Zoonotic Toxoplasmosis through Meat Consumption

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Abstract

Toxoplasma gondii is a protozoan parasite that causes toxoplasmosis, which can infect both humans and animals. The three life stages of this parasite include tachyzoite, bradyzoite, and sporozoite. This parasite has a complex life cycle, which involves multiple interconnected stages. Consumption of raw or undercooked meat is the primary cause of transmitting this infection in humans, as well as exposure to contaminated seafood, fresh produce, water, soil, and the environment. Parasites spread through contact with cat faeces and contaminated water in animals. *T. gondii* is a significant zoonotic parasite of public health concern, and currently, no standardised method exists for its detection in food. Cat population management and the implementation of proper sanitation and hygiene practices play a vital role in controlling this parasite. It can also be inactivated by using the various processing methods like heating, freezing, cooking, high-pressure processing, curing, ionising radiation, and the application of chemical and biochemical treatments. There is a need to develop strong control strategies and standardised detection methods because the parasite's prevalence is increasing in various food products. All potential risk factors and modes of transmission of the parasite, along with available detection techniques and inactivation methods, are discussed. Furthermore, the impact of this infection in animals on human health should be acknowledged and addressed.

Keywords: Protozoan, Tachyzoite, Bradyzoite, Sporozoite, Parasite, Cat faeces, Ionising radiation

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Introduction

An intracellular obligate protozoan, *T. gondii,* which belongs to the genus Toxoplasma and in nature is zoonotic. This parasite has affected many communities worldwide and causes zoonotic toxoplasmosis (Almeria & Dubey, 2021). The transmission occurred across the Atlantic, As Recent studies suggest that *T. gondii* originated in South American felines and spread primarily through the migratory birds (Lehmann et al., 2006). The intermediate host for *T. gondii* is humans, birds and animals; besides these, cats, wild or domestic, are the main hosts that are capable of releasing oocytes in their faeces (Dubey, 2016; Marín-García et al., 2022). Progression of the infection and its pathogenicity depend on factors such as the genetics of the parasite, the dose of inoculated parasites, as well as the host's genetics and immune status. Because *T. gondii* is an exceptionally well-adapted parasite, capable of infecting a wide range of animal species & various types of cells. As a result, these parasites can persist in their hosts for extended periods, potentially for the whole life (Habegger de Sorrentino et al, 2005; Hunter & Sibley, 2012). Thirty per cent population of the world is seropositive, which tells us that 1 of 3 persons is infected due to *T. gondii,* as geographically, toxoplasmosis has spread globally (Khan et al., 2017; Marín-García et al., 2022).

People with weak immunity, like pregnant women and HIV patients, are at major risk as this infection is a major health concern (Almeria & Dubey, 2021). There will be no symptoms in people who have strong immunity, but there are chances that they can still be infected by *T. gondii,* which can be ocular, acute or chronic. One third of the pregnant women were found infected with toxoplasmosis globally which was found in a review conducted recently, beside this in France chances of toxoplasmosis reduced because of their hygienic condition, improved sanitation conditions and change in food habits during last thirty years (Yazdani et al., 2018; Picone et al., 2020; Rostami et al., 2020). The main host of spreading parasites is the cat through its faeces to people of all ages. Which can easily spread in the warm and humid areas (Senderowski et al., 2010). Besides this, toxoplasmosis can also be spread or transmitted to humans through raw or undercooked meat that is contaminated with the tissue cysts and raw vegetables contaminated with the oocysts of cat faeces and drinking contaminated water, which is considered a primary source. Secondary sources include the transplant of organs and blood transfusion; the placenta is one of the routes of transmitting the infection from mother to child during pregnancy (Ali et al., 2020). So, toxoplasmosis is an important emerging or re-emerging zoonotic disease globally. Toxoplasmosis has an injurious effect on the national economy and poses various risks to the health of humans. So, awareness about *T. gondii* is very important because it is connected to several illnesses and serious clinical outcomes.

Life Cycle & Transmission of *T. Gondii*

Among all the parasites, *T. gondii* has a specific life cycle (Figure 1) that can undergo asexual and sexual reproduction. 3 primary stages for infection to spread to the host, which include sporozoite (environmental stage), bradyzoite (divides slowly) and tachyzoite (divides rapidly). A host is required in sexual reproduction, like a cat and for asexual reproduction intermediate host is required (Smith, 2009; Al-malki, 2021).

