Control and Detection of Toxoplasma Gondii in Meat Chain

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ABSTRACT

Toxoplasmosis caused by infection with the Toxoplasma gondii which can infect all warm-blooded hosts including humans is estimated to be present in one-third of the world population. Human infection with T. gondii has different routes by ingesting sporulated oocysts present in water, soil, or vegetables. Food-borne toxoplasmosis in humans may result from exposure to different stages of T. gondii, especially from the ingestion of tissue cysts or tachyzoites contained in meat, offal, and meat-derived products of many different livestock animals. Although the prevalence of viable T. gondii in retail meat was very low, consumers can acquire T. gondii infection from ingestion of undercooked meat. Thus, raw or undercooked meat containing viable cysts has been suggested to be a major source of T. gondii infection in humans. Prevention of toxoplasmosis transmission in humans depends on meat safety strategies. Most realistic solutions to control T. gondii in the meat chain come in the form of a risk-based meat safety assurance system that incorporates risk-categorization of farms and slaughterhouses, as well as education of all interested parties. The difficulty of toxoplasmosis monitoring in farm animals is due to the asymptomatic and chronic expression of the disease. Vaccine development and vaccination of farm animals against T. gondii are traditionally being implemented. On the other hand, carcass surveys present several advantages and control plans to detect and take measures for this pathogen. After systematic meat inspection, some treatments must be applied to the meat found to contain cysts, such as freezing, cooking, irradiation, or high pressure. Laboratory analyses to detect T. gondii are very important in terms of protecting public health. The gold standard for detecting T. gondii in meat samples is a bioassay using either mice or cats. Molecular methods are used as fast and realiable in addition to conventional serological methods for the diagnosis of toxoplasmosis.

Key Words: Toxoplasma gondi, Farm Management, Meat Inspection, Vaccination.

CITATION

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1. INTRODUCTION

Toxoplasma gondii is a protozoan parasite of the Apicomplexa Phylum (Opsteegh et al. 2011). Its biology, natural life cycle and epidemiology have many aspects. Everything depends upon the factors by which Toxoplasma is spreading and how it's affecting living beings, leading to the fatalities caused by it in worldwide cases (Tenter et al. 2000). *T. gondii* reproduction occurs solely in species of the Felidae family, its ultimate hosts, and ends up in the shedding of numerous oocysts following initial infection (Opsteegh et al. 2011). All warm-blooded animals, including livestock and humans, to will likely be its intermediate hosts (Tenter et al. 2000).

Toxoplasmosis caused by infection with *T. gondii* is estimated to be present in that one-third of the world's human population (Kijlstra and Jongert 2008a; Hussain et al. 2017) and is responsible for approximately 8% of all hospital stays and 24% of all deaths resulting from foodborne illnesses in America annually (Guo et al. 2015; Rani and Pradhana 2020). Recent assessments on foodborne diseases rank toxoplasmosis in the globe and it is on par with salmonellosis or campylobacteriosis (Kijlstra and Jongert 2008a). *T. gondii* is rated second place out of 14 pathogens transmitted by food in the United States and top in the Netherlands in terms of illness burden (Almeria and Dubey 2021).

Toxoplasmosis has a vast distribution in a range of cattle and wild species (Sousa et al. 2010). Because of this, the major transmission routes of toxoplasmosis to humans are the consumption of infected raw or undercooked meat harboring bradyzoites cysts (Rahdar et al. 2012; Zoua et al. 2017; Rani and Pradhana 2020). The largest proportion of specimens positive for the protozoa or antibodies was found in the EU, which was reported for sheep and goat meats (Opsteegh et al. 2016). Therefore, nowadays *T. gondii* is regarded as one of the most significant veterinary and medical parasites of food and water (Almeria and Dubey 2021).

Despite the fact that it can spread very easily by affecting many species and creating very important clinical findings in humans, toxoplasmosis continues to be seen as a minor and ignored illness. And also, vaccines for humans are not present and current specific antiparasitic treatment application is not at the desired level yet (Kijlstra and Jongert 2008b). Because of this fact, pre-harvest control programs to struggle with toxoplasmosis in farm animals and post-harvest detection and destruction methods to ensure/promise toxoplasma-safe meat are very important (Kijlstra and Jongert 2008a).

2. LIFE CYCLE OF *TOXOPLASMA GONDII*

T. gondii is an organism that lives inside cells and infects animals (Ragozo 2018). Its medicinal significance was unknown until 1939 and its life cycle was not found until 1970 (Dubey 2008). *T. gondii*'s life cycle is a heterogeneous structure alternating between sexuality and asexual phases (Tenter et al. 2000; Ragozo 2018) (Fig. 1). Felines and wild felines are the only definite hosts that play a significant role in *T. gondii*'s epidemiology (Dubey 1996; Roqeplo et al. 2011). Both pets and wild cats shed environmentally resistant oocysts in their feces for only 1-2 weeks after primary infection (Roqeplo et al. 2011; Opsteegh et al. 2016) and they frequently become resistant to oocyst re-shedding (Dubey 1996). Warm-blooded species and humans are mid-hosts (Tenter et al. 2000; Rani and Pradhana 2020), allowing parasites to multiply in two stages (Opsteegh et al. 2016).

