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REVIEW ARTICLE

Flow of Zoonotic Toxoplasmosis in Food Chain

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A B S T R A C T

Toxoplasmosis is a disease caused by a protozoan parasite known as *Toxoplasma gondii*. This parasite infects both human and animal species. In humans, it is transmitted by consumption of raw and under cooked meat. The other risk factors include infected sea foods, fresh foods, water, soil and environment. While in animals, it is transmitted through cat feces and contaminated water. *T. gondii* is an important zoonotic parasite of public health concern and still there is no standard detection method for its detection in foods. The mostly used detection methods include molecular techniques, cell culture methods, animal bioassays, microscopic and serological methods. The parasite can be controlled by controlling cat population and proper sanitation and hygienic measures. Parasite is inactivated by different processing methods such as heating, freezing, cooking, high pressure processing, curing, ionizing radiations and use of chemicals and biochemicals. As, the prevalence of the parasite is increasing in different food products, so the strong control measure strategies and standard detection methods need to be developed. This review covers all the possible risk factors and modes of transmission for the parasite along with detection methods and inactivation techniques.

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INTRODUCTION

Zoonotic toxoplasmosis is a disease affecting the communities globally and is caused by a protozoa named as *Toxoplasma gondii* that is the sole species from the genus of *toxoplasma* (Almeria and Dubey, 2021). This parasite infects humans, domestic animals, wild animals, livestock and birds (Shani *et al*., 2012; Shapiro *et al*., 2019). Animals, humans and birds (Marín-García *et al*., 2022) act as intermediate host for *T. gondii* while cats either domestic or wild act as final host and they are the only host that can release oocysts from their feces (Dubey, 2016). As symptoms do not appear in most of the cases but still it causes a massive disease burden and deaths in humans and animals (Jones *et al*., 2012). Toxoplasmosis is very important water and food born parasitic zoonoses and has importance in veterinary and medical field (Havelaar *et al*., 2012). This parasite is transmitted via three basic transmission passages including direct transmission from mother to newborn through placenta, consumption of food, water and soil contaminated with cyst and the consumption of animal tissues infected with oocyst (improperly cooked meat). Transmission of the parasite (oocyst) through the environment is not studied properly since the discovery of the parasite in 1908 (Ferguson, 2009) because of the unavailability of the methods to detect the cyst in the environmental matrix (Dumètre and Darde, 2003; Shapiro *et al*., 2019).

Toxoplasmosis is distributed geographically all over the world and 30% of the world's population is seropositive (Marín-García *et al*., 2022) suggesting that one of the three persons is infected with *T. gondii*, globally (Khan *et al*., 2017). This parasite is major pathogen of food in America. From the CDC reports, it is evident that *T. gondii* along with Salmonella and Listeria accounts for 70% of all deaths caused by foodborne pathogens in the United States of America (Scallan *et al*., 2011). This report suggests that almost 24% of all deaths occurring due to foodborne illness are due to *T. gondii* and it costs $2,973 million because of disease (86,686 diseased and 327 deaths every year in America). Epidemiological studies show that eating raw or improperly cooked meat infected with *T. gondii* is the main cause of the illness in the United States of America (Guo *et al*., 2015).

T. gondii is an important public health concern in women during pregnancy and the individuals with compromised immunity (HIV patients) (Almeria and Dubey, 2021). Symptoms of toxoplasmosis do not appear

in healthy individuals, but it can cause adverse health outcomes and illness in neonates and individuals with weak immune systems (HIV individuals, use of corticosteroids for long periods and individuals with transplant) (Robert *et al*., 2018; Mcleod *et al*., 2020). Although, symptoms are absent in individuals with strong immunity but still they can develop chronic, acute and ocular toxoplasmosis (Yazdani *et al*., 2018). In systematic review and metanalysis conducted recently, 33.8% of women in pregnancy found infected with latent *T. gondii* worldwide, South American countries contributing largest ratio (56.2%) and the region of Western Pacific shares the smallest ratio (11.8%) of this disease (Rostami *et al*., 2020). The burden of toxoplasmosis has decreased in the countries with high economies like France during last 30 years. This is because of the reduced contact with the parasite due to change in food habits and improved sanitation and hygienic conditions (Picone *et al*., 2020).

40-60% transmission of toxoplasma illness is from food sources (European, 2019). Reports indicating the positive food samples such as meat, uncooked shellfishes and mollusks, fish, honey and portable water and this parasite was added in the third category for the monitoring of zoonotic parasites (European, 2021). There is an influence of socio-economic factors also in the progression of this disease (Reiling and Dixon, 2019). The factors involved in the positive seroprevalence of this disease include infection of domestic and wild host reservoirs, availability of safe drinking water, rural and urban living conditions, variety of foods, cooking methods of foods (uncooked versus dried and freeze) and hygienic conditions (hand washings) (Dubey and Frenkel, 1972).

Number of studies have been conducted on different aspects of toxoplasmosis. This review is carried out on the foodborne transmission of *T. gondii.* The aim of this study was to discuss all the possible foodborne risk factors for the transmission of the parasite, detection of the parasite in different foods and how we can control and reduce the burden of this disease in community.

Life cycle of *Toxoplasma gondii*: *Toxoplasma gondii* has special life cycle among all the parasites and consists of sexual and asexual methods of reproduction (Al-malki, 2021). This parasite has three main stages (Fig. 1) that infect the host and include tachyzoite, bradyzoite and sporozoite. Sexual reproduction of the parasite occurs only in the definitive host cats (member of Felidae family) while asexual cycle of the parasite takes place in the intermediate host (Dubey, 2009; Smith, 2009). The process of infection starts by infecting the cats (domestic or wild) by consuming the prey contaminated with tissue cyst or oocyst. After the consumption, cyst lining/wall is ruptured in the intestine of definitive host because of the activity of some enzymes (proteolytic enzymes) and low pH (Freppel *et al*., 2019) liberating sporozoite or bradyzoite (Attias *et al*., 2020). In both cases, either sporozoites or bradyzoites they invade the epithelial cells of intestine and are converted to schizonts (asexual reproduction stage). This stage produces merozoites and release these parasites from enterocytes. These merozoites then infect further epithelial cells and schizogony occurs. There are five different types of *T. gondii* before the start of gametogony in the intestinal cells of cats (Dubey and Frenkel, 1972).

Macrogametes and microgametes are produced after three to four days of initial infection (Frenkel *et al*., 2006). Macro and micro gametes fuse and form immature oocysts that are converted to mature forms on environmental exposure after secretion in the feces. Four sporozoites are present in each sporocyst and these sporocysts are more resistant to the environmental conditions (Dubey, 1986; Dubey *et al*., 1998).

After the ingestion of oocysts by the intermediate host the release of sporocysts occurs in the intestine and epithelial cells are invaded. Sporocysts are converted into tachyzoites causing acute infection. Vertical transmission of the tachyzoite parasite occurs by crossing the placenta while first infection of the intermediate host (McAuley, 2014). Conversion of tachyzoites into bradyzoites occurs due to the immune response. Bradyzoites cause slow infection and are present for longer period in the skeletal muscle, heart muscles and in the central nervous system in the form of tissue cyst (Ferguson *et al*., 1989; Sokol-Borrelli *et al*., 2020). Tissue cyst containing bradyzoites are source of infection for definitive host (cat). When they are consumed, the tissue cysts are transformed into sexual stages and produce thousands and millions of oocysts that are ready to infect other hosts and thus completing the cycle (Dubey and Frenkel, 1972).

Transmission to Humans and Risk Factors: *Toxoplasma gondii* is at second number responsible for the most deaths due to food borne infections and illnesses in America (Gao *et al*., 2016). Initially disease is a symptomatic and may be acute or chronic and symptoms may appear at any stage of life (Boyer *et al*., 2011). Undetected *T. gondii* oocysts in the environment are the leading cause of its transmission in the animals and humans (Van *et al*., 2016). The main risk factors for the distribution and transmission of *T. gondii* are consumption of contaminated raw or improperly cooked meat; consuming polluted soil, vegetables, water and any substance that is contaminated with *T. gondii* oocysts from the feces, organ transplants, blood exchange and contaminated milk if it is unpasteurized (Aguirre *et al*., 2019).