Tachyzoites, a highly virulent parasitic form, are capable of infecting nearly any type of vertebrate cell. A subsequent stage, known as bradyzoites, induces the formation of cysts in various tissues. In muscle cells, cysts tend to be elongated, whereas brain cells typically harbour fewer, spheroid cysts. There is a notable size discrepancy between younger and older cysts. Juvenile cysts generally measure around 10 m, although early-stage cysts have been documented to reach sizes up to 100 m. The mature cysts are composed of thousands of densely packed bradyzoites. Additionally, several invaginations have been observed (Guevara et al., 2020). There will be long-term survival of cysts in the bradyzoite stage within the intracellular environment because of their well-adapted mode of life. Bradyzoites exhibit resistance to the host's pepsin enzyme, enabling them to endure for 1–2 hours in the presence of pepsin-HCl (Figure 1). 12-13 meter oocytes contain sporozoites, and the oocyst wall is a multilayer structure that shields the organism from both chemical and mechanical stress, which enables the parasite to withstand environmental conditions for over a year (Mai et al., 2009).

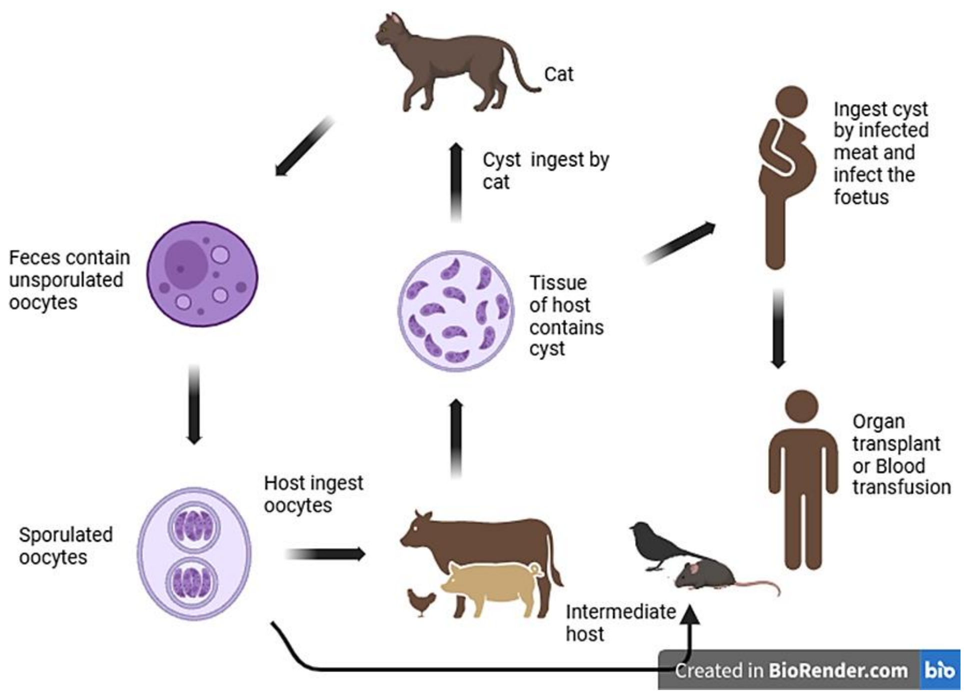

Fig. 1: Life Cycle of Toxoplasma Gondii

Transmission Ways

T. gondii spreads normally by water, organ transplant, food which is contaminated by oocytes and tissue cysts (Figure 2) (Al-Malki, 2021). According to (Jones and Dubey, 2010; Reiling & Dixon, 2019), this pathogen is transmitted to humans in three ways:

1. When ingesting sporulated oocysts, which can persist in water and soil for months or even years, this is considered the main source of infection and transmission.
2. Consuming undercooked or raw meat and organs from infected animals can lead to the ingestion of tissue cysts.
3. Congenital transmission, when tachyzoites pass from mother to fetus through the placenta.