T. gondii develops asexually in two stages in intermediate hosts (Tenter et al. 2000). Tachyzoite is the parasite's earliest, rapidly replicating stage. Tachyzoites transform into bradyzoites that are contained in tissue cyst (Opsteegh et al. 2011). Tissue cysts with a strong affinity for neurological and muscular tissues are primarily seen in areas such as the eye, the skeletal and cardiac muscles, and the lungs, liver and kidneys (Tenter et al. 2000).

T. gondii has three infectious stages: tachyzoites, bradyzoites located inside tissue cysts, and spores included in spore-forming oocysts. All three stages are infectious to intermediate and definitive hosts, who

can become infected with *T. gondii* primarily by one of the following methods as shown in Fig. 2. *T. gondii* can be transferred from definitive to intermediate hosts, interim to definitive hosts, and between definitive and intermediate hosts (Tenter et al. 2000).

Most of this parasite collected from humans as well as animals in North America has been classified as one of three clonal lineages, including types one, two, and three, and varies physiologically and genetically from isolates obtained in some other countries but are similar to isolates from Europe. Recent genotyping investigations of pig, lamb, and goat isolates show a Type II line prevailing in animals used for food in the United States, followed by Type III strains and atypical genotypes (Hill and Dubey 2013).

3. *T. GONDII* IN HUMANS

Toxoplasmosis is still a public health issue. An estimated 1 million persons are infected with *T. gondii* annually in the USA, (Dubey et al. 2011) and almost 400 cases of toxoplasmosis are clinical in England and Wales (Plaza et al. 2020). Daryani et al. (2014) reported a 39.3% total frequency rate of toxoplasmosis in Iran's population. According to a recent comprehensive review and a meta-anlysis in pregnant women, the worldwide incidence of latent disease in pregnant women is 33.8 % (Almeria and Dubey 2021). However, considering the lack of pathological indications in a lot of cases and the fact that they are not reportable, the real prevalence of toxoplasmosis is likely to be underestimated (Plaza et al. 2020).

Infection of humans with *T. gondii* can occur in a variety of ways, including the eating of uncooked or raw meat from chronically infected animals, the ingestion of sporulated oocysts found in water or on vegetables (Rani and Pradhana 2020), by handling contaminated soil or cat litter trays (Opsteegh et al. 2016) and by vertical transmission (congenital toxoplasmosis) (Rani and Pradhana 2020).

