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***Toxoplasma gondii* in Meat and Food Safety Implications – A Review**

Susana Bayarri, María Jesús Gracia, Regina Lázaro,
Consuelo Pérez-Arquillué and Antonio Herrera
*University of Zaragoza, Veterinary Faculty,
Spain*

1. Introduction

In contrast to the important global advance in the last decades in the food availability, hundreds of million people anywhere in the world suffer diseases caused by the food consumption, maintaining effective today, an important problem for the public health and an extraordinary cause of reduction of the productive economy.

Foodborne diseases are caused by a number of agents, varying their severity from weak to chronic or acute disturbances that can affect or compromise the life of the consumer, and being the agents of biological origin (bacteria, viruses, parasites) the major cause of these diseases.

The important advances accomplished regarding personal hygiene, basic cleaning, potable water supply, food control structures, and the increase of the systems of technological control that assure the elimination or destruction of food pathogens to safe limits, have led to a practically null number of cases of classic food diseases at the present time in the industrialized countries.

Nevertheless, there are many reasons for foodborne disease remaining a global public health challenge. As some diseases are controlled, others emerge as new threats. New agents of risk have occupied the ecological niche of those on which control pressure has been exerted, replacing the previous ones. The proportions of the population who are elderly, immunosuppressed or otherwise disproportionately susceptible to severe outcomes from foodborne diseases are growing in many countries. Globalization of the food supply has led to the rapid and widespread international distribution of foods. This fundamental fact has motivated a radical change in the modern systems of management of the food safety, forced to the search of new methods of risk assessment, more effective preventive systems, permanent research of new systems of identification of these agents, to the development of the epidemiology applied to the food hygiene and the application of much more effective methods in the complex world of the decision making.

A recent publication from the United States concludes that more than 90 percent of the health burden is caused by five pathogens: *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Toxoplasma gondii* and norovirus. *Toxoplasma gondii* is not a “front page”

foodborne pathogen, but it is very important from a public health standpoint (Batz et al., 2011). The CDC (Centers for Disease Control and Prevention) estimates that foodborne toxoplasmosis is surpassed only by *Salmonella* in the number of annual deaths it causes. Although toxoplasmosis is conventionally associated with cats and kitty litter, currently it is estimated that 50% of cases are foodborne (Scallan et al., 2011).

Parasites, including *Toxoplasma gondii*, are reported less frequently in humans, and have caused fewer outbreaks than bacteria and viruses in the European Union (EU). However, in many instances, their impact (severe illness, disability, death, and costs related to diagnostic procedures, hospitalization and treatment) on vulnerable groups of the population, and often in immunocompetent persons, has been considerable (EFSA, 2007).

Toxoplasmosis is a common infection in animals and humans. It is caused by an obligate intracellular protozoan parasite, *Toxoplasma gondii*. The life cycle of *Toxoplasma* includes asexual multiplication in the intermediate host and sexual reproduction in the definitive host. Many species of warm-blooded animals can act as intermediate hosts and, seemingly, most animal species may be carriers of tissue cysts of this parasite. Cats and wild felids are the only definitive hosts that may pass oocysts with their faeces and these need to sporulate in the environment before becoming infective.

In intermediate hosts, *Toxoplasma* undergoes two phases of asexual development. In the first phase, tachyzoites multiply rapidly in many different types of host cells. Tachyzoites of the last generation initiate the second phase of development which results in the formation of tissue cysts containing bradyzoites, which multiply only infrequently. All hosts, including humans, can be infected by three different life cycle stages: tachyzoites, bradyzoites contained in tissue cysts and sporozoites contained in sporulated oocysts (Dubey, 2007).

The organotropism of tissue cysts varies in different intermediate host species. In many hosts, tissue cysts have a high affinity for neural and muscular tissues. They are located predominantly in the central nervous system, the eye as well as skeletal and cardiac muscles. However, to a lesser extent they may also be found in visceral organs, such as lungs, liver, and kidneys (Dubey, 1993; Dubey et al., 1998). Tissue cysts are the terminal life-cycle stage in the intermediate host and are immediately infective. In some intermediate host species, including most livestock, they may persist for the life of the host, being consumption of raw or undercooked meat products containing tissue cysts a major risk factor associated with human toxoplasmosis (Dubey & Beattie, 1988; García et al., 2006; Hill, 2007; Mie et al., 2008). *Toxoplasma* has historically been associated with pork meat but a recent case-control study by CDC found the leading foodborne risks to be eating raw ground beef, rare lamb or locally-produced cured, dried or smoked meat (Jones et al., 2009).

