Review Article

Review: Potential Transmission of Toxoplasmosis through Consumption of Raw Goat's Milk

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Abstract | Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*. The definitive host of *T. gondii* is the cat, while the intermediate hosts are all mammals. Livestock become infected by ingesting sporulated oocysts in the environment, while humans can be infected by *T. gondii* due to environmental contamination by *T. gondii* oocysts, consumption of foods and milk containing cysts or tachyzoites of *T. gondii*. Raw milk is a risk factor for toxoplasmosis in humans. This paper discusses the presence of *T. gondii* in raw milk from various animals in both artificial and natural infections, the survival of *T. gondii* in milk and its derivatives and raw milk as a risk factor for toxoplasmosis in humans.

Keywords | Toxoplasmosis, Raw milk, Environmental contamination

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INTRODUCTION

Toxoplasmosis is a zoonotic parasitic infection both in human and animals caused by the obligate intracellular protozoan *Toxoplasma gondii*. *Toxoplasma gondii* infects almost all warm-blooded animals including humans, livestock, and marine mammals (Dubey et al., 2011; Schlüter et al., 2014). Maharana et al. (2010), reported that one third of the human population has been infected with *T. gondii* and the socio-economic losses due to toxoplasmosis include high costs for patient care, mental disorders, and blindness in children. Infections of *T. gondii* are considered the main causes of reproductive disorders and economic losses in small ruminants worldwide. Toxoplasma investigation on sheep and goats showed that these diseases become an important agent as abortion disease. Toxoplasmosis is a relevant zoonosis and concern for public health. *T. gondii* infection in small ruminants may play a major role in its transmission to humans (Stelzer, 2019).

Transmission through food is considered to be the main role of *T. gondii* transmission. *T. gondii* tissue cysts and tachyzoites are responsible for infection through meat and milk, respectively, and sporulating oocysts in the environment can be successful in fresh produce, shellfish, and water and infect humans after consumption (Figure 1). One of the main routes of transmission of toxoplasmosis is the faecal-oral route. The oocysts of *T. gondii* shad out with the feces of the definitive host, the felids, including domestic cats (Stelzer, 2019) and in environment oocysts become infective for hosts accidentally ingesting contaminated products (Belluco et al., 2017). Since cats defecate in soil and sand, contact with an environment contaminated with oocysts is a risk factor for infection (Maharana et al., 2010). Livestock become infected by ingesting sporulated oocysts in the environment (Dubey et al., 2014; Al-Malki, 2021), while humans are infected by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or accidentally ingesting oocysts (Dubey et al., 2011).

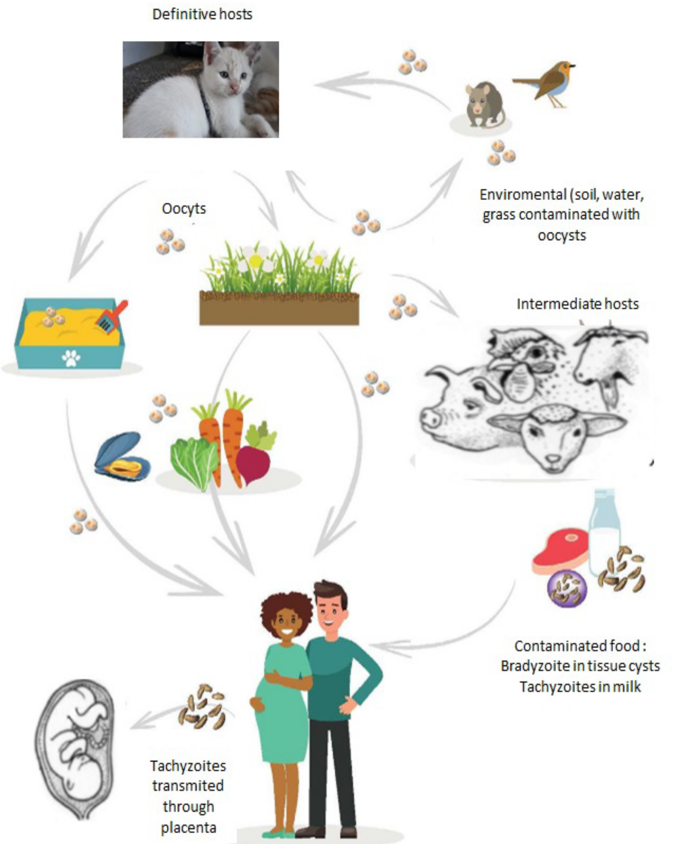

Figure 1: Foodborne transmission pathways of *T. gondii* (Koutsoumanis et al., 2018; Hill and Dubey, 2002) modified.