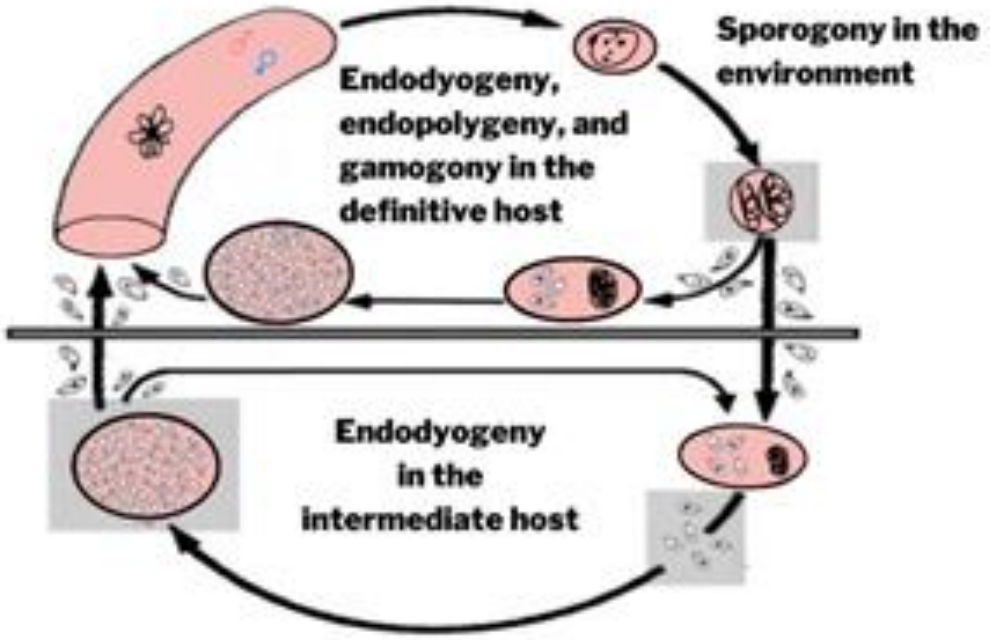

Fig. 1: Life cycle of *T. gondii*

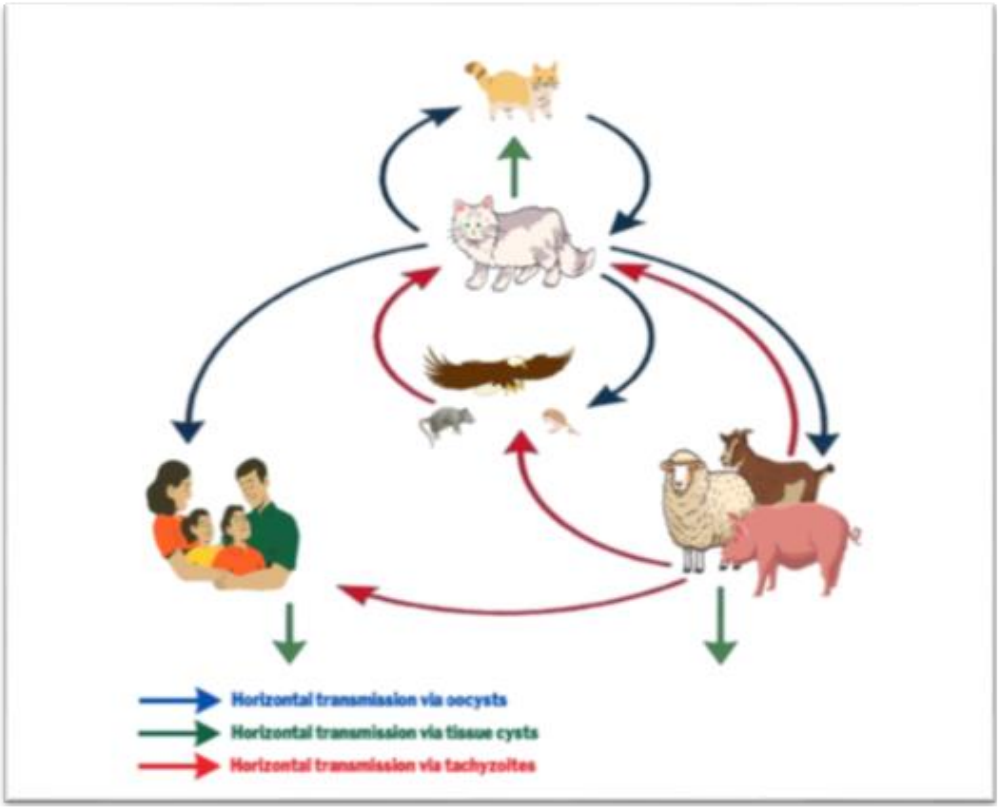

Fig. 2: Major routes of transmission of *T. gondii*

Inhalation of dust-containing oocysts has also been implicated as a transmission route (Kijlstra and Jongert 2008a). A European multicenter case-control research found that undercooked or raw meat caused 30% to 63% of acute infections in pregnant women in diverse European cities, while soil exposure caused up to 17% of infections (Opsteegh et al. 2016). Similar to these results, *T. gondii* infection in strict vegetarians demonstrates that oocyst invasion by dust still plays a key role in infection (Kijlstra and Jongert 2008a). Jones et al. (2009) evaluated 148 patients and reported that several raw or undercooked foods, as well as kittens, are hazards for *T. gondii* illness. Some important recommendations for pregnant women to prevent infection by *T. gondii* include: ingest well-cooked (67° C for 10 minutes) meat, do not experiment with the ingestion of raw or uncooked meat, freeze (-18°C for 7 days) all products, protect all food from flies and cockroaches, not feed the cat with raw meat and prevent the animal from hunting (Navarrol 2018).

In mankind, infection is typically asymptomatic or causes mild flu-like symptoms (such as fever, lymphadenopathy, headache, myalgia, stiff neck, sore throat, arthralgia, rash, confusion, nausea, eye pain, and abdominal pain) (Hill and Dubey 2002; Halos et al. 2010). Although most healthy people remain asymptomatic, severe clinical illness, including death, can be seen in infants and immunocompromised patients, such as HIV infection, long-term corticosteroid therapy, hematologic malignancies, and transplant recipients (Almeria and Dubey 2021). If an infection occurred at an early stage of pregnancy, toxoplasmosis can induce serious disease in the fetus (Tenter et al. 2000; Zoua et al. 2017) and can induce miscarriage or congenital abnormalities of the fetus's brain, eyes, or other organs (Halos et al. 2010). If an initial infection occurs 46 months or earlier before pregnancy, protective immunity typically prevents vertical transfer to the unborn on future exposures (Tenter et al. 2000). If the patient is under investigation or with a confirmed infection, spiramycin (under investigation and first trimester), pyrimethamine, sulfadiazine, and folinic acid (after the 18th week, until parturition) could be used (Navarrol 2018).