Across the different regions, the development of toxoplasmosis in immunocompetent individuals appears consistent. The prevalence among immunocompromised patients is higher in certain countries, largely due to factors such as HIV/AIDS infections, organ transplants, and the use of immunomodulatory medications (Ahuja J & Kanne J P, 2014). Poverty and socio-economic factors play a role in the transmission of this parasite. Key points include limited access to safe drinking water, lifestyle differences between urban and rural areas, cooking methods, dietary habits, and overall hygiene conditions (Reiling & Dixon, 2019). Among parasitic zoonoses, *T. gondii* is considered a priority hazard in the meat production chain (Foroutan et al., 2019).

Effects of Toxoplasmosis

According to (Tenter et al., 2000), Raw meat and undercooked meat containing tissue cysts by which humans got infected after consumed that infected meat. And that meat is contaminated by oocytes that are present in soil or by polluted water, and vegetables or fruits that are contaminated by these parasites. *T. gondii* has been linked to various illnesses and serious clinical outcomes. This parasite can invade multiple organs in the human body (Figure 2). *T. gondii*-related human disorders include:

1. Heart disease
2. Encephalitis in AIDS patients
3. Prostate cancer

4. Asthma
5. Autoimmune diseases

Epidemiological studies have shown that *T. gondii* is present in pigs worldwide, with prevalence varying based on factors such as age, pig category, geographical region, and management practice. 12.3% off *T. gondii* occurrence was found in direct identification of pigs, with a 95% confidence range varying from 0% to 55% (Belluco et al., 2018).

