livestock since it is a frequent cause of early embryonic death and resorption, fetal death and mummification, abortion, stillbirth and neonatal death in sheep [3, 4].

Clinical toxoplasmosis in humans has been associated with consumption of unpasteurized goat milk [5-8]. However, it must be emphasized that any type of milk, especially raw, is a potential source of infection [9]. *T.gondii* tachyzoites have been found in the milk of several intermediate hosts [10]. Toxoplasmosis transmission by unpasteurized or inadequately processed milk or fresh cheese, important food sources in rural areas, can be a significant means of contamination by this agent [11].

Pasteurizationis a processing method by heating below the boiling point for extending the shelf life of fresh milk. There are 2 ways of pasteurizing milk, namely, low temperature long time (LTLT) at low temperature of 62.8 °C for 30 minutes and at high temperature short time (HTST) at a temperature of 71.7 °C for 15 seconds [12, 13]. There have been no research reports on the living ability of tachyzoite inpasteurized milk, as Dubey [14] only found *T.gondii* DNA in the milk through a polymerase chain reaction test (PCR). The examination of tachyzoite in milk was conducted by PCR through the DNA detection and the tachyzoite contained in milk was unknown whether it was alive or dead. Based on the description above, this study was conducted to determine whether tachyzoite

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could still live in low temperature and long time pasteurized milk (63°C for 30 minutes) because the living tachyzoite may still be able to cause infection and harmfulness to public health.

MATERIALS AND METHOD

Place and Time: The study was conducted at the Indonesia Research Center for Veterinary Science (BBalitvet) Bogor, Laboratory of Veterinary Public Health (Kesmavet), Faculty of Veterinary Medicine, Bogor Agricultural University (FKH IPB), Integrated Laboratory of Faculty of Veterinary Medicine andAnimal Hospital from April to December 2012.

Research Design: This study was conducted *in vivo* using DDY strain male mice aged 5-6 weeks as experimental animals. Mice were kept for a week before treatment and fed and watered by ad libitum. Mice were infected with a liquid mixture between goat milk with a particular treatment and *T.gondii* tachyzoite RH strain and tachyzoites concentration in goat milk was $2.76x10^6$ tachyzoite/ml. The liquid mixture of goat milk and tachyzoite was injected intraperitoneally as much as 0.3ml. A total of 15 mice were used in this study and were divided into 3 groups, the group that was given (1) pasteurized milk and tachyzoite heated at a temperature of 63°C for 30 minutes (T), (2) pasteurized milk and tachyzoite without being heated as a positive control (PC)