4. *T. GONDII* IN FOOD ANIMALS

Toxoplasmosis' symptoms differ based on the animal type. Early embryonic expiring and resorption, abortion, stillbirth, and infant death, in most ruminants, are the main symptoms (Almeria and Dubey 2021). *T. gondii* can be identified more frequently in the muscles of pigs, sheep, and goats compared to tissues from other edible animals. Although the incidence of *T. gondii* within sheep is unknown, alive *T. gondii* is detected in several edible tissues of *T. gondii*-infected lambs (Dubey 1996). Seroprevalence in pigs and chickens from nonbio-secure management systems is high. Clinical disease and high seroprevalence are seen in sheep and goats (Hill and Dubey 2013). In sheep up to 92% seroprevalence has been seen in some European countries (Tenter et al. 2000). According to a recent poll, 62.2% of sheep in the US are pasture-raised. Roaming sheep are surrounded by soil and water, both of which can be a source of *T. gondii* (Guo et al. 2015). *T. gondii* infection was found in hogs and wild boars reared and slaughtered in the Czech Republic (Slany et al. 2016). According to some researchs, cattle are immune to infection and are not thought to be the key carriers for *T. gondii*. (Sroka J et al. 2019).

5. TRANSMISSION OF *T. GONDII* WITH DIFFERENT KINDS OF MEAT

The use of undercooked/raw meat with viable tissue cysts (Plaza et al. 2020; Mancusi et al. 2023) is thought to be a significant cause of infection due to *T. gondii*'s propensity to spread to a wide range of animals and remain in their structures for years (Tenter et al. 2000; Kuruca et al. 2023). Human toxoplasmosis has been documented following the intake of raw and uncooked flesh and organs, and live parasites have been identified from fresh, frozen, and cured meat (Liu et al. 2015; Kuruca et al. 2023). Sheep and goats had the greatest proportion of positive samples for the pathogen or antibodies amongst every single one of the Member States in the EU. Beef, sheep, pork, and combined meat products account for 68%, 14%, 11%, and 7% of meat-borne diseases in the Netherlands, respectively (Opsteegh et al. 2016). Beef is one of the most popular foods in the United States, and diverse *T. gondii* seroprevalences were found in a cow study (Guo et al. 2015). *T. gondii* prevalence in cattle, on the other hand, did not match the incidence of cysts in tissues in cattle. Dubey et al. (2005) examined the incidence of live *T. gondii* in 6,282 data acquired from 698 retail meat outlets in 28 main geographic regions of the United States. They found no bioassay or ELISA-positive results that were positive in any one of the 349 pools or 2,094 specific edible beef samples. Rahdar et al. (2012) collected tongue, heart, and muscle samples from 50 lamb and 50 beef wholesalers, as well as 90 meaty product samples from local Ahvaz city merchants. They discovered *T. gondii* cysts in seven lambs (14%) and two beef (4%), however, the infection did not originate from a single of the meat samples. Plaza et al. (2020) acquired 300 meat samples from butchers, farmers' markets, farm stores, and supermarkets. They found DNA in 0/39 (0%) cattle samples, 19/67 (28.4%) venison samples, and antibodies to *T. gondii* in the meat and juice of 2/38 (5.3%) beef samples.

T. gondii seroprevalence in raw sheep meat ingested in France was examined by Halos et al. (2010). The proportion of French carcasses bearing live parasites was estimated to be 5.4%. They cautioned that all case-control investigations have indicated mutton/lamb meat intake as a significantly major danger factor among pregnant women. Likewise, Villena et al. (2012) stated that ovine meat has been linked to an increased risk of Toxoplasma infection in specific parts of France.

Little knowledge on the presence of viable *T. gondii* in goats' tissue is available (Villena et al. 2012). Although *T. gondii* has been isolated from caprine tissues in investigations, no enormous scale prevalence data on the detection of parasites in goat flesh and products are known (Kijlstra and Jongert 2008a; Dubey et al. 2011). Dubey et al. (2011) obtained the hearts of 234 goats from a market in the United States. They discovered the antibodies to *T. gondii* in 125 of 234 goat hearts.

Pig meat constitutes one of the most common forms of meat involved with human-caused toxoplasmosis, and consumer cooking temperatures may not be adequate for deactivating *T. gondii* cysts in carcasses and products (Guo et al. 2015). Hamilton et al. (2015) detected antibodies from meat juice in 55% of pig hearts. The transmission of *T. gondii* in pigs has been nearly eradicated, and the amount of *T. gondii* in pork products has reduced considerably (Tenter et al. 2000; Kijlstra and Jongert 2008a). As a result, in many regions of the world, pork meat is no longer considered the primary source of infection (Kijlstra and Jongert 2008a).