There are three main ways (Fig. 2) of transmission of this pathogen to humans (Reiling and Dixon, 2019).1. Ingesting sporulated oocysts that have the capacity to persist for months and years in the water and soil (the main source of transmission and infection). 2. Ingesting tissue cysts by consuming undercooked and raw meat or organs of infected animals. 3. Can be transferred congenitally through placenta (by tachyzoites) from mother to fetus (Jones and Dubey, 2010).

After the completion of initial infection cycle in humans, replication of the parasite becomes slow, and the condition is known as latent toxoplasmosis. When the individuals become immunocompromised, the dormant tissue cysts start replicating and repeating the life cycle. Showing harsh flue like signs and symptoms, blurred vision and encephalitis toxoplasmosis (Reiling and Dixon, 2019). It also alters the cell signaling process leading to nervous system disorders such as epilepsy, Alzheimer's disease, schizophrenia and Parkinson's disease (Burgdorf *et al*., 2019). *T. gondii* infections also because behavior change in humans (Sutterland *et al*., 2019). Toxoplasmosis is the cause of premature birth and other neurological disorders when transmitted congenitally (Reiling and Dixon, 2019).

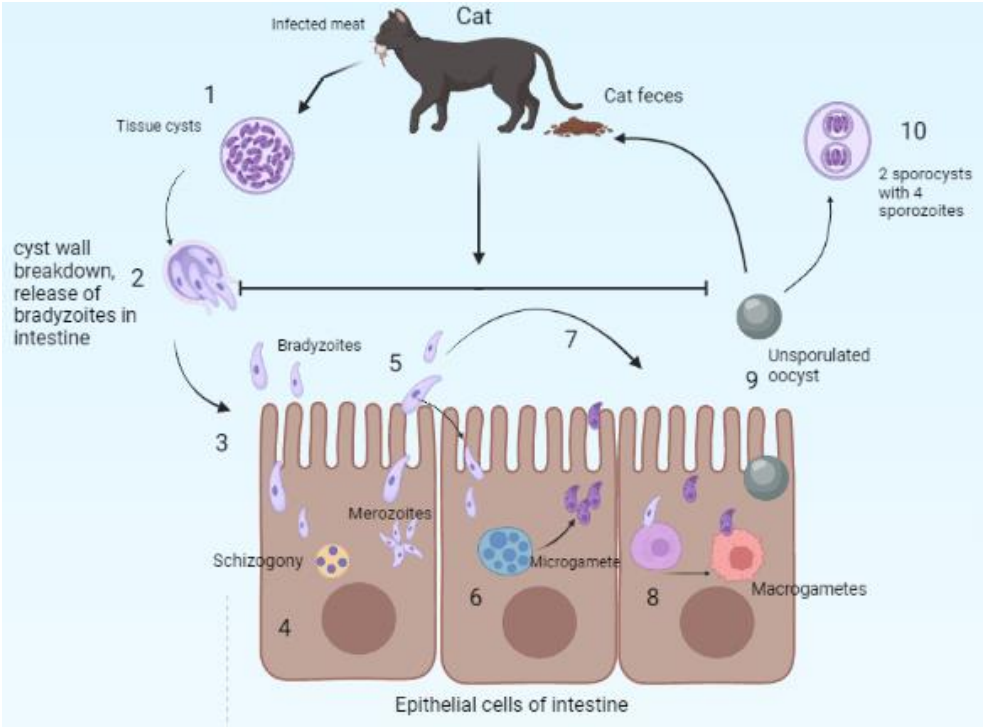

Fig. 1: Life cycle of *Toxoplasma gondii*. 1 Infected meat ingestion. 2 Digestion of cyst wall in stomach and intestine. 3 Invasions of bradyzoites on epithelial cells in intestine. 4 Division of bradyzoites and formation of merozoites. 5, 6 Formation of micro and macro gametes from merozoites. 7-9 Formation of unsporulated oocysts by fertilization. 10 Formation of sporocysts in environment.

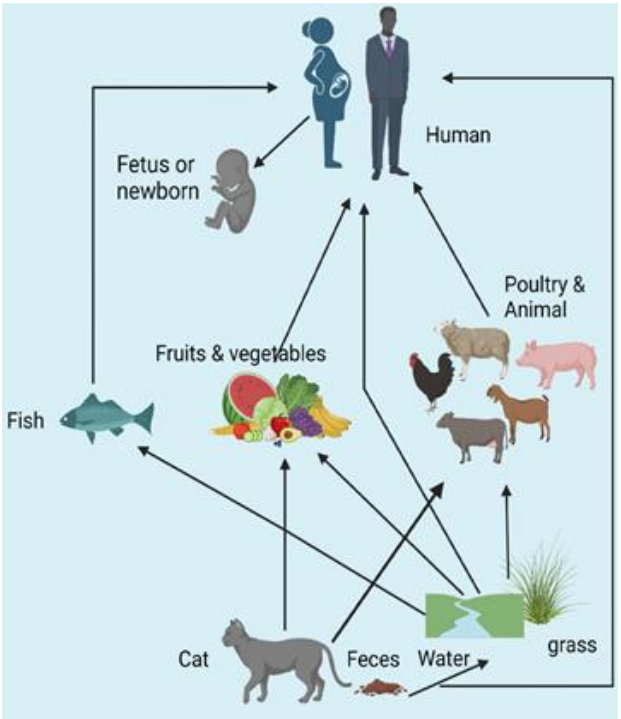

Fig. 2: All possible routes of transmission of *Toxoplasma gondii* to humans

Poverty and socio-economic factors also play a significant role in the transmission of this parasite. These factors include unavailability of safe drinking water, closeness to wild or domestic infected reservoir, differences in lifestyle in cities and villages, methods of cooking foods, variety of foods consumed and hygienic conditions (Reiling and Dixon, 2019).

Following are possible risk factors for the transmission of *T. gondii* to humans.

Poultry: Chicken, ducks and goose do not develop clinical signs and symptoms of toxoplasmosis. Chickens are important hosts for *T. gondii* and are source of infection for cats that shed oocysts in the environment with their feces. Thus, chicken plays an important role in the epidemiology of toxoplasmosis. This parasite is present most commonly in the muscles than in brain so chicken meat becomes an important source of infection for humans (Dubey and Beattie, 1988).

In America nearly 8.5 billion chickens are slaughtered yearly for human use and 37.2kg of chicken meat is consumed per capita every year. Studies showed that *T. gondii* was absent in the meat samples taken from the retail shops (Dubey *et al*., 2005). All the samples collected from the shops were *T. gondii* free. This is because of the reasons such as the processing of meat in the processing plant kills the parasite. Freezing and application of salt solutions reduce the parasite in the chicken meat (Guo *et al*., 2015).

Chicken raised in the confined and controlled environment (farms) has low prevalence of the parasite as compared to the free ranging chicken and chickens kept in backyards (Schares *et al*., 2017). Free living chickens have greater chances of ingesting parasite from soil, water and environments (Stelzer *et al*., 2019). Chicken raised in homes has free access to grazing environments and age is longer compared with chickens raised in industrial level, so they have greater chances of contamination with parasite (Aboelhadid *et al*., 2013). Chickens kept in confined environments have minimum chances of contact with parasite so having no contributions for the transmission of *T. gondii* to humans (Aboelhadid *et al*., 2013).

In developing countries hygienic conditions at slaughterhouse are not so good and viscera is left openly that may be source of transmission. Parasite can be transmitted if hands are not washed properly after cutting

and cooking of meat. Pakistan is one of those countries which are larger poultry meat producers and poultry is the cheap and common source of meat contributing 28% of all the meat produced every year (Sohaib and Jamil, 2017). These shifts towards high poultry meat consumptions in Pakistan and all over the world can be the source of *T. gondii* transmission in humans (Khan *et al*., 2020).