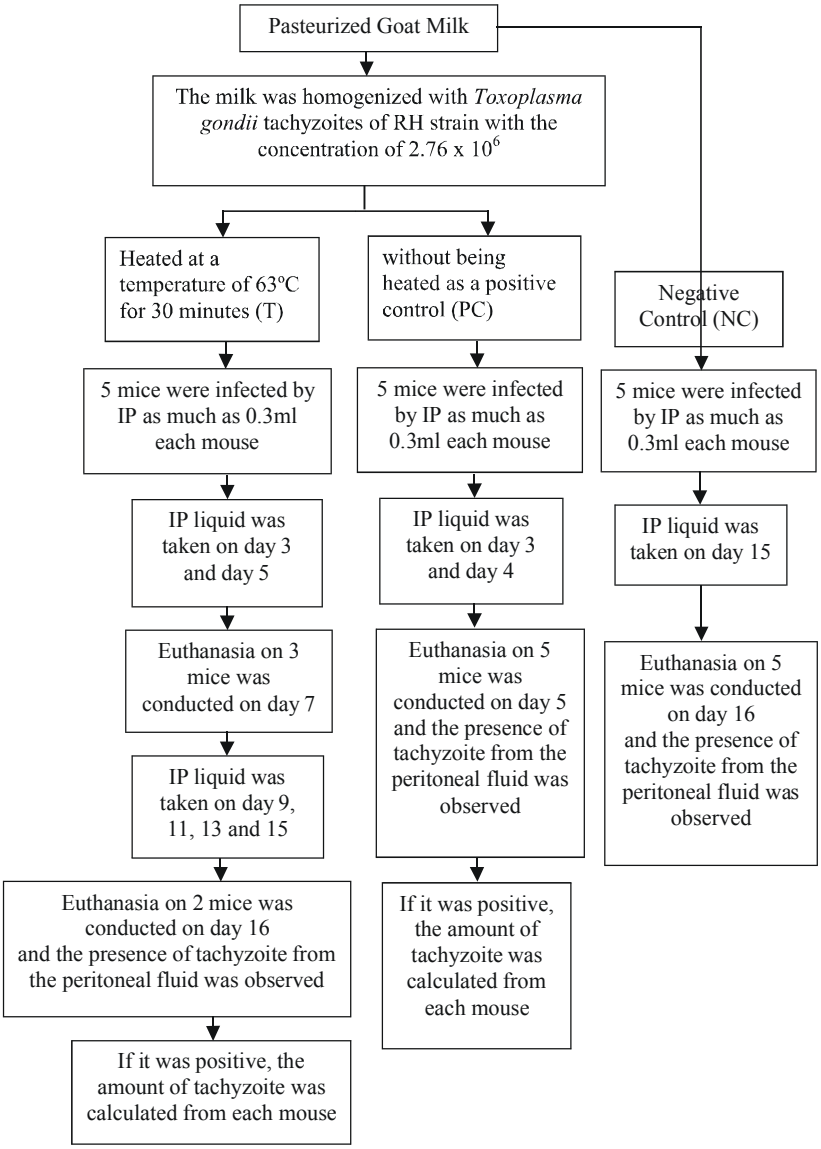

Fig. 1: The flowchart of the research method

and (3) pasteurized milk without tachyzoite as a negative control (NC). The pasteurization process was verified through a Storch test and total plate count test (TPC).

Mice that had been injected were raised for 16 days and observed their clinical symptoms everyday. On day 3, 5, 7, 9, 11, 13, 15 and16, the peritoneal fluid was taken from 1 mouse in each treatment group and then the fluid was examined under a microscope natively with magnification of 400x and observed for the existence of tachyzoite. If the amount of tachyzoite was more than the number of normal cells, euthanasia was conducted on the mouse with Ketamine HCl (0.03 ml). Once it was dead, a necropsy without opening the peritoneal was performed. A total of 5ml of PBS was inserted using a syringe into the stomach cavity through the peritoneal and the mouse was shaken carefully. The peritoneal fluid was then aspirated using a syringe and put into a test tube and centrifuged at a speed of 3500 rpm for 20 minutes. The supernatant was discarded and sediment was mixed with 1ml of PBS and it was then dropped in the Neubaur counting and counted under a microscope with the magnification of 400x using the leukocyte counting room. The flowchart of the study method can be seen in Figure 1. The above testing procedure was approved by the IPB Animal Ethic Committee, IACUC Number 09-2012AH-BAU.

The making of T.gondii Isolates: The isolate of *T.gondii* tachyzoite of RH strain at the laboratory of BBalitvet was separated from the liquid Nitrogen by *thawing* and was diluted with PBS and this liquid was then injected to the mouse intraperitoneally. Following this, every mouse was injected by 0.3ml of isolat intraperitoneally.

Goat Milk Pasteurization before it was given *T.gondii*: The milk pasteurization was conducted in a water bath in the Laboratory of FKH IPB at 63 °C for 30 minutes and at 72°C for 15 seconds. The milk was cooled at room temperature and then were subjected to the examination of total plate count, presence of pathogenic bacteria and peroxidase (Storch test). Total plate count test was performed using a plate count method by pouring using plate count agar. The existence of pathogenic bacteria was detected using blood agar.

RESULTS AND DISCUSSIONS

The result of the observation from the mouse group infected by milk and *T.gondii* tachyzoites showed that in Group PC was found the tachyzoite in the peritoneal fluid on day 4, Group T and NC, tachyzoite was not found until day 16. The result of the observations in detail can be seen at Table 1.