Zoua et al. (2017) collected tissue from muscle specimens of 414 poultry birds (257 chickens, 115 ducks, and 42 geese) and discovered that 32 (7.37%) examples of meat from poultry were *T. gondii* B1 DNA positive, with chicken having the highest *T. gondii* incidence (8.17%), followed by ducks (7.83%), and geese (4.76%). This means that out of 257 chickens, 8.17% were infected, and of the 115 ducks mentioned, only 7.83% were affected. At the end of the study, the lowest rate was found in the geese; with a flock of 42, only 4.76% of them were affected.

Silva et al. (2003), examined *T. gondii* frequency in allowed-to-roam hens and reported that seroprevalence of free-ranging chickens was up to 65% and the present parasite in flesh obtained from seropositive was 81%. Although *T. gondii* is prevalent throughout chicken meat, the danger of spreading from infected chicken meat to humans is minimal since chicken flesh is normally properly cooked, and purchasing frozen chicken flesh may lessen the risk (Almeria and Dubey 2021).

T. gondii DNA was detected in 43% of 231 horse carcass samples in France by Aroussi et al. (2015), who also reported that there was no strain solitude in mice obeying inoculation of over 100 horse meat examples suggesting a low prevalence of cysts in the muscles of the skeleton and a small chance of infection with *T. gondii* related to horse meat consumption.

6. CONTROL OF *T. GONDII*

6.1. PRE-HARVEST PREVENTION

Preventive medicine practices in the fight against diseases in livestock are very important for both economic and public health (Dubey 1996). Many studies have found risk variables that are cat-related, although those connected with risk factors include contamination of feed or water, having access to a severely polluted environment, age, gender, and geographic and regional characteristics (Stelzer et al. 2019). During pre-harvest production, to control all these risks and to lessen the propagation of *T. gondii*, there are two important strategies: scientific and realistic management practices and vaccination (Dubey 1996).

a- FARM MANAGEMENT

Management of a farm can greatly help in *T. gondii* prevention in animals from which we get the meat by decreasing the animals' interaction with infectious phases from the environment. Farm type (indoor or outdoor production systems), feeding practices, decontamination of animal feed and bedding, cat, rodent, and bird control methods, water source, and quality are important farm management practices (Guo et al. 2015; Hussain et al. 2017). It is also critical to develop biosecurity policies on traditional farms and to apply laboratory tests to various kinds of flocks to detect farms that may have too much risk and to utilize them all across the chain of food information systems to improve food safety (Guo et al. 2015).

One of the drawbacks of outdoor farming is the lack of biosecurity. As a result of being around infected rodents in particular nature, and animal feed, water, or ambient surfaces carrying infectious oocysts, *T.*

gondii prevalence is greater in conventionally raised pigs, sheep, and fowl than in cattle (Guo et al. 2015). In order to be successful in these matters or in reducing the levels of infection in animals, monitoring and surveillance programs must be implemented (Kijlstra and Jongert 2008a).

The incidence of *T. gondii* severity in swine varies according to the animal's age, farm management techniques, and farm location (Guo et al. 2015). Natural pig farms are gaining popularity in many regions of the world. Pigs usually eat infectious rodents or rodent cadavers, as well as small animals. Because of this fact to reduce *T. gondii* seropositivity in pigs, monitoring and surveillance programs are vital (Burrells et al. 2015). The rate of seroprevalence is high in pigs and hens from non-biosecure management practices (Hill and Dubey 2013). The quantity of smaller swine farms in the United States is dropping, which might be one of the primary causes for the reduction in *T. gondii* seroprevalence in pigs during the previous decade (Dubey 1996).

b- VACCINATION OF CATS AND LIVESTOCK

Vaccine applications are very important in protecting humans and animals, especially against various diseases that cannot be treated with drugs. Because of the fact that there is no medicine that can combat the *T. gondii* tissue cyst stage (Innes and Vermeulen 2006), vaccination of farm animals against *T. gondii* is traditionally being implemented (Kijlstra and Jongert 2008a) and have two objectives; to reduce abortions in small ruminants, especially goats and sheep, and to decrease the risk of mankind's exposure to infected meat (Dubey 1996).