Research on risk assessment linking *T. gondii* infection to consuming raw meat from livestock has been widely carried out. Some livestock besides producing meat also produce milk, this paper will review the potential of milk as a transmission of toxoplasmosis. Reviews are carried out on articles published online starting in 2000, both original articles and reviews.

The presence of *Toxoplasma gondii* in raw milk

Toxoplasma gondii can infect almost all nucleated cells (Laliberte and Carruthers, 2008) including cells in the mammary gland (Costa and Langoni, 2010). *Toxoplasma gondii* is also known as an obligate intracellular parasite (Gupta et al., 2021). One of the infective stages of *T. gondii* is tachyzoites, which can be transmitted through animal fluids containing infected cells. During acute animal infection, circulating tachyzoites can be transferred from blood to milk (Tenter et al., 2000). In chronic conditions, *T. gondii* tachyzoites can also be found in the milk of infected animals. Abdel-Rahman et al. (2012), described the presence of *T. gondii* tachyzoites in the milk of chronically infected goats caused by bradyzoites in tissue cysts turning into tachyzoites and being able to recirculate and be excreted in milk during the physiological decline of peripartum immunity. Experimental research by (Pereira et al., 2010) found that the *T. gondii* cysts strain ME 49 was infected in raw milk and could live for 20 days in the refrigerator. This paragraph shows that tachyzoites and bradyzoites can be found in milk. The difference between these two stages is the speed of multiplication. The status of acute and chronic infection is related to the circulation of the parasite and depends on the condition of the infected or exposed animal. So even though tachyzoites or bradyzoites were found in milk, it did not show the level of infection in goats.

The existence of *T. gondii* in milk has been proven by researchers, both in artificial and natural infections. Powell et al. (2001), detected *T. gondii* DNA in the milk of cats experimentally infected at different gestational stages. With the same method, by PCR *T. gondii* was found in the milk of experimentally infected rats, both in chronic and acute infections (Costa and Langoni, 2010). Dubey et al. (2014), performed an artificial infection in goats and proved that *T. gondii* can be detected in milk either by bioassay (infection in mice and cats) or molecularly (PCR). The presence of *T. gondii* was detected in goat milk samples depending on the dose of infection and breed of goat, respectively high dose infection (10000 oocysts) in Boer goats, infection with 1000 oocysts in mixed breed goats and infection with 3000 and 300 oocysts in Toggenberg goats, *T. gondii* was detected in goat's milk on day 4, 12-13 and 8-9 after infection. They concluded that the detection of viable *T. gondii* in goat's milk was intermittent. Ishag et al. (2006) conducted experimental study in camel and found tachyzoites in camel milk.

T. gondii were detected in the milk of naturally infected cows (Abadi et al., 2020; Gharekhani et al., 2021). Using B1 specific gene of *T. gondii*, (Abadi et al., 2020) detected 60 samples raw milk of cows and resulted that 5% (3/60) samples were positive. Based on the ELISA and Molecular methods on 149 milk samples from Iranian dairy farms, the prevalence rates of *T. gondii* infection in milk were 5.4% and 10.7%, respectively (Gharekhani et al., 2021).