In the positive control group, tachyzoites started to emerge on day 4, although the peritoneal fluid was taken on day 3. On day 3, the mice in Group PC had shown clinical symptoms, i.e., they had inactive movement, lack of appetite, were more gathered in silence in the corner of the tub and their hair began to stand up. Based on these clinical symptoms, the peritoneal fluid collection was performed on the following day, i.e., on day 4. The results of this study showed that mouse mortality was estimated to take place on day 5 or day 6 after the infection. The results obtained are consistent with the [15] whereas the tachyzoite infection 10^6 samples were taken on day 4. Similar report was also stated by [16] stating

Table 1: The result of the observation of the mouse group infected by pasteurized milk

	Treatment Groups		
Day	Group T	Group PC	Group NC
3	Tachyzoite was not found	Tachyzoite was not found	The peritoneal fluid was not taken
4	The peritoneal fluid was not taken	Tachyzoite was found in small amount of quantity i.e. the number of normal cells was still higher than the amount of tachyzoite	The peritoneal fluid was not taken
5	Tachyzoite was not found	The amount of Tachyzoite was higher than the number of cells so that Euthanasia was conducted on the five mice to calculate their amount of tachyzoite	The peritoneal fluid was not taken
7	Euthanasia of the 3 mice and Tachyzoite was not found		The peritoneal fluid was not taken
9	Tachyzoite was not found		The peritoneal fluid was not taken
11	Tachyzoite was not found		The peritoneal fluid was not taken
13	Tachyzoite was not found		The peritoneal fluid was not taken
15	Tachyzoite was not found		Tachyzoite was not found
16	Euthanasia of the 2mice and Tachyzoite was not found		Euthanasia of the 5mice and Tachyzoite was not found

Table 2: The amount of tachyzoite obtained from PC (Positive Control)

Mice	The amount of tachyzoite (ml)
1	2.6×10^6
2	6.0×10^6
3	1.0×10^7
4	3.0×10^6
5	7.0×10^6

that *T.gondii* could cause the death of mice within a period of 6-9 days and the death was dependent on the post-infective dose.

The mouse groups infected with pasteurized goat milk did not indicate the presence of tachyzoites in the peritoneal fluid and this is due to the heating of pasteurization that can kill tachyzoites. World Health Organization [17] stated that meat heated at a temperature of 65 °C for 4-5 minutes could kill active cysts of *T.gondii*. The same thing is also expressed by [18] stating that the cysts of *T.gondii* in tissues could be destroyed by cooking at the temperature of 66°C.

The amount of post-infective tachyzoite obtained from the peritoneal fluid of the mice from Group PC did not vary much with the amount of infected tachyzoites (Table 2).

T.gondii tachyzoite of RH type I strain is used in this study has the ability of cytokine Type I induction and its destruction is very high compared to other types; as a result, it can have LD100 in a very short time. If living tachyzoiteis found in milk and consumed by humans, it can cause infections in humans who consume the milk. The pasteurization in this study used a temperature of 63°C for 30 minutes because heating at this temperature can kill pathogenic bacteria and nutrients in the milk can be maintained.

Oocysts survive and remain infective in water and feces for months at a temperature of 20°C to 37°C [19]. Bradizoits in meat is deactivated by cooking, freezing, preservation, irradiation and pressure. Temperature of 66 °C could inactivate cysts in meat [20] while there have been no data on the temperature that can kill tachyzoites in milk.

Goatsis an important source of mea (very popular in some ethnic groups, especially from Asia) and milk in developing countries but can act as a source of infection for humans who live in the area [21]. In addition, the consumption of unprocessed goat milk and dairy products derived from this unprocessed milk has been linked to cases of toxoplasmosis in humans [5, 6, 8, 22].

Excretion of *T.gondii* tachyzoites through milk can occur naturally and cause the transmission of toxoplasmosis in humans due to their consumption of milk. This transmission mechanism takes place because tachyzoite entering the body will go into the blood vessels and occupies the nucleated cells and subsequently flows throughout the body. Milk produced from the blood; as a result, tachyzoites which are in the blood are also likely to be excreted through the milk.

CONCLUSION

T.gondii tachyzoites RH strain died in pasteurized milk with a temperature of 63°C for 30minutes.

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