Understanding the defensive immune system response throughout *T. gondii* penetration and infection will be critical to producing a safer, more effective vaccine for toxoplasmosis in both people and cattle (Innes et al. 2019). The first generation of vaccines was elaborated with live, live attenuated, or inactivated antigens, the second generation consisted of the subunit vaccines (recombinant), and finally in third generation immunogens are based on genetic vaccines (Garcia 2018). Live and inactivated vaccinations that the immune system perceives as alien cause the creation of antibodies that can prevent infection (Garcia 2018). This vaccine is a tachyzoite vaccine with a live mutant strain (S48) that is easily available commercially. A live vaccination employing a nonpersistent variant of *T. gondii* is being researched in the United States to prevent abortion in sheep as is available in the country of New Zealand, the United Kingdom, and Europe to minimize oocyst shedding by cats (Dubey 1996). The researcher believes that developing single or multi-epitope-based antigens expressing possible B or/and T cell epitopes of both tachyzoite and bradyzoite-specific particular antigens would significantly enhance *T. gondii* vaccination techniques (Bastos et al. 2016). Vaccines made of DNA have various advantages, including ease of production, ease of administration, immunogenicity, and the possibility for long-term protection (Kim et al. 2012). Manufacturing vaccines by eliminating gene mutants provides a unique strategy for toxoplasmosis control (Zhang et al. 2022).

Burrells et al. (2015) wanted to see how immunization (incomplete S48 strain) affected the production of infective tissue cysts in pigs. According to the findings, the parasite preferentially inhabits the brain and extremely vascular skeletal muscles (such as the tongue, diaphragm, heart, and masseter) of swine, whereas meat cuts used for human consumption, including chop, loin, left triceps, along with left semitendinosus, possessed a smaller percentage of *T. gondii* cysts in the tissue in pork.

7. POST-HARVEST PREVENTION

a- MONITORING

The World Health Organization has frequently recommended collecting reliable epidemiological information on *T. gondii*. However, only a few nations monitor toxoplasmosis in people on a regular basis, and even fewer countries track the infection of *T. gondii* in animals (Tenter et al. 2000). To effectively prevent *T. gondii* infection in meat, an extensive carcass assurance system from 'farm to fork' is required (Felin et al. 2016). On the other hand, veterinary leadership is very important and adds value to the process (Huey 2012). Because toxoplasmosis surveillance in livestock farming is challenging due to the disease's silent and chronic manifestations, carcass surveys conducted by veterinary teams present several advantages and control plans for *T. gondii* (Villena et al. 2012). Despite the fact that monitoring systems for Salmonella and Campylobacter have begun in numerous nations, *T. gondii* in meat is not checked at the slaughterhouse because there are no defined benchmark sera or additional sources of information available, and there is no testing certification scheme (Kijlstra and Jongert 2008a). If a slaughterhouse monitoring program could be used for *T. gondii*, it offers an important opportunity to prevent transmission from meat to humans (Villena et al. 2012).

B- KILLING THE PARASITE

T. gondii tissue cysts are not resistant to environmental factors (Dubey 1996) and in some treatments, applied to the meat, such as freezing, and cooking, the elevated pressure is favorable for the elimination of *T. gondii* (Dubey 1996, Kijlstra and Jongert 2008a).

Dubey described the impact of cooling on *T. gondii* cyst survivability in 1966. Freezing to -12°C can kill tissue cysts in meat and the most feasible approach to risk control would be a freezing operation (Dubey 1996). *T. gondii* tissue cysts were alive for several days at -1°C and -7°C but were frequently declared unprofitable by freezing at -12°C by Kotula et al. (1991). Nevertheless, consumer attitudes towards freezing shows less demand. Garcia et al. (2021) found that dry-cured bacon contaminated with 4,000 *T. gondii* oocysts stored at -20°C for 7-14 days failed to inactivate *T. gondii*. Based on the results of the Monte Carlo computerized test, the cells in the cysts can survive at 4°C for at least 30 days (Rani and Pradhan 2021).

Heat treatment is an especially secure way to kill the parasite (Kijlstra and Jongert 2008a). Tissue cysts in meat can be killed by heating to an interior temperature of 67°C (Dubey 1996). The safe temperature for cooking is determined as 64°C (Rani and Pradhan 2021). Aroussi et al. (2015) recommended that cooking of horse meat is an important stage to avoid any risk of toxoplasmosis. Lunden and Uggla (1992) investigated the changing acts of microwave cooking over the infectivity of *T. gondii* and reported that the parasite remained infective in steaks processed in a microwave oven, maybe due to proper heat spreading. When tissue cysts are exposed to dosages of 0.4 to 0.7 kg of gamma radiation, the parasite can become inactive (Kijlstra and Jongert 2008a). *T. gondii* tissue cysts could also be inactivated utilizing a 300 MPa high-pressure (Kijlstra and Jongert 2008a).