T. gondii is identified in the ovaries and oviducts of the egg laying hens but there is no evidence for the presence of the parasite in shelled eggs (Peixoto and Lopes, 1990). In another experiment 2214 eggs were laid by experimentally infected hens and no egg was found infected with parasite (Boch *et al*., 1966). In a false experiment to examine the presence and survival of this parasite, it was injected into the egg yolk and albumin and yolk was fried for three minutes and albumin boiled for three minutes, and the parasite survived these conditions (Pande *et al*., 1961). Although there is no evidence of *T. gondii* from egg but still consumption of raw egg should be avoided (Dubey, 2010).

Beef, mutton and pork: Toxoplasmosis is reported in all the meat producing animals globally. Meat and meat products from these infected animals contain tissue cysts of the parasite (Marín-García *et al*., 2022). The parasite is transmitted to humans when raw and improperly cooked meat and meat products are consumed, obtained from these infected farm animals (Innes *et al*., 2019). Data from European Food Safety Authority reveals that nearly 60% of the toxoplasmosis in humans is transmitted through consumption of pork, beef and mutton (Innes *et al*., 2019).

The prevalence of toxoplasma in pork and mutton is higher than beef and poultry and meat obtained from sheep has the highest risk of obtaining infection in humans as compared to all other livestock (Belluco *et al*., 2018). In a systematic review, the prevalence of *T. gondii* was found 2.6% in cattle, 12.3% in pigs and 16.7% in sheep globally (Belluco *et al*., 2016). Recently a study was conducted in Scotland to observe the prevalence of *T. gondii* in meat from retail shops and it showed the presence of *T. gondii* DNA in 4.2% pork samples, 6.9% of mutton samples and no presence of DNA of parasite in beef obtained from cattle (Plaza *et al*., 2020).

In spite of the fact that cattle are resistant clinically to toxoplasmosis but still they have the potential to transmit the infection in humans if meat obtained from cattle is consumed raw or improperly cooked (Almeria and Dubey, 2021). The chances of transmission of infection through beef are minimum. In research conducted in France, only 2 heart samples out of 200 seropositive cattle were found infected with *T. gondii* parasite (Blaga *et al*., 2019). Another study using bioassay method of detection of parasite revealed that only 6 liver samples out of 385 samples were found infected with this parasite (Opsteegh *et al*., 2019). There is no presence of tissue cyst in the meat of water buffalo and hence there are minimum chances of human infection from this meat (Barburaș *et al*., 2019).

According to the USDA classification, sheep are one-year-old while those without permanent teeth are known as lamb (U.S. 2013). American people use only lamb meat for their own consumption while meat of sheep is used in feeds of pets or exported to other countries. The prevalence of *T. gondii* infection in sheep and lamb is high. Another study in America showed 62% prevalence of the parasite in sheep (U.S. 2011). Sheep in the outdoor environments are exposed to soil and water that is main source of parasite. *T. gondii* can infect sheep at any stage of their life and the chance of infection increases with the age (Dubey, 2009). A study shows 37.7% seroprevalence of parasite in one-year-old sheep while 73.8% in 6-year-old sheep (Katzer *et al*., 2011). Another study in America showed 74% seroprevalence of *T. gondii* infection in sheep (Huong and Dubey, 2007). This data suggests that the meat from lamb and sheep can be a source of infection for humans. In France, 17.7% of diaphragm and heart samples out of 433 samples were tested positive for *T. gondii* in slaughtered lambs (Guo *et al*., 2015). In a survey conducted in United Kingdom, six samples out of nine lamb cut samples collected from merchant sources were found positive for the DNA of *T. gondii* (Aspinall *et al.*, 2002).

People use goat milk and meat all over the world and from last few years its demand has increased due to the increasing populations of religious and ethnic groups as they use meat of goat for this purpose (Geisler, 2014; Solaiman, 2007). *T. gondii* is transmitted through goat meat when raw or improperly cooked goat meat and raw milk are consumed. The prevalence of this parasite in goat is lower than sheep (Kamani *et al*., 2010; Asgari *et al*., 2011; Munoz-Zanzi *et al*., 2012; Lopes *et al*., 2013). The reason behind the difference in prevalence among sheep and goat is not clearly identified but some factors such as breed, feeding behavior and susceptibility may induce this difference (Sharif *et al*., 2007; Ramzan *et al*., 2009; Tzanidakis *et al*., 2012). Prevalence of the infection is higher in those animals kept on grazing lands than the animals feeding on stalls. Goats consume upper part of the grass and trees while sheep consume lower parts and lower parts may have greater number of the parasite due to which chances of infection in sheep are higher than goat. The prevalence of *T. gondii* in goat milk is greater than in goat meat and contributing factors for this difference may be age and gender (Guo *et al*., 2015).

In the past, some outbreaks occurred because of the consumption of raw and unpasteurized goat milk (de Andrade and Neves, 1984). In a study, *T gondii* was isolated from goat milk and it remained alive when treated with cold enzyme in fresh cheese (Dubey *et al*., 2014). In another study, parasite was absent in traditionally made cheese in limited quantity from raw milk of sheep obtained from the animals infected naturally (Ranucci *et al*., 2020). However, an outbreak was reported in recent years by consuming traditionally made cheese from cow milk. The possible source of the *T. gondii* may be the contaminated water used during the manufacturing of cheese (da Costa *et al*., 2019).

Pork meat is source of infection for humans when it is contaminated with *T. gondii* parasite. Research conducted in America indicted that nearly 41% of food base toxoplasma infections were due to the consumption of pork meat (Batz *et al*., 2012). Pigs are carnivorous and can get sporulated oocysts of parasite from contaminated water, soil and foods. The other possible source of *T. gondii* for pigs is the consumption of the animals (rodents and birds) that are infected with tissue cysts of parasite. Pigs raised outside or in the open environment have more chances of getting infection than those raised in confined places because in open environments they have greater probability

of contact with parasite (van der *et al*., 2007). Recently, the reduction of the contamination of pork is observed but still the production of outdoor pigs may impact the trend (Limon *et al*., 2017). Commercial pork is observed highly infected with the parasite in China (Wang *et al*., 2012). Toxoplasma parasite was isolated from the meat of the pigs raised in natural environments and used for human consumption, 92 out of 914 heart samples were found infected (tested by MC-qPCR) and 9 out of 14 samples were positive when assayed by mouse biopsy (Gisbert *et al*., 2020).

The system where the animals are raised is important as the prevalence is high in those animals that are raised in organic ways as compared to the animals raised in intensive care (Gomez *et al*., 2021). The processed products were found free of the parasite so the proper processing methods can kill the parasite in all kinds of meat. The prevalence may increase when meat is not treated in industrial procedures (Marín-García *et al*., 2022).

Sea foods: Polluted water containing *T. gondii* oocysts enters in the aquatic habitats and contaminates these habitats and environments with the parasite. Different mollusk species use their gills for the filtration of foods and preying phytoplankton. Oocysts of *T. gondii* accumulate in the tissue cells due to the process of food filtration and these oocysts can remain in fresh and salty water sources for longer time spans (Lindsay and Dubey, 2009). PCR is the most commonly used method for the detection of parasite in the organ and whole tissue samples of mollusks (Ghozzi *et al.,* 2017). Different molecular techniques known as RT-PCR, PCR and qPCR are used for the detection of parasite in fish samples (Coupe *et al.,* 2019). ELISA is an example of serological method used for the identification of *T. gondii* in fish by detection of IgM and IgG antibodies in the blood of fish and this technique suggests that fishes not only act as a host for the parasite like in shellfish but are really infected with the parasite (Taghadosi *et al.,* 2010).