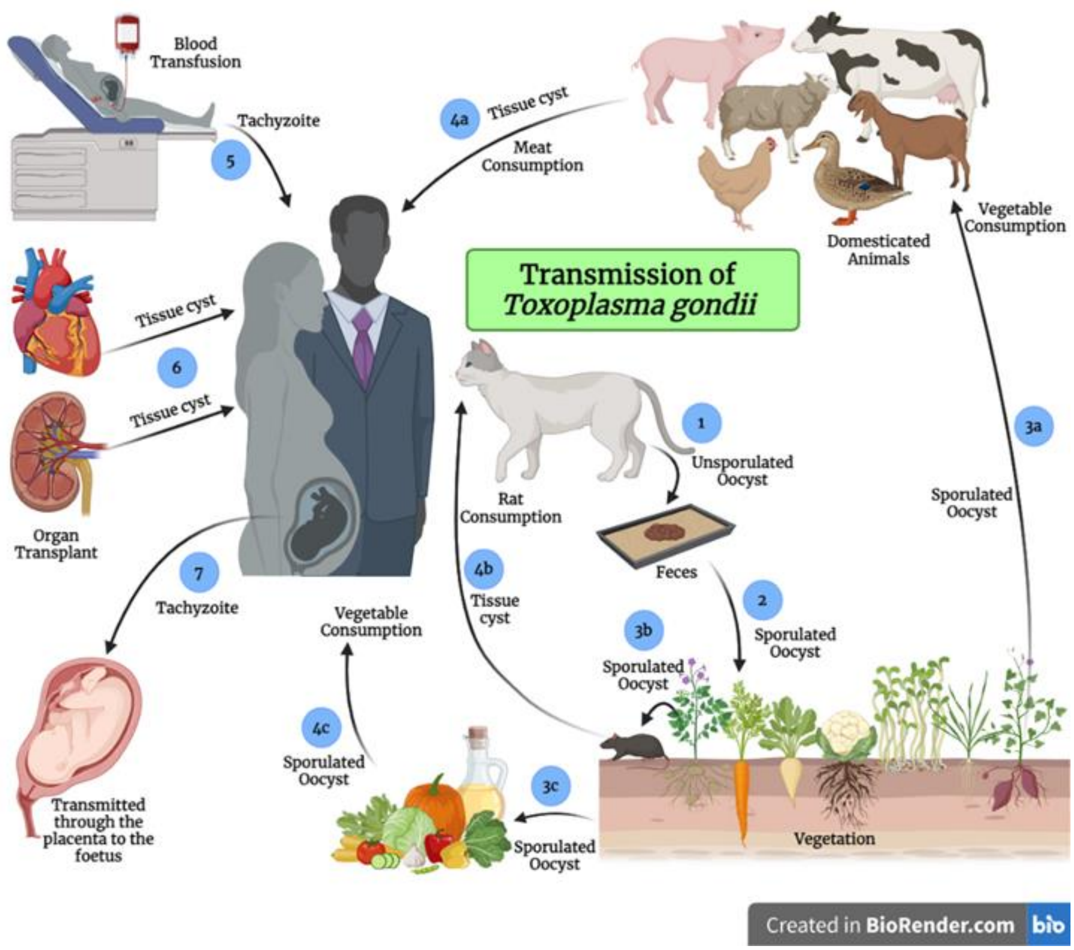

Fig. 2: Transmission of *T. gondii*

Toxoplasmosis in Meat

Toxoplasmosis has been reported in animals which produce meat worldwide. Meat and meat products from animals which are infected may contain tissue cysts of the parasite. Humans can become infected by consuming raw or improperly cooked meat from these farm animals. According to the European Food Safety Authority, approximately 60% of human toxoplasmosis cases are attributed to the consumption of beef, pork, and mutton (Innes et al., 2019; Marín-García et al., 2022).

The number of cysts found in meat is generally low, almost about one cyst per 100 grams. The cysts in musculature and viscera vary among hosts. Cysts are more commonly found in the tissues of swine, sheep, and goats, whereas they are less commonly found in chickens, horses, and rabbits. Infected cattle and buffaloes rarely have tissue cysts (Sroka et al., 2020; Smith et al., 2021).In a recent Scottish study, the occurrence of *T. gondii* in meat from retail shops was investigated, and *T. gondii* DNA was found in 4.2% of pork, 6.9% of mutton, and none in beef from cattle.

Toxoplasma prevalence is also greater in mutton and pork than in beef and poultry, and sheep meat is the most dangerous for infection among all the livestock (Belluco et al., 2018; Plaza et al., 2020). Raw or undercooked meat obtained from cattle, if consumed by humans, there is chance of transmission of infection, besides the fact that cattle are clinically resistant to *T. gondii* infection. Bioassay method used for the detection of parasites in a study, which showed that out of 385 samples, only 6 samples were infected with toxoplasmosis. There is almost no chance of infection from the meat of water buffalo because there was no tissue cysts were found in that meat (Bărburaș et al., 2019; Opsteegh et al., 2019; Almeria & Dubey, 2021).

Slaughterhouse hygiene is often inadequate, with viscera left exposed, potentially serving as a source of transmission in developing countries. The parasite can spread if hands are not properly washed after handling or cooking meat. Pakistan, one of the largest poultry meat producers, where poultry is considered an economic and common and easily available meat source, accounting for 28% of total annual meat production. Increasing consumption of poultry meat in Pakistan and also globally, due to its availability and affordability, may contribute to the transmission of *T. gondii* in humans (Sohaib & Jamil, 2017; Khan et al., 2020).

In China, commercial pork has been found to have a high prevalence of *T. gondii*. The parasite was isolated from pigs that were raised in natural environments for human consumption. Testing using MC-qPCR revealed that 92 out of 914 heart samples were infected, while mouse biopsy assays detected the parasite in 9 out of 14 samples, which proves the high prevalence of infection in pork (Gisbert-Algaba et al., 2020).

The rearing system of animals plays a crucial role in *T. gondii* prevalence, because organically raised animals show higher infection rates compared to those raised under intensive care. Parasite-free processed meat products have been found, which indicates that proper processing techniques and methods effectively eliminate it from all types of meat. However, when meat is not subjected to industrial processing procedures, the prevalence may increase (Gomez-Samblas et al., 2021; Marín-García et al., 2022).