The presence of *T. gondii* DNA in milk from small ruminants has been reported by Hussain et al. (2017) and most reports are of the presence of *T. gondii* DNA in goat and sheep milk. *T. gondii* DNA also found in milk from sheep (Camossi et al., 2011; Fusco et al., 2013; Saad et al., 2018; Gazzonis et al., 2019). Camossi et al. (2011) detected 139 milk samples by PCR and found 7 milk samples were that positive. The results of research conducted by Fusco et al. (2007), reported that 4 from 117 milk samples positive with *T. gondii* DNA and Ossani et al. (2017), in Bahia, reported 10.5% (21/200) of milk samples from sheep with the presence of the parasite. While Ossani et al. (2017), detected *T. gondii* in milk of dairy sheep in the western mesorregion of Santa Catarina Brazil by bioassay (inoculation in mice) and PCR methods and found that in 12.04% (13/108) samples were detected the presence of DNA of *T. gondii*. Detection of *Toxoplasma gondii* DNA in 625 samples of Sheep's and Goat's Milk in Northwest of Iran by PCR-RFLP produced 19 (3.04%) with details of 16 (4.63%) sheep's milk and 3 (1.07%) goat's milk showed the presence of specific *T. gondii* B1 fragments (529 bp) (Tavassoli et al., 2013). With real-time PCR, *T. gondii* DNA was detected in 7 of milk samples of sheep, 5 (20%) from the IgM+ group (25 samples) and 2 milk samples (3.6%) from the IgM- group (55 samples) (Luptakova et al., 2015). Evidence for the presence of *T. gondii* DNA in milk was also reported by Bezerra et al. (2013), that in 15 milk samples from 248 goats exposed naturally there was *T. gondii* DNA. Saad et al. (2018), found 1 goat's milk and 1 sheep's milk positive for *T. gondii* DNA from each of the 30 samples examined. Research on the molecular prevalence of *T. gondii* in goat's milk was also carried out in Northwest Tunisia (Jendouba Governorate) and showed that in goat's milk an estimated 7.8% (±0.03) was positive for *T. gondii* (Amairia et al., 2016). Molecular analysis by amplifying the sequence in the ITS1 region of *T. gondii* on 63 milk samples from naturally infected dairy goats in Italy showed that 20.6% (13/63) of milk samples revealed the presence of *T. gondii* DNA. In a previous study, in Italy, Mancianti et al. (2013), found *T. gondii* DNA in 13% (10/77) of milk samples from naturally infected goats. In the Slovak Republic, *T. gondii* DNA was confirmed in 32.56% (14/43) of goat milk samples and based on DNA polymorphisms at the SAG2 *T. gondii* locus identified goats infected with *T. gondii* genotype II (Spišák et al., 2010). Research in Egypt, also reported the presence of *T. gondii* in naturally infected goats and sheep (Abdel-Rahman et al., 2012; Sadek et al., 2015). Detection of T gondii in milk can be done using a bioassay or by PCR technique. The primers used for PCR were different target genes ranging from rRNA, ITS 1, B1, SAG 1, SAG 3, TOX 4, TOX 5. The percentage found in milk was T gondii with a low value of between 3.7% (Saad et al., 2018); 4.63% (Ossani et al., 2017); 7.8% (Aimaira et al., 2016). Although then, the results of serological tests using ELISA showed a relatively high prevalence of 90%, 38.9%, and 31.2%. It shows that even though goats get exposure to *T. gondii*, the infective phase of *T. gondii* does not necessarily reach the milk. In addition, it is necessary to evaluate the sample preparation method to detect *T. gondii* in milk. The average milking of goat's milk is one liter but preparing a PCR sample needed only 50 ml. Then does it represent the presence of *T. gondii* in milk? The methodology for preparing the milk sample needs to be re-examined.

In addition to cow, goat and sheep milk, Abadi et al. (2020), also detected the presence of *T. gondii* in buffalo and camel milk. They explained that the presence of *T. gondii* in the milk of the sampled animals was related to the season and age of the animal. Milk that detected the presence of *T. gondii* was milk collected in summer and autumn and from animals over the age of 2 years. Iacobucci et al. (2019) reported *T. gondii* DNA was detected in samples from five camels in Mongolia. Based on DNA sequence analysis from camel milk, it was revealed that two were from the potentially virulent *T. gondii* genotype. The presence of *T. gondii* was also found in donkey milk (Mancianti et al., 2014; Martini et al., 2014). Abadi et al. (2020) also found DNA of *T. gondii* in dairy products, namely cream, cheese, and butter.

Toxoplasma gondii survival in milk and its derivatives

Several researchers conducted studies that proved that *T. gondii* was alive and infective in milk or its derivative products by inoculation on experimental animals. Research by Hiramoto et al. (2001), assessed the infectivity of *T. gondii* cyst strain ME-49 which was intentionally infected in cow's milk and processed the infected milk into cheese. The results showed that the cyst infectivity of ME-49 strain *T. gondii* was maintained in milk even after storage for 20 days at refrigeration temperature and the cysts were also able to survive during the production process, both in fresh cheese and cheese stored for 10 days under conditions that same. Dubey et al. (2014), conducted an experimental infection of cats with cheese from an artificially infected goat's milk sample. The results of their research showed that the cat was infected with evidenced by the production of oocysts on days 14-18 after feeding of cheese.