The *T. gondii* infecting fish and shellfish is risk for both aquatic life and humans. Consumption of raw fish and shellfish by humans is the main source of infection transmission from seafoods to humans (Jones *et al*., 2009). The human activities and changes in climate are increasing the number and burden of pathogens in water habitats and similarly the burden of *T. gondii* is also increasing in different species of sea foods consumed by humans and wildlife (Van *et al*., 2016). Minimizing the infection of *T. gondii* at domestic sources (cat feces management) and preventing the polluted wastewater flow to aquatic habitats is the ultimate possible strategy to decrease the chances of exposure of *T. gondii* by consuming the sea foods (Shapiro *et al*., 2019).

In 2008, the first research was published that identified the genotype X of the parasite in a mussel collected from California (Miller *et al*., 2008). Further eight other studies from 2008 to 2015 found the infection in different sea foods (Bahia *et al*., 2017). In China, Brazil, Turkey and USA, the *T. gondii* is identified in different mollusk and fish species obtained from commercial sources. After 2015, further two studies tested the *T. gondii* in mussels and wild clams' samples obtained from retail shops in New Zealand and Tunisia (Shapiro *et al.,* 2019).

Fresh foods and water: Cat stools and polluted water containing *T. gondii* contaminate fresh foods such as fruits and vegetables and these fresh foods become transmission source of parasite to humans. There is no specific and optimized lab technique for the detection of oocysts of this parasite in the samples of environment and foods and it is because of the difficulties occurring in separation and concentration of the oocysts present in complex matrix like raw vegetables (Temesgen *et al*., 2019). But some studies have used methods to detect oocysts of this parasite in samples of foods, soil and water on the basis of those methods that are used in the detection of other protozoan (Dumètre and Darde, 2003). Some other techniques such as Immunomagnetic Separation Assay (IMS), LAMP test and RT-PCR technique are also used in the detection of *T. gondii* oocysts present in food samples (Marín-García *et al*., 2022).

The DNA of the *T. gondii* was detected in vegetables and fruits for the first time in 2012 (Lass *et al*., 2012). Recently some studies have found the relation between *T. gondii* outbreaks in humans and consumption of green vegetables polluted with the irrigation water containing *T. gondii* oocysts (Cabral *et al*., 2020). Another meta-analysis identified that in the future the most frequent way of transmission will be the vegetables consumption (Pinto *et al.,* 2019). In a study 9.7% prevalence was found in the vegetables collected from home base gardens and shops in Poland (Cabral *et al*., 2020). The prevalence rate of 3.6% was found in China, 21.2% in Morocco and 0.8% in Italy in fresh foods (Marín-García *et al*., 2022). 0.8% of ready to eat raped salads were found contaminated with oocysts and load of the parasite was high in these positive samples (Barlaam *et al*., 2021).

Fresh foods are contaminated with parasite primarily at production site (where they are placed on land polluted with cat stools) and with polluted water used in cleaning, processing and irrigation. Contamination occurs directly or indirectly by these sources in poor and developing countries due to compromised quality of water, bad sanitary and hygienic conditions (Dixon, 2016). But still there are chances of parasite transmission in developed countries due to the import of foreign and off-season vegetables and fruits. As the population of domesticated cats is increasing, the chances of environmental contamination with *T. gondii* are also increasing ultimately imposing higher risks of foods and water-based transmissions in humans (Bahia *et al*., 2017).

Because fresh foods are used in raw forms so strong control strategies and physical removal of the oocysts is necessary to minimize the contact of parasite in consumers. Before harvesting, the primary measure to minimize the exposure of parasite is to restrict the entry of cats to crops and farms and the use of safe water for the irrigations. Oocysts can withstand harsh environmental conditions and remain on fresh products for longer periods (weeks and months) (Robertson, 2016). Another study identified that oocysts attached with berries can withstand refrigeration temperature for minimum eight weeks or longer (Kniel *et al*., 2002).

The initial measure taken after harvesting the crops is the use of safe water for cleaning and processing the products and in washing of utensils. Spread of oocysts through foods can also be reduced by the use of disinfectants (physical or chemical) on the utensils, foods

and lands where produce is placed (Erickson and Ortiga, 2006). Physical methods to disinfect the parasite oocysts on fresh foods are more effective than chemical methods. In a study beneficial impacts of gamma radiations were found in decontamination of fresh vegetables and fruits (Shapiro *et al.*, 2019). Another study found that the application of 0.4kilogray radiations inactivates the oocysts present on raspberry (Dubey *et al*., 1998). Recently an experiment found a significant reduction in the number of *T. gondii* oocysts by the application of 0.2kilogray radiations (Lacombe *et al*., 2017). High pressure processing technique is also beneficial in reducing the oocysts on fresh foods and juices. In a study, mice developed no infection when feed on raspberry treated with pressure of 340MPa for 60 seconds (Lindsay *et al.*, 2008).

Reduction of parasite exposure at consumer's level include the removal of outer layer of vegetables and fruits. Safe handling of foods like clean hands, distinct boards for cutting, separate cutter and other equipment decrease the chances of cross contamination of fresh foods. Freezing at domestic level is not advised as the only way to inactivate the parasite in fresh produce because the oocysts of *T. gondii* can bear the temperature of -20°C for 28 days (Frenkel and Dubey, 1973).

Soil and environment: Soil is contaminated with *T. gondii* when the parasite is released in the feces of infected cats. Felids are the only definitive host for *T. gondii*, and the parasite is not present everywhere in the soil but at the sites of cat defecations and these oocysts are spread by the rain, winds and some vectors (arthropod and earthworm). Contact with this polluted soil can transfer the oocysts to humans and may be the important way of transmission in food producing animals and hosts (Egorov *et al*., 2018; Shapiro *et al*., 2019).

Soil can be the important reservoir for the parasite because of the toughness of oocysts due to which they can remain in the soil environments for longer periods (Dabritz and Conrad, 2010). The extent of the contamination of public places is not properly understood due to the fact that a smaller number of researches are available on soil contamination globally. *T. gondii* present in social and public places may the source of infection for the elder individuals and small children and they can get oocysts from clothes, arms and from the toys after touching the contaminated soil (de *et al*., 2021). Environmental contaminations are a danger for health of people mainly for the individuals at high risk such as women in pregnancy, children, workers in gardens and individuals having weak immunity (Maleki *et al*., 2021).

In a study conducted in China, samples of soil were collected from six provinces and 10.9% (230/2100 samples) samples were tested positive with the oocysts. In the individual soil samples, the highest prevalence was detected in farmlands such as chicken 16%, 14.7% in livestock and 15% in parks while the minimum prevalence was found in high schools 4.3% and elementary schools 5.3% due to the low access of cats to the schools (Cong *et al*., 2020). Soil samples were collected from different places in Brazil, 8.33% (10/120) samples tested positive for DNA of *T. gondii* (de *et al*., 2021). Research conducted in China to check the prevalence of *T. gondii* in soil samples collected from pig farms with high and low number of cat population found 11 farms positive and 1 farm having less cat population negative for *T. gondii* (Fen *et al*., 2012).

Tick bites: Few studies have discussed the role of ticks in the distribution of *T. gondii*. Ticks feed on the blood of hosts and while sucking blood they can transfer many disease-causing pathogens to the host and in the same way there may be a possibility of transmitting *T. gondii* through tick bites (Sroka *et al.*, 2003; Ben, 2019). All the intermediate hosts of *T. gondii* can be the blood source for the ticks at any stage (larvae or adult) and in this way infection may spread in different intermediate hosts populations and apparently, they create a possible way of transmission of *T. gondii* in humans by transfusions (Esch and Peterson, 2013).

Role of ticks in the spread of toxoplasmosis is explained by many experiments in the laboratory. Transmission occurred by *A. Americanum, D. andersoni* and *D. variabilis* while these ticks were feeding blood on the experimental animals (Woke *et al*., 1953). Mouse can get infection by feeding the infected *I. Ricinus* on its blood. *H. longicornis* can live in the ticks for nearly 10 days and some studies show the transmission of parasite by the ingestion of infected ticks (Kim *et al*., 2020). The DNA of *T. gondii* was isolated in *I. ricinis* and results of this study revealed that these ticks can play a vector role in the transmission of parasite suggesting the alternate way of transmission than oral transmissions of the parasite (Skotarczak, 2016).