Consumption of food and water contaminated with sporulated oocysts and congenital infection, are another main modes of transmission of *T. gondii*. In humans, the majority of infections is asymptomatic or cause mild flu-like symptoms. However, infection may produce a severe disease in immunocompromised people, and abortions in pregnant women, as well as adverse effects such as perinatal death, fetal abnormalities, or reduced quality of life in children who survive a prenatal infection. *T. gondii* can cause permanent and devastating damage to developing fetuses, including stillbirths, serious hospitalization during infancy, and permanent, lifelong mental and physical disabilities (EFSA, 2011 b).

Batz et al. (2011) estimate that congenital toxoplasmosis acquired through food results in 16 stillbirths or neonatal deaths annually in USA, as well as in 216 infants born with mild to serious permanent impairments, ranging from blurry vision, mental impairment and neurological problems such as partial paralysis and abnormal movement.

There is a globalisation in the trade of animals and food worldwide, so rules for trade of meat and meat products seek to guarantee that all imports and exports fulfil high standards to guarantee food safety. This should also be addressed to the animal health status and high standard of meat and meat products in order to avoid human toxoplasmosis. Besides measures focussing on pre-harvest food safety (e.g. surveillance and monitoring in animals), post-harvest strategies at slaughter and during food processing have become more and more important in recent years.

With regard to meat processing, demands of consumers for pathogen free meat products have focused the attention of the meat industry on food safety and the necessity to produce meat that is wholesome, safe, and of high quality, using the appropriate technological treatments. Scientific studies have indicated that *T. gondii* tissue cysts in meat are susceptible to various physical procedures such as heat treatment, freezing, irradiation or high hydrostatic pressures, and some authors have suggested that tissue cysts are killed by commercial procedures of curing with salt.

Improved surveillance is needed to better estimate the true incidence of foodborne toxoplasmosis, and significant increases in data collection, epidemiologic studies and scientific research are needed to understand the relative importance of routes of toxoplasmosis transmission. Addressing the risk of the foodborne disease requires the implementation of a well functioning and integrated food control system, and this necessitates collaboration among all the components of a food control system, including food law and regulations, food control management, inspection services, epidemiological and food monitoring (laboratory services), education, and communication with the consumer (WHO, 2008). Under this consideration, surveillance and monitoring in food and food products could provide important information for a better risk assessment on *Toxoplasma* and toxoplasmosis from the consumer protection point of view (EFSA, 2007).

Considering the importance for the risk assessment process, the aim of this review is to show the state of the art for data implicating meat as a source of infection and to describe studies focused on the effect of technological processes of meat products in the inactivation of *Toxoplasma gondii*.

2. Surveillance and monitoring in animals used for human consumption. Meat as a source of *Toxoplasma gondii* infection in humans

There is a widespread distribution of *Toxoplasma* infection in a variety of livestock, wild animals and pets. Ingestion of environmentally robust stages (sporozoites in oocysts) or eating raw or undercooked meat or meat products containing tissue stages (tachyzoites or bradyzoites in tissue cysts), are the main transmission routes for *T. gondii* to humans (EFSA, 2007).

Some authors assume that about 50% of all human toxoplasmosis cases are related to foodborne infection (Slifko et al., 2000), and retrospective epidemiological analyses of

human toxoplasmosis outbreaks suggest that many are associated with consumption of raw or undercooked meat or other edible parts of animals. Tenter et al. (2000) and Schlundt et al. (2004) estimated that the percentage of meat-borne cases was approximately 30% to 63%, depending on eating habits.

However, the relative importance of the risk factor and the type of meat associated with it varied among different countries (Cook et al., 2000). For example, in France and Norway consumption of undercooked lamb was a stronger risk factor than consumption of undercooked pork (Kapperud et al., 1996; Baril et al., 1999), whereas in Poland consumption of undercooked pork was the principal risk factor identified in the study (Paul, 1998). These findings may reflect differences in eating habits of consumers or different prevalences of infection in meat producing animals in these regions.