Toxoplasmosis in Seafood, Fresh Food & Water

Fresh foods like fruits and vegetables can be contaminated by cat faeces, and polluted water contains *T. gondii* and which can be transferred to humans. There's no specific laboratory technique to detect the oocysts in food samples and the environment, because it is difficult to isolate and concentrate oocysts from complex matrices like raw vegetables (Temesgen et al., 2019). Immunomagnetic Separation Assay (IMS), Loop-Mediated Isothermal Amplification (LAMP) test, and Real-Time Polymerase Chain Reaction (RT-PCR) are the techniques that are used for detecting *T. gondii* oocysts in food samples (Marín-García et al., 2022). Recent studies showed that there is a link between *T. gondii* outbreaks in humans and the consumption of green vegetables contaminated with irrigation water (Cabral-Monica et al., 2020). And recent meta-analysis showed that vegetable consumption has become the most frequent route of *T. gondii* transmission in the future. In Poland, 9.7% of toxoplasmosis prevalence was found in vegetables that are collected from home gardens and local shops (Pinto-Ferreira et al., 2019; Cabral-Monica et al., 2020). *T. gondii* occurrence in fresh foods was reported: 3.6% in China, 21.2% in Morocco, and 0.8% in Italy. Moreover, 0.8% of ready-to-eat salad samples were found to be contaminated with oocysts with high parasite load in the positive samples (Barlaam et al., 2021; Marín-García et al., 2022).

T. gondii infection in fish and shellfish is a risk factor for both aquatic life and humans. Consumption of raw or undercooked fish and shellfish is the main route of transmission from seafood to humans. Human activities and climate change are increasing the number of pathogens in aquatic environments, which leads to a rising prevalence of *T. gondii* in various seafood species consumed by both humans and wildlife (Jones et al., 2009; VanWormer et al., 2016). Minimising *T. gondii* infection from its source, like managing cat faeces properly and preventing the discharge of polluted wastewater into aquatic habitats, is are essential strategy to reduce the risk of exposure through seafood consumption (Shapiro et al., 2019).

Detection Methods of *T. gondii*

T. gondii is a significant foodborne pathogen transmitted through zoonotic sources, yet no comprehensive strategies have been implemented for its control (Bouwknegt et al., 2018). For the detection of *T. gondii* in food samples, the ISO has to establish standardised methods and specific guidelines. However, various detection techniques are available as the most advanced and reliable approach for identification of the parasite's DNA in diverse food samples (Marín-García et al., 2022).

Serological methods: For the detection of *T. gondii* in animals and humans, serological methods are known as indirect techniques. They can be applied to food samples, like meat and meat juice, for the identification of the presence of parasites. Primarily used as screening tools to detect infections in animals, these methods require confirmation through bioassays performed on tissue samples (Marín-García et al., 2022). The most commonly employed serological methods for detecting *T. gondii* include the Latex Agglutination Test (LAT), Indirect Fluorescent Antibody Test (IFAT), Western blot, and ELISA. These techniques play a crucial role in the identification of infections by detecting specific antibodies that work against the parasite (Ismael, 2021). These techniques are utilised to distinguish between the chronic and acute phases of *T. gondii* infection. In the initial stage of infection, Igm antibodies are produced while Igg antibodies take time to appear, like 7–14 days. And will reach its peak concentrations between 30 and 60 days post-infection. Since Igm is indicative of an acute infection and Igg signifies a chronic infection, these methods detect the respective antibodies, which enables the identification of both chronic and acute toxoplasmosis (Molaei et al., 2022). For the confirmation of the results obtained from serological tests, additional confirmatory methods are necessary (Robert et al., 2021).

Animal bioassay: Effective methods for detecting infections caused by *T. gondii* bradyzoites and oocysts. The cat bioassay is considered the most efficient technique among all because cats serve as the definitive host for the parasite. The mouse bioassay is a reliable alternative which frequently used for the detection and isolation of *T. gondii* from various samples (Marín-García et al., 2022; Dubey, 1998). Cats are fed approximately 500g of the test meat sample to assess contamination with *T. gondii* cysts in the cat bioassay. After three weeks, the presence of oocysts in the cat's faeces is examined, and in serum samples, specific antibodies against the parasite are also detected. Due to ethical concerns and the high costs associated with this method, the cat bioassays are conducted only in a limited number of laboratories (Opsteegh et al., 2016; Dubey et al., 2021).

Thermal treatments: For the prevention of toxoplasmosis in both animals and humans, effective control measures are taken which inactivate the parasite in animal tissues and eliminate oocysts from the environment. Methods like chilling, heat treatment, and thorough cooking have proven to be effective methods in lowering the risk of infection and also ensuring food safety (Mirza-Alizadeh et al., 2018). In all categories of food, high temperatures are not universally effective for inactivating *T. gondii*. While cooking at high temperatures can efficiently eliminate the parasite in meat and in certain foods, such as fresh fruits and vegetables, which require alternative methods like refrigeration, freezing, or other low-temperature treatments to reduce the risk of contamination while preserving their quality (Pinto-Ferreira et al., 2021). While considering the effect of lowered temperatures on the inactivation of tissue cysts in meat, freezing is an effective method. Research indicates that *T. gondii* cysts can be inactivated at a minimum temperature of -20°C when maintained for at least three days. And making freezing a practical approach to reducing the risk of infection (Djurković-Djaković & Milenković, 2000).