Asexual reproduction of the parasite occurs in intermediate host and the parasite can penetrate in the blood cells (monocytes and neutrophils) moving to other tissues of the body. Hence, the parasite is present in the blood so it can transmit to other animals by arthropods feeding on the blood (Esch and Petersen, 2013). All the intermediate hosts of *T. gondii* can be the blood source for *I. ricinus* and different domestic and wild animals are also the hosts for ticks. So, the ticks play a role in the transmission and distribution of *T. gondii* in the linkages of food chain. These linkages are intermediate hosts and include birds and rodents. Cats feed on these infected birds and small rodents and thus the life cycle of the parasite is completed. This is the possible way by which ticks can increase animal and human infected populations (Skotarczak, 2016).

DNA of *T. gondii* was isolated from 2.8% (Sroka *et al*., 2003) of female mature tick of *I. Ricinus* in Poland collected from forests in the eastern areas while 12.7% (Sroka *et al*., 2008) in nymph and mature ticks in northwestern areas and 64.9% in the ticks collected from upper areas (Asman *et al*., 2015). A study conducted in China also detected *T. gondii* (11.3% in adults and 6% in nymph) in *Haemaphysalis longicornis* (Ben, 2019). *Amblyomma cajennense* ticks were also detected positive for *T. gondii* in research carried in Brazil (Deane, 1958). In Poland ticks' species named as *Dermacentor reticulatus* were detected for the DNA of *T. gondii* (Wojcik *et al*., 2015).

Detection methods: Toxoplasma is important food borne pathogen obtained from zoonotic sources and no systematic strategies are available for its control (Bouwknegt *et al*., 2018). Still the ISO have no specific guidelines and standard methods to detect *T. gondii* in food materials. Some available techniques for the detection of

Table 1: Table shows detection of *T. gondii* in different food samples in different countries

Country	Sample	Detection Method	No. of Samples	Positive Samples	Year	Reference
Pakistan	Goat blood	PCR	898	48 (5.3%)	2019-2020	(Aziz *et al*., 2022)
Pakistan	Cattle	PCR	190	23 (12.2%)	2019	(Taalay *et al*., 2022)
India	Pig ham muscle	ELISA and (nested) PCR	810	54 (6.7%)	2019	(Thakur *et al.,* 2019)
China	Chicken (heart muscle)	Nested PCR /mouse bioassay	1653	204 (12.3%)	2015-2018	(Chen *et al.,* 2022)
Spain and Portugal	RTE vegetables and berry	RT-PCR, qPCR	35	14 (40%)	2018-2019	(Marques *et al*., 2020)
USA	Retail pork and lamb	Mouse bioassay	1500	3 (0.2%)	2013-2017	(Dubey *et al.,* 2020)
Italy	Blue crabs	PCR	11	6	2020	(Marangi *et al.,* 2022)
Norway	Berries (blue berry, raspberry and strawberry)	qPCR	820	24 (2.9%)	2019-2020	(Temesgen *et al*., 2022)
Colombia	Water	Nested PCR	55	5 (9.1%)	2019-2020	(Pinto-Duarte *et al.,* 2022)
Colombia	Soil	Nested PCR	50	14 (28%)	2019-2020	(Pinto-Duarte *et al*., 2022)
Serbia	Pig samples (hearts)	Mouse bioassay, MAT, PCR	825	16.5%	2017-2019	(Betić *et al*., 2022)
Italy	Serum of roe deer	ELISA	323	130 (40.2%)	2017-2018	(Crotta *et al*., 2022)
Iran	Organ meat (sheep and cattle)	Nested PCR	179	38 (21.2%)	2109	(Gorgani-Firouzjaee *et al.,* 2022)
Iran	Chicken (heart) free living	Nested PCR	75	30 (40%)	2017-2018	(Abbaszadeh *et al.,* 2022)
Iran	Chicken (heart) industrial	Nested PCR	75	29 (28.7%)	2017-2018	(Abbaszadeh *et al*., 2022)
China	Pig serum samples	ELISA	1170	244 (20.85%)	2020-2021	(Liu *et al*., 2022)
Iraq	Goat milk	ELISA	80	17 (21.25%)		(Madi and Al-Samarai, 2022)
Iraq	Goat milk	Nested PCR	80	23 (28.75%)		(Madi and Al-Samarai, 2022)
Tunisia	Breast meat (chicken)	PCR	140	28 (20%)	2019-2020	(Zrelli *et al*., 2022)
East Azerbaijan, Iran	Milk (cow, buffalos and camels)	PCR	100 (55 cows, 30 buffalos, 15 camels)	5 (2 cows, 1 buffalo and 2 camels) (5%)	2019	(Asiyabi *et al.,* 2022)
Brazil	Pig serum	IFA	84	21 (25%)	2020-2021	(Espindola *et al*., 2022)
Brazil	Pork sausages and tissues	PCR	53	7 (13.2%)	2020-2021	(Espindola *et al*., 2022)
Morocco (Marrakesh)	Leafy green vegetables (coriander, lettuce and parsley)	RT-qPCR	152 (51 from wholesale market, 50 supermarket, 51 from vendor)	49 (22 lettuce, 17 coriander, 10 parsley) (32.2%)	2018-2019	(Berrouch *et al*., 2022)
Japan	Serum sample (wild boars)	ELISA	180	12 (6.6%)	2020-2021	(Kaneko *et al*., 2022)
Africa (Kiambu)	Brain tissue (chicken)	Mouse bioassay	48	38 (79%)		(Njuguna *et al*., 2022)
USA (Michigan)	Tongue tissue (otter)	PCR	124	35 (28%)	2018-2019	(Cotey *et al*., 2022)
Israel	Serum (sheep)	Nested PCR	138	33%	2018-2019	(Mazuz *et al.,* 2023)
Israel	Serum (cattle)	Nested PCR	249	29%	2018-2019	(Mazuz *et al.,* 2023)
Greece	Serum (chicken)	ELISA	934	88 (9.4%)	2016-2017	(Andreopoulou *et al.*, 2022)
Greece	Heart (chicken)	mc-PCR	20	8 (40%)	2016-2017	(Andreopoulou *et al.*, 2022)
Switzerland	Serum (sheep)	ELISA	653	428 (66.3%)	2017-2018	(Basso *et al.,* 2022)

parasite in foods include microscopical and immunological methods. These methods are used for the detection of oocysts, tissue cysts and tachyzoites. The most recent techniques are the molecular techniques used for the detection of DNA of the parasite in different food samples (Marín-García *et al*., 2022). Further, these methods are discussed in detail below.

Molecular methods: Molecular methods for the detection of *T. gondii* include PCR and LAMP techniques and PCR is used to detect *T. gondii* directly in humans (Khan and Noordin, 2020). In PCR, the amplification of a particular piece of genome occurs and many (millions) copies of specific molecule of the DNA are prepared. For the detection of *T. gondii* with PCR, technique B1 gene of the parasite is used and amplified by 35 repetitions (Veronesi *et al*., 2017). Samples of 25mg are used when using DNA separation method commercially while the number of tissue cysts in such small samples is low and chances of detection of parasite in these samples are low. To analyze the samples larger in size and to increase the sensitivity of detection, Percoll gradient isolations, homogenizations, artificial digestions and magnetic captured sequencing-based methods have been used (Dubey, 2010). In this way, sample is concentrated and allows the analysis of more tissues. The incubation of sample is done at temperature of 27ºC for an hour and pepsin, sodium chloride and hydrolytic acid are added, they liberate bradyzoite by breaking the walls of cysts of *T. gondii* (Bayarri *et al*., 2010).