8. DETECTION OF *T. GONDII*

a- MEAT INSPECTION

Meat inspection is an important stage in the healthy meat production chain. Monitoring animals during slaughter might be utilized to identify *T. gondii*-infected flesh and remove cysts from tissue in the meat using particular techniques, as well as to make farm management adjustments (Kijlstra and Jongert 2008a). The existence of this threat in asymptomatic animals during antemortem inspection impedes controlling it in the meat chain. Traditional meat inspection cannot identify minute cysts of *T. gondii* in

slaughter animal tissues, and existing laboratory techniques are still not sensitive enough to identify the infection in individual carcasses (Kuruca et al. 2023). As a result, during the current post-mortem assessment of pig carcasses, mainly *Trichinella* spp. are detected as meat-borne biological risks (Zdolec and Kis 2021).

Developing the Quantitative Microbial Risk Assessment model has helped to look into the presence and amount of feasible bradyzoites in cattle to implement farm management changes, to assess the impact of muttering flesh on bradyzoite amounts using real batch sizes, and to estimate the percentage of meat that has been still prior to purchase (Opsteegh at al. 2011). When compared to traditional slaughter and meat inspection, the meat factory cell allows for customized chilling regimes for various sections, focused cleaning or pathogen-killing procedures, and reduced energy usage, which will benefit public health (Alvseike et al. 2018).

Although numerous indirect and direct techniques for detecting *T. gondii* have been established (Rani and Pradhana 2020) yet three types of approaches for detecting *T. gondii* in flesh and other goods have been created: (i) mouse or cat bioassays, (ii) serological tests, and (iii) PCR-based approaches. While bioassays in mice and cats are regarded as the gold standard for detecting live *T. gondii* in animal products, but these are time-consuming, costly, and need an extensive number of animals (Rani and Pradhana 2020).

Gazzonis et al. (2020) sought epidemiological and molecular information on *T. gondii* contamination in small ruminants slaughtered and sold in Italy. They discovered antibodies in 28.6% of sheep and 27.5% of goats. *T. gondii* DNA was found in fifteen sheep and three goats based on DNA testing of positive muscle samples. Hosein et al. (2016) gathered samples of 305 animals slaughtered throughout the UK, and 1.6% animals tested positive for *T. gondii* after real-time PCR.

b- SEROLOGICAL DETECTION

The present approaches for detecting *T. gondii* in meat-producing livestock, animal products, or the environment are inadequate since they do not allow for the quantification of infectious phases (Tenter 2009). Most people with *T. gondii* infection have no or few clinical signs, and their diagnosis is based mostly on serological testing. To detect distinct antibody classes or antigens, a variety of serological tests have been developed, including dye tests, modified agglutination tests (MAT), enzyme linked immunosorbent assays (ELISA), immunosorbent agglutination assays, indirect fluorescent antibody tests, and indirect haemagglutination assays (Liu et al. 2015). Serological screening for toxoplasmosis in slaughterhouse has shown to be the most effective approach. Based on their findings, Felin et al. (2015) proposed that using meat juice serology during slaughter is a valuable technique for controlling this pathogen.

The indirect ELISA proved to be a simple, quick, low-cost, and more sensitive assay for detecting *T. gondii* in livestock juice, and it might be regarded as an attractive test to track toxoplasmosis in cow meat and meat products. The meat juice was used as an appropriate sample for detecting *T. gondii* antibodies using ELISA, and diaphragmatic tissues could be used as a matrix for parallelism seropositive diagnosis (Shaapan et al. 2021).

At a slaughterhouse, Villena et al. (2012) gathered flesh specimens and muscular secretions from 419 ovine carcasses. On cardiac fluids, an industrial ELISA plus a MAT were used. They discovered that ventricular fluids appear to be a useful matrix for toxoplasmosis detection in meat. Wang et al. (2012) collected 416 freshly slaughtered pork samples from various sites in Anhui province, Eastern China, and utilized ELISA to identify *T. gondii* antibodies in the fluid from the tissues. Similarly, Mecca et al. (2011) collected effusion from meat packaging owing to their blood content in order to identify infections that occur naturally in rabbit meat for sale. In addition, they acquired chops from Botucatu strain rabbits during

slaughter and discovered 1.35% (1/74) positive samples in commercialized Brazilian rabbit meat cuts using this technique.

Sousa et al. (2010) collected blood samples from 11 free-range hens and fifteen pigs in order to isolate *T. gondii* genotypes. Serotyping findings from three chicken serum samples and two pig serum samples were consistent with genotyping. Roqueplo et al. (2011) collected samples (serum or meat juice) from 205 animals in New Caledonia and detected *T. gondii* in 2% of the pigs, 3.3% of the cattle, 13.8% of Rusa deer, 16% of the horses, 32.8% of the dogs, and 50% of cats.