Microscopic techniques: Microscopic examination is a valuable method for identifying the parasite in various samples, by which *T. gondii* can be detected. This technique allows the detection of *T. gondii* in matter, water, environmental samples, shellfish, and fresh food products. This supports the evaluation of contamination and potential transmission risks associated with it (Liu et al., 2015; Marín García et al., 2022). *T.*

gondii can be visualised but cannot be reliably distinguished from other apicomplexan parasites when non-specific stains such as eosin, Giemsa, and hematoxylin are used. To enhance specificity and improve the detection sensitivity, specific staining techniques are used, which include the use of antibodies and fluorescence-conjugated enzymes. *T. gondii* can now be more precisely distinguished from another similar organisms due to these advanced techniques (Dubey, 2010; Bajnok, 2017). A significant drawback of the microscopic detection method is that it can produce false-negative results even when the sample is positive for *T. gondii*. This happens mainly due to the small sample size, as the specific area which is examined may not contain the parasite, and other parts of the sample could be contaminated. This limitation highlights the need for additional detection techniques to improve the precision of diagnostics. (Marín-García et al., 2022).

Ionising radiations: This is a non-thermal method which utilises the radiation to pasteurise the food and effectively inactivate, reduce, or eliminate pathogens like fungi, insects, and pests. The most commonly used radiation types include gamma rays and ultraviolet rays. Gamma radiation sources typically include X-rays, cobalt-60, caesium-137, electron beams, and electron beam accelerators. Studies on the effects of radiation on *T. gondii* have shown that it is effective in inactivation the parasite, which makes it a promising method for enhancing food safety (Mirza-Alizadeh et al., 2018).

Curing methods: Meat preservation is achieved through curing methods that utilise the nitrites, nitrates, salts, and sugars (such as sucrose) in conjunction with low-temperature smoking. These methods not only extend shelf life but also improve flavour and inhibit the growth of pathogens, including *T. gondii,* which contributes to improving food safety and quality (Bayarri et al., 2012). Salts are crucial in inactivating *T. gondii* cysts. The effectiveness of this method depends on various factors, which include the maturity of the parasite, the temperature at which the meat is stored, and the concentration of salt applied. Higher salt concentrations and optimal storage conditions enhance the inactivation process, making salting an effective preservation and parasite control strategy in food products (Hill et al., 2004; Kijlstra & Jongert, 2008).

Conclusion

Toxoplasmosis is a zoonotic disease and has different routes of transmission. 30% population is infected with *T. Gondii,* and the main routes of toxoplasma infection spread are by eating undercooked or partially cooked meat and through cats, cattle, and roaming animals that are infected with *T. gondii*. Other factors that are responsible for transmission to humans include exposure to seafood, water, soil, and the surrounding environment. Due to the complex nature of food, such as fresh meat or food, the detection of parasites is difficult. The majority of pregnant women lacked awareness regarding toxoplasmosis, including its preventive measures, risk factors, symptoms, and the timing of infection. However, their attitudes and practices were generally more favourable, likely due to local knowledge that instilled confidence in their ability to prevent various infections.

Parasites can be managed at both the farm and domestic levels through the proper hygiene practices during food handling and processing, as well as by controlling cat populations. Its transmission can be reduced by implementing preventive measures. Additionally, various processing techniques can effectively destroy or inactivate the parasite in food, like thermal methods (such as heating, chilling, and cooking), non-thermal methods (including high-pressure processing, ionising radiation, curing, and cold plasma), and chemical or biochemical treatments. However, detection of parasites in the samples of food is both costly and challenging, highlighting the need for the development of affordable and rapid detection methods. Further research is required to improve parasite control and inactivation across domestic, production, and consumer levels. Educating the local population could play a crucial role in preventing toxoplasmosis.

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