Another technique such as quantitative PCR is also used for the identification of the DNA of the *T. gondii* in the samples that are analyzed, and this method is more sensitive, precise and fast (Marín-García *et al*., 2022). In this method, there is no need of gels as compared to conventional PCR methods. The TaqMan and SYBR Green probes are used in the qPCR techniques. The TaqMan probe has more specificity and reduced sensitivity while SYBR Green probes have greater sensitivity but less specificity and therefore they cannot identify *T. gondii* DNA when it is in low concentrations (Lemmon and Gardner, 2008).

Another type of PCR known as nested PCR is a technique having high specificity for DNA of the parasite and can also detect the parasite in low quantities. In a study, this method was used to detect the *T. gondii* in the samples of water obtained from sea, river and well (Kourenti and Karanis, 2006). To detect the parasite in food and animal samples, this technique with high sensitivity was used targeting the regions in rDNA (Vitale *et al.,* 2013). Multiplex PCR is another technique that is used for the

identification of different pathogens in a single sample (Rostami *et al*., 2018). In a recent study, this method was used to detect *T. gondii* with different other pathogens in a fruit (berry). This method is very fast having increased precision and specification (Temesgen *et al.,* 2019).

Loop-mediated isothermal amplification (LAMP) is a new technique for the detection of *T. gondii* in different food and water samples (Sotiriadou and Karanis, 2008; Zeedan *et al.,* 2022). This is rapid method and has high specificity and sensitivity for the *T. gondii* in complex samples. The DNA is amplified in isothermal environment. LAMP is more efficient technique compared with other molecular methods because it does not require expensive thermal cycler, amplifies DNA in less than one hour, no need of probe labelling and assists multiplexing (Durand *et al.,* 2020). In a comparative study between PCR and LAMP methods, 76.9% of lymph node pig samples were found positive with PCR while 85.7% samples were detected positive by using LAMP technique (Zhang *et al.,* 2009). In another study, water samples were tested for *T. gondii* with different methods and 48% of samples were tested positive with LAMP, 13.5% with nested PCR and no sample was tested positive with immunofluorescence test (IFT). These comparative studies give the clarity about the increased specificity, sensitivity and rapidity of LAMP method as compared to other methods (Sotiriadou and Karanis, 2008).

These are the main molecular methods used for the detection of *T. gondii* in different samples on the basis of genotyping (Fernández *et al.,* 2022). The important limitation of these detection methods is that it only detects the DNA of parasite and viability of the parasite is not known. To check that identified DNA belongs with viable parasite, other techniques are also needed (Opsteegh *et al.*, 2016). To overcome this limitation of the molecular methods, another method such as Propidium monoazide based qPCR (PMA-qPCR) is developed (Rousseau *et al*., 2019). In a recent study, this method was used to identify the parasite on viability basis in green leafy foods (Kim *et al.,* 2021).

Serological methods: Serological methods are classified as indirect methods for the detection of *T. gondii* in humans and animals and can also be used for the detection of parasite in food samples such as meat juice and meat. These methods are used as screening test to identify the infection in animals and the results of these tests are confirmed by bioassays in tissue sample (Marín-García *et al.,* 2022). In a study, *T. gondii* is identified by these methods in meat juices (Guo *et al*., 2015). The mostly used serological methods include latex agglutination test (LAT), indirect hemagglutination antibody (IHA), indirect fluorescent antibody test (IFAT), enzyme linked immunosorbent assay (ELISA), modified agglutination test (MAT) and Western blot (Ismael, 2021).

These techniques are used to identify the acute and chronic phase of infection. During the start of infection, the IgM antibodies are produced, and the IgG antibodies are produced after 7-14 days of infection and reach at maximum concentrations in 30-60 days after the infection. As, IgM are produced in acute phase of infection and IgG in chronic phase, so these methods detect these antibodies and acute and chronic toxoplasmosis is identified (Molaei *et al.,* 2022). For the confirmation of these results, other tests are required (Robert *et al.,* 2021).

Latex agglutination test (LAT) is the first serological test used mostly and has application as screening test for the identification of *T. gondii* antibodies. This test is specific, easy to use, sensitive and cheap. LAT has some limitations also and should be used carefully while performing the screening of pregnant women and individuals with compromised immunity living in the areas in which the disease is rare because of its average positive predictive values (Rostami *et al*., 2018). Another test known as dye test (DT) or Sabin Feldman dye test was used for the identification of almost all the antibodies produced in response to *T. gondii* and is known as gold standard test (Weiss and Kim, 2011). The test has specificity and sensitivity for humans but in animals the test may not be reliable enough. This test requires the alive parasite and sera of healthy individuals that limits its availability is the main problem in this method (Ashburn *et al.,* 2001).

ELISA works by identifying the antibodies against the *T. gondii* in the meat tissues and serum samples. This is simple and easy method but needs conjugates that are specie-specific and require plate reader. Later this problem was solved by creating a conjugate protein such as A/G which is not species specific and can be used in the testing of many species (Al-Adhami and Gajadar, 2014). ELISA, IFAT and MAT are the main serological test that are used in most of the cases for the identification of *T. gondii* in sera and food samples. The modified agglutination test (MAT) is better test as compared to other agglutination test but has limitations while performing in slaughterhouses and fields due to the fact that it needs intact tachyzoites in huge amounts (Jones and Dubey, 2012). These tests may be different on the basis of specificity and sensitivity and their results may differ from the results of bioassays, this difference is observed in cattle. The parasite may have 90% seroprevalence in cattle but the separation of the parasite in cattle may not be successful (Guo *et al*., 2015). In a study, comparison of these tests was performed and results showed that with the bioassay 70 samples from pig tissues were found positive for *T. gondii* while 60 samples (85.7%) identified positive by MAT test and 62 samples (88.6%) tested positive with ELISA (Gamble *et al*., 2005).

Animal bioassay: Infections caused by the bradyzoites and oocysts are detected by these methods. In these methods, the cat bioassay method is the most efficient method and mouse bioassay is also a good detection method for *T. gondii* (Marín-García *et al*., 2022; Dubey, 1998). While performing cat bioassay, they are fed on sample meat, nearly 500g to check the contamination of the cysts. After the three weeks of feeding, oocysts of the parasite are identified in the cat stools (if present or not) and the particular antibodies for the parasite can also be checked in the serum samples of these cats (Dubey *et al*., 2021). Cat bioassays are the efficient techniques because all forms of the parasite (bradyzoites, tachyzoites and oocysts) can be identified with this method (Lindsay and Dubey, 2009). Due to the ethical issues and very high cost of cat bioassays, these are performed in very few laboratories (Opsteegh *et al.,* 2016).

Due to the limitations of cat bioassay, the infection by oocysts are evaluated by mouse bioassay techniques mainly. Tissue sample of 50-200g is taken and pepsin/trypsin is applied on the sample for its digestion and a minute quantity is injected in subcutaneous or peritoneal layers and mice can also fed samples containing *T. gondii* orally (Jones and Dubey, 2012). Then to check the *T. gondii*, peritoneal fluids or brain samples are taken after the death of infected mice and these samples are examined by microscopic methods or by PCR techniques to see the particular antibodies in sera. Usually, 2-5 mice are taken in each sample and are kept under the clinical monitoring. While performing this bioassay, the drugs (Dexamethasone) that suppress the immune system of mice can be provided with drinking water to obtain the efficiency of the bioassay (Liu *et al*., 2015; Dubey, 1998).

Size of the sample that is inoculated or fed in mice is less than that of used in cat assay. Mouse bioassay is cost effective as compared to cat bioassay but still both have ethical limitations because of the use of animals in these techniques (Lindsay and Dubey, 2009). With the help of these techniques, oocysts of the parasite were detected in the samples of shellfish and water (Esmerini *et al*., 2010). These methods were also used to check the influence of temperature and duration of storage on the infectivity of the oocysts in blueberry and raspberry fruits (Kniel *et al.,* 2002). Limitations of these assays include a high number of screenings which cannot be performed and not efficient for the chilled samples because these assays give viability of the *T. gondii* and the severity of the disease cannot be analyzed by these assays (Juránková *et al.,* 2015). But these methods are still efficient for the identification of viable parasite in other kind of samples (Marín-García *et al.,* 2022).