In response to natural infection, most farm animals are seropositive for *T. gondii* and serological studies have found evidence of widespread *T. gondii* infection in meat-producing animals. It is important to note that the organotropism of *Toxoplasma* and the number of tissue cysts produced in a certain organ vary with the intermediate host species. In livestock, tissue cysts of *Toxoplasma* are most frequently observed in various tissues of infected pigs, sheep, and goats, and less frequently in infected poultry, rabbits, and horses (Tenter et al., 2000). Although tissue cysts are less resistant to environmental conditions than oocysts, they are relatively resistant to changes in temperature and remain infectious in refrigerated (1 to 4°C) carcasses or minced meat for up to 3 weeks, *i.e.* probably as long as the meat remains suitable for human consumption (Dubey, 1988; Tenter et al., 2000).

Experimental infections of food animals such as cattle, pigs, sheep and goats, have shown that these animals are susceptible to *T. gondii* contamination by intake of oocysts or tissue cysts, and that following experimental infection *T. gondii* can be isolated from their tissues, with the exception of beef (Dubey et al., 1980, 1984; Dubey, 1983, 1986a, 1988; Blewett et al., 1982; McColgan et al., 1988; Lunden & Uggla, 1992; Dubey & Thulliez, 1993; Esteban-Redondo et al., 1999; Tenter et al., 2000; Zia-Ali et al., 2007).

It is very important the impact of farming systems on the risk of *Toxoplasma* infections. Recent data show that it is possible to significantly reduce the risk of *Toxoplasma* infection in livestock using intensive farm management with adequate measures of hygiene, confinement, and prevention. These measures include: (A) keeping meat-producing animals indoors throughout their life-time, (B) keeping the sheds free of rodents, birds, and insects, (C) feeding meat producing animals on sterilised food, and (D) controlling access to sheds and feed stores, *i.e.* no pet animals should be allowed inside them. On the contrary, production of free-ranging livestock will inevitably be associated with *Toxoplasma* infection. Animals kept on pastures with an increased pressure of infection due to contamination of the environment with oocysts, such as goats and sheep, show a high level of seropositives in many areas of the world, *i.e.* up to 75% to 92% respectively (EFSA, 2007).

The observed decline in *Toxoplasma* seroprevalence as noted in many developed countries over past decades has been attributed to the introduction of modern farming systems resulting in a lower prevalence of *Toxoplasma* cysts in meat in combination with an increased use of frozen meat by consumers (Tenter et al., 2000; Kortbeek et al., 2004; AFSSA, 2005; Diza et al., 2005; Jones et al., 2007).

Another important aspect is the age of the animals. In a recent study (Berger-Schoch et al., 2011), a P-30-ELISA was used to detect *T. gondii*-specific antibodies and to determine seroprevalences in meat juice of slaughtered animals in Switzerland. The study included pigs, cattle, sheep and wild boar of different age groups and housing conditions. The results show that the seropositivity increased with the age of the assessed animals. Independent of the age-group, the overall seroprevalence was lowest in wild boars (6.7%), followed by pigs (23.3%), cattle (45.6%) and sheep (61.6%). Conventional fattening pigs and free-ranging pigs surprisingly had comparable seroprevalences (14.0% and 13.0%, respectively). Unlike in other European countries, where generally a decrease in the number of seropositive animals had been observed, these authors found that the prevalence of seropositive animals, when compared with that of 10 years ago, had increased for most species/age groups. Conclusively, the results demonstrated a high seroprevalence of *T. gondii* in animals slaughtered for meat production and revealed that increasing age of the animals is a more important risk factor than housing conditions in this country.

Sheep, rather than pigs, are the main source of infected meat in Southern European countries (EFSA, 2007). Data from a number of different European countries showing that, whilst seroprevalence of *Toxoplasma* amongst pigs has fallen in the last three decades, to around 1%, the measured values for sheep have remained significantly higher at 20–30% (Tenter et al., 2000). Relevant to this apparent increase in the potential of sheep to serve as a route of human infection is the fact that, like pigs, they do not generally develop overt clinical symptoms upon *T. gondii* infection (Owen & Trees, 1999).

In sheep and goats, seropositives reported in different countries vary widely. Dubey (2009) reviewed serological surveys in