c- MOLECULAR DETECTION

The gold standard bioassay methods for identifying *T. gondii* in samples of meat (Opsteegh et al. 2011; Rani and Pradhana 2020) are tedious and time-consuming approaches that are not suitable for screening large quantities of samples due to animal ethical concerns (Opsteegh et al. 2011). Toxoplasmosis is diagnosed using molecular approaches in addition to traditional serological methods (Liu et al. 2015). *T. gondii* may be readily defined by detecting distinct and unique genes, and the amount of *T. gondii* may even be counted using a real-time PCR experiment (Guo et al. 2015). Mancusi et al. (2023) set out to create a droplet digitally polymerase chain reaction-based test for detecting and quantifying *T. gondii* in meat samples, to achieve absolute quantification of the target DNA, QuantaSoft software was utilized for determining the PCR + and PCR - droplets. Each target's quantification values were represented as the number of genome copies per 1 l of reaction. They observed that this novel method might be highly beneficial for detecting tiny quantities of *T. gondii* in meats. If the sample had two drops, it was declared affirmative. Positive droplets were not seen in the negative control samples. The reaction produced droplets in the range of 8985 to 13,940, with a median of 11,384 droplets. For analysis, reactions with over 8000 accepted droplets per well were employed. The ddPCR results showed a strong separation of both positive and negative droplets with few interface droplets, indicating a high primer specificity and reaction efficiency. Drops were positively saturated at high concentrations (> 10,000 GC/l), rendering the Poisson method incorrect and resulting in a substantially lower variability than qPCR. Wang et al. (2012) attempted to provide the first study on the distribution and genetic type of *T. gondii* in pork in Chinese retail meat outlets. They discovered that 75 out of 416 specimens (18.03%) had a positive real-time PCR result. Aspinall et al. (2002) collected 71 meat specimens from retail establishments in the United Kingdom. Using particular primers that targeted the *T. gondii* SAG2 locus, they discovered the parasite in 27 of the flesh samples and 21 of the infected foods. Franco-Hernandez et al. (2016) used PCR to examine a total of 120 specimens from the first group of herbs and 60 samples from class II herbs in Colombia. They discovered that 79 (43%) of the samples were positive for B1 nested PCR tests (33 from chicken, 22 from beef, and 24 from pig) and advised that comprehensive PCR research can assist to implement effective methods to limit the risk of getting this parasite through meat intake.

Pastiu et al. (2015) gathered sera and cardiac tissue from 82 horses killed in the northern part of Romania for trade and human consumption. Antibodies anti-*T. gondii* were found in 32 (39%) of the serum and 31 (37.8%) of the cardiac tissues. *T. gondii* DNA wasn't found in any of the cardiac samples, contrary to the PCR results. Despite these breakthroughs in identifying *T. gondii* using PCR, it is stated that the substantial salt content in certain cured foods reduced the PCR assay's sensitivity. Warnekulasuriya et al. (1998) found alive *T. gondii* in one of 67 ready-to-eat meat cure samples, although they suggested that new methods for identifying the protozoa to infiltrate of cured meats were needed. It's quite a challenge to get rid of this protozoa easily and completely by using the old and known techniques.

9. CONCLUSION

Food-borne toxoplasmosis in humans can occur as a result of exposure to various stages of *T. gondii*, including eating or drinking cysts of tissue or tachyzoites found in meat, offal (viscera), or the intake of sporulated oocysts found in the atmosphere and water. Because of *T. gondii*'s propensity to spread through a broad spectrum of livestock hosts and stay within their tissues for years raw or undercooked meat carrying live cysts has been acknowledged as an important cause of infection with *T. gondii* in humans. Although the percentage of live *T. gondii* in meat sold at retail was very low, customers can get *T. gondii* infection by eating raw meat. However, many people are unaware of ways to avoid *T. gondii* infection, particularly the hazards related to ingesting or handling undercooked or raw meat. Understanding these risk variables can assist to focus preventative efforts.

Monitoring programs for toxoplasma infection in livestock animals is desirable for human toxoplasmosis prevention. Good Farming Practices to develop efficient and sustainable control measures and better rodent management towards infection with *T. gondii* in farms, such as severe confinement housing and stringent biosecurity laws, is critical. Vaccine development and immunization of livestock on farms against *T. gondii* have traditionally been used to prevent miscarriages in small livestock and the danger of human exposure to contaminated meat. Despite the fact that toxoplasmosis is recognized as one of the most underappreciated biological dangers in the meat supply chain, there is no legal mandate for *T. gondii* surveillance or control in livestock-producing animals and their products. The presence of the parasite inside slaughtered animals suggested that the danger of food-transmitted toxoplasmosis remains. Unfortunately, routine meat inspection cannot identify minute cysts of *T. gondii* in slaughtered animal tissues, and modern laboratory techniques are still not sensitive or specific enough to detect contamination in individual carcasses. As a result, developing and standardizing assays for identifying the infection of *T. gondii* in meat-producing species and their products with equivalent specificity and sensitivity is critical.

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