Koete et al. (2017), conducted a study on the survival and infectivity of *T. gondii* tachyzoites in simulated gastric fluid (SGF) of various pH values representing different constitutions. To prove the oral consumption of milk contaminated with *T. gondii* tachyzoites, they also added different milk to SGF (25, 50, 75%) as well as in pure milk and evaluated the survival of the tachyzoites. The results showed that tachyzoites were still infective in SGF pH 5.0 and 6.0 for at least 90 minutes and sensitive to lower pH values. The addition of milk prolongs the survival of the tachyzoites in SGF. Asgari et al. (2011), showed that Toxoplasma tachyzoites are resistant to milk media and still remain infective even when heated at 37°C for 30 minutes. This is evidenced by infecting mice orally, and mice are infected. *T. gondii* cysts are relatively resistant to temperature changes and will survive at a temperature of 1-4°C. Storage at -12°C or lower allows some strains of *T. gondii* to survive. However, the cyst can die by heating to 67°C. Tachyzoites are more sensitive and can survive in pepsin acid solution for 2 hours, while in the milk, they can last between 1 to 2 hours. T. gondii Strain of ME 49 can survive in milk for up to 20 days if stored in the refrigerator. It showed that raw milk consumption is the source of toxoplasmosis infection. Milk pasteurization could avoid the transmission of toxoplasmosis through milk consumption. The heating process will kill the parasite, but It's was possible to find the DNA particle. It also confuses PCR testing that traces the particle of DNA parasite in milk. The T gondii PCR test results on milk do not mean that it is a source of infection in humans.

On the contrary, Ranucci et al. (2020), the cheese-making procedure was able to inactivate the parasite, as evidenced by the absence of *T. gondii* RNA by RT-PCR after 5 days of ripening. However, this study did not provide quantitative data regarding the amount of *T. gondii* in milk samples, so further research is recommended to determine the viable fate of *T. gondii* in the case of tachyzoites in milk, prior to cheese making. Takizoites of *T. gondii* in pasteurized milk will be inactive after being heated at 72°C for 15 seconds (Saridewi et al., 2015).

Raw milk as a risk factor for human toxoplasmosis

Unpasteurized milk and dairy products could be an important source of human infection with *T. gondii*. (Pereira et al., 2010). The same opinion was also conveyed by Deng et al. (2016), that the incidence of toxoplasmosis was due to consumption of raw goat's milk. According to Weiss (2020), cases of toxoplasmosis with risk factors for consuming raw goat's milk have been reported in 1978 in the United States. Ten of 24 people who consumed raw goat's milk were positive for toxoplasmosis but only 1 person showed pathognomonic clinical symptoms and nine others were asymptomatic.

However, in a systematic review of the risk of toxoplasmosis due to food consumption, unpasteurized milk was considered not to be a risk factor (Belluco et al., 2017). In a meta-analysis conducted by Boughattas (2017), the milk-borne route of infection in humans showed that the risk factors for the milk-borne *T. gondii* infection pathway were consumption of goat's milk and dairy products, immunocompromised, and living in North America, the Middle East, and the Latin region.

Rehman et al. (2020), assessed unpasteurised milk consumption as a potential risk factor for toxoplasmosis in females with recurrent pregnancy loss from the province of Khyber Pakhtunkhwa, Pakistan. The results demonstrated high seroprevalence of *T. gondii* antibodies in females of child bearing age that have consumed unpasteurised milk and it is a potential risk factor for developing toxoplasmosis.

CONCLUSIONS AND RECOMMENDATIONS

In both artificial and natural infections, *T. gondii* can be detected in the milk of lactating animals. The prevalence of toxoplasmosis in milk has been studied more in goats and sheep. Both cysts and tachyzoites are resistant in milk and dairy products. Raw goat's and sheep's milk is a risk factor for toxoplasmosis in humans and milk processing such as pasteurization with temperature can reduce the potential for transmission of *T. gondii* through milk consumption. However, it is necessary to re-examine the technique of testing T. gondii in milk through the PCR test. A positive result on the PCR test did not indicate the level of infectivity of T gondii through the consumption of raw milk.

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NOVELTY STATEMENT

The authors declare that thereis no conflict of interest in regards with the publication of this manuscript.

AUTHOR'S CONTRIBUTION

Conseptualisation: Mira Fatmawati, Lucia Tri Suwanti. Writing draft, review and approval of the manuscript: Mira Fatmawati, Lucia Tri Suwanti, Mufasirin.

Conflict of interest

The authors have declared no conflict of interest.

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