Cell culture methods: Molecular methods for the detection of *T. gondii* are efficient methods having specificity and sensitivity but with these methods we can only detect the DNA of the parasites and viability of the *T. gondii* cannot be analyzed. This problem is resolved by cell culture methods in which viability of the parasite is possible. In this method, the sample is added with the culture of cells. Samples having viable *T. gondii* will cause the infection in cell cultures and tachyzoites will multiply that can be seen with reverse microscopy after 3 to 10 days (Genchi *et al*., 2017; Molaei *et al*., 2022). As animal model bioassays are expensive methods and are ethically not good due the use of live animals so instead of these methods cell culture techniques are efficient ways to detect the *T. gondii* (da Costa *et al.,* 2012). But the sensitivity of cell culture methods is less than that of bioassays and also, they need extensive care of the samples to remove the contamination of the samples (Zintl *et al.,* 2009). Furthermore, these culture techniques give quick results than bioassays which help in the recall and disposal of the foods that are contaminated with the parasite during food born outbreak (Rousseau *et al.,* 2018). Meals digested by artificial ways and homogenate of sediments were tested successfully with these methods. In an experiment, *T. gondii* was detected in milk samples obtained from various cattle species by tissue culture methods (Dehkordi *et al.,* 2013).

Microscopic techniques: *T. gondii* can also be detected by microscopic method. This method can be used for the detection of parasite in fecal, water, environmental, shellfish and fresh food samples (Liu *et al.,* 2015; Marín-García *et al*., 2022). When the non-specific stains (Giemsa, eosin and hematoxylin) are used, the parasite is only visible and is not differentiated from other apicomplexan parasite, so for the differentiation from other parasites and to increase the sensitivity, the specific stains (antibodies and fluorescence conjugated enzymes) are used (Dubey, 2010; Bajnok, 2017). The important limitation of this method is that it shows the negative results while sample is positive actually. The appearance of false negative results occurs because the sizes of the samples are small and the area of the sample that is analyzed may not be contaminated and other areas may be infected with the parasite (Marín-García *et al*., 2022).

Breaking the chain of infection: The chain of infection of *T. gondii* can be broken and its spread can be controlled at different levels. There are many risk factors that increase the spread of this disease in humans and animals. But the main three factors that should get special attention to control infection include: decrease of environmental contamination by oocysts released by cats, avoiding the contamination of soil, water and food with oocysts and physical reduction or inactivation of parasite in food and water (Shapiro *et al*., 2019). Some factors are involved in the spread of the oocyst in the animals kept in farms. For the control of the parasite in the farm, proper hygiene measurements should be taken and avoid the entry of the felids (cats) in animal's feeds, garden and crops (Wu *et al*., 2017). The burden of oocysts of the parasite in the environment is related to the cat population, chances of the cats getting infected, and number of the parasite released with the feces of infected cats and the amount of the parasite survived in the environment (Dabritz and Conrad, 2010). For the control of populations of cat, the reproductive organs of male and female cats should be removed. Cats get infections by preying the infected birds and raw meat consumption, so they should be kept in cages and indoor during night. Vaccinating the cats can also help in the control of parasite but still no vaccine is available. Specific box should be used to manage the feces of cat and should dispose with the waste of house where heat treatments on oocysts can be applied (Opsteegh *et al*., 2015).

Humans can get infection by consuming the raw milk and dairy products, fresh vegetables, fruits and meat. In these foods, the parasite can be controlled by proper hygienic measures, proper cleaning and washing of fruits and vegetables, pasteurization of milk and proper cooking of meat (Almeria and Dubey, 2021). Some physical methods are used for the control of the parasite in food industries during processing and production of foods. The parasite can also be inactivated with the help of some chemical methods. With the help of these methods, the spored and non-spored forms of the oocysts are inactivated and also the inactivation of bradyzoites and tachyzoites occurs (El-Nawawi *et al*., 2008). These methods are explained in detail below.

Thermal treatments: For the prevention of the Toxoplasmosis in animals and humans, the inactivating the parasite in tissues of animals and oocysts present in

environment by chilling, heat and cooking are the beneficial ways to control the parasite (Mirza *et al.,* 2018).

Treatment of foods with heat is the essential way to increase the storage time of the foods and inactivation of the parasite and other microbes. *T. gondii* is vulnerable to heat treatments and some research proved the inactivation of spored and non-spored stages of the *T. gondii* (Ito *et al.,* 1975). Another research proved that the heat treatment at 58°C is enough for the inactivation of all kinds of oocysts (Kuticic and Wikerhauer, 1994). It was shown that minimum of 60°C temperature for one minute may not always kill the parasite and the volume of water, time required for heating and cooling of water need to be considered during inactivation of the parasite (Wainwright *et al.,* 2010). Tachyzoites are inactivated at low temperature provided for short time while bradyzoites need high temperature and longer time as compared to tachyzoites. Oocysts present in fresh foods, water and intestine of cat need higher temperature for longer time for inactivation (Mirza *et al.,* 2018). *T. gondii* can be inactivated in meat by heating at 49°C for 5 to 6 minutes, at 55°C when heated for 44 seconds and at 61°C for 6 seconds (Hill *et al.,* 2018). Various meat products need varying temperatures for the deactivation of the parasite, such as in beef requires temperature of 63°C; minced, pork and bush meat 67°C and poultry meat 82°C. Generally, meat should be treated at 67°C prior to consumption. For the inactivation of parasite present in milk and dairy products, the pasteurization of milk at 63°C for 30 minutes is required (Saridewi *et al.,* 2013).

High temperature is not efficient for the inactivation of the parasite in all kinds of foods and some foods such as fresh foods (fruits and vegetables) require reduced or low temperatures for the inactivation of parasite (Pinto-Ferreira *et al.,* 2021). If we consider the role of reduced temperatures in the inactivation of the tissue cysts present in meat, then freezing is also beneficial for the inactivation of these cysts. These cysts are inactivated at the minimum temperature of -20°C provided for three days (Djurkovic´-Djakovic and Milenkovic, 2000). People in the different regions of the world freeze foods for some time before consumption but they are conscious because by freezing the food may lose the texture, flavor and other good qualities (Bayarri *et al.,* 2012). Different studies have explained the impact of freezing on the infectivity of *T. gondii* in different foods. In 1965, the impact of freezing on the inactivation of parasite was studied first time, this study revealed that the temperature of -20°C for two days was enough to kill the *T. gondii* (Mirza *et al.,* 2018). A study concluded that temperature of -20°C for 21 days is required for the inactivation of sporulated oocysts while -7°C temperature for 4 days is needed for the inactivation of tissue cysts (Kuticic and Wikerhauer, 1994).

Non-thermal techniques: From the last few years, non-thermal methods have attained great attention in food processing and control of the pathogens in foods as they do not have significant effects on nutritional and sensory characteristics of the foods. These methods help in the increase of shelf life of the foods by inactivating the pathogens present in the foods (Morris *et al.,* 2007). Some new non thermal methods that can inactivate *T. gondii* and other pathogens of food include pulse light treatment (PLT), cold plasma (CP), pulse electric field (PEF), high pressure processing (HPP) and ionizing radiation (IR) (Mirza *et al.,* 2018).

High pressure processing: In this non thermal technique, a pressure of 200-600MPa (mega pascal) is provided to kill pathogens. A liquid is used to generate a constant pressure and the time temperature required during compression depends on the food product and its nature and composition (Gérard *et al.,* 2019). In this technique, a constant pressure is provided with minimum changes in temperature irrespective of shape and volume of foods (Rendueles *et al.,* 2011). Temperature increases 3°C for increase of each 100MPa pressure depending on the nature of food material. This method does not impact the mineral and vitamin content of food and maintains the color and flavor of the products (Considine *et al.,* 2008).

A study revealed that the tissue cysts of the *T. gondii* can be inactivated by applying pressure of 340 to 550MPa in laboratory (Lindsay *et al.,* 2006). Another study concluded that the pressure of 340MPa-400MPa for 60 seconds can be beneficial for the control or inactivation of *T. gondii* in foods but still there is need of studies to estimate the time and pressure required to inactivate each stage of *T. gondii* (Lindsay *et al.,* 2008). A pressure of 550, 480, 400 and 340MPa for 60 seconds inactivated the oocysts form of *T. gondii* present in distilled water and they showed no infection in mice (Lindsay *et al.,* 2005).

Ionizing radiations: This is non-thermal method that pasteurizes the food by using radiations and may inactivate, reduce or eliminate the pathogens, fungi, insect and pest in foods. The mostly used radiations include gamma rays and ultraviolet rays. Common sources for gamma rays include x-rays, cobalt-60, electrons, cesium 137 and electrons beam accelerator. Impact of radiations on *T. gondii* has been studied and it has shown beneficial effects by inactivation of the parasite (Mirza *et al.,* 2018).

In a study, use of 40krad gamma radiations inactivated the *T. gondii* tissue cysts (Dubey and Thayer, 1994). Another study reported the impacts of gamma radiations on blueberries by using low doses (20krad) of these radiations and successfully killed the *T. gondii* oocysts without significant changes in the color, moisture, composition and anthocyanin level in these fruits (Lacombe *et al.,* 2017). Use of ultraviolet radiations also inactivated the tachyzoites of the parasite, but they require longer time as compared to gamma radiations (Kannan *et al.,* 2014). Ultraviolet radiations (>20mJ/cm^2) were found efficient and destroyed the infectivity of sporozoites (Dumetre *et al.,* 2008). Gamma radiations are high energy rays as compared to ultraviolet rays because the gamma rays are emitted from nucleus of the atom while ultraviolet rays are generated by the orbits of the atoms and these orbits have low energy. As gamma rays are high energy so they go deeper in the foods than ultraviolet rays and kill *T. gondii* more effectively in same circumstances. In a study, continuous and pulsed low frequency (75Hz) electromagnetic fields were applied on the *T. gondii* and results showed reduction in the number of parasites as compared to the number of parasites in control groups (Ozlem-Caliskan *et al.,* 2016).

Curing methods: Meat is preserved by curing methods using nitrites, salts, nitrates and sugars (sucrose) and smoking at reduced temperatures (Bayarri *et al.,* 2012). Salts are important ways to kill the *T. gondii* cysts and the extent of the deactivation of the parasite depends on the maturity of the parasite, storage temperatures and concentrations of the salt (Hill *et al.,* 2004; Kijlstra and Jongert, 2008). In an experiment, solution of 2% of NaCl or 1.4% of lactate salts were injected into the pork for eight hours and it showed efficient results by inactivating the parasite (Hill *et al.,* 2004). In another research, salts and sugars were applied on meat of lamb for 64 hours at temperature of 4°C and in another test, salts were injected in the lamb meat and smoked for 24 to 28 hours at 50°C and parasite was killed effectively in both conditions (Bayarri *et al.,* 2012). When 2 and 2.5% of salts solutions were applied on pig meat for 48 hours, that was injected with the parasite, the results showed the inactivation of the parasite (Navarro *et al.,* 1992). Hams preserved with sodium ascorbate, NaCl, 4% nitrite and 7% nitrates for the duration of 9 to 12 months, the concentration of viable parasites were greater in the hams that were preserved for nine months as compared to those preserved for 12 months (Herrero *et al*., 2017).

Chemical and biochemical techniques: The food chain gets infected with *T. gondii* mostly by food sources obtained from infected animals and its control is important to break the chain of transmission of the parasite. Many techniques are used to minimize the contaminations of foods and feeds and they include biochemical and chemical methods. The mainly used agents in these treatments include enzymes, plant essential oils, alcohols, disinfectants, oxidant compounds, additives of the foods, organic acids and solvents (Ito *et al.,* 1975; Sharif *et al*., 2016).

The most frequently used chemicals are ozone and chlorine that preserve foods and water and having the ability to kill or inactivate the oocysts of *T. gondii* (Betancourt and Rose, 2004). Both these chemicals are oxidizing agents and kill the *T. gondii* by inhibiting the activity of the enzymes, destruction of RNA and DNA and changing the permeability of the cells (U.S. 1999). The main benefit of the use of these chemicals is that they are easy to handle and use as compared with gaseous chemicals (gaseous chlorine, calcium hypochlorite and sodium hypochlorite) and also require short time and doses. Still there is a disadvantage in the use of the disinfectants due to their hazardous nature and they should be used carefully in food preservation to avoid cross contaminations (Mirza *et al.,* 2018).

In a study, *T. gondii* was controlled when applied with high concentrations of ammonia (28%) in ten minutes and iodine took 30 minutes to show similar results (Frenkel and Dubey, 1972). In another study when 5 and 10% of doses of ammonia were applied for 30 and 10 minutes respectively, they killed the oocysts of the parasite (Dubey *et al.,* 1970). A study found that all the *T. gondii* oocysts are inactivated when 7% concentrations of iodine are used with 5% concentrations of potassium iodide for 30 minutes and same results are obtained when 1-10% concentrations of formaldehyde are applied for 24 hours (New Zealand, 2001).

If we compare between different treatments used to control the *T. gondii* parasite in different foods, we can see that the physical methods are more effective methods of control as compared to chemical treatments. It can be seen that the chances of getting infections from public places (restaurants, swimming pools) where chlorine is used as disinfectant and from fruits and vegetables on which aqueous solutions of chlorine are used for disinfection are higher (Pinto-Ferreira *et al*., 2021). In a study, *T. gondii* was detected in drinking water samples that were treated with chlorine showing the inefficacy of chlorine to inactivate the parasite (Dumètre *et al*., 2013). Oocysts can withstand the stress of the environment and salts (Shapiro *et al*., 2019). A research concluded that the oocysts of *T. gondii* can withstand seawater at the temperature of 4°C for two years and go for sporulation (Lindsay and Dubey, 2009). So, the salting techniques are not so efficient methods to control *T. gondii*. The techniques that use radiations and high-pressure processing to control the parasite are beneficial methods (Pinto-Ferreira *et al.,* 2021).

Conclusions: *T. gondii* is a zoonotic parasite and is transmitted to humans by different foods that are contaminated with this parasite. It is distributed all over the world and infects 30% of the population. The main source of transmission to humans is the consumption of undercooked meat from infected animals and fresh and raw foods. Ticks can also play a role in the transmission of the parasite in food animals. The other risk factors for the transmission to humans include sea foods, water, soil and environment. The detection of parasite in foods is difficult because of the complex nature of foods such as fresh foods. The main detection methods are molecular techniques (PCR) that detect the DNA of the parasite in different foods. These techniques do not give the viability of the parasite. Animal bioassays and cell culture techniques are used to check the viability of the parasite in different food samples. Serological methods such as ELISA, IHA, IFAT, LAT and MAT are the indirect methods used to detect the antibodies against parasite in animals and humans.

The parasite can be controlled at domestic and farm level. Proper hygiene measures during handling and processing of foods and control of cat populations at domestic and farm levels can reduce the transmission of the parasite. Furthermore, the parasite in foods can be destroyed and inactivated by different processing techniques. These techniques include Thermal methods (heating, chilling and cooking), non-thermal methods (high pressure processing, ionizing radiations, curing, cold plasma etc.) and chemical and biochemical methods. The detection of the parasite in food samples is expensive and difficult so cheap and quick detection methods needs to be developed. Further work is needed on the control and inactivation of parasite on domestic, production site and consumer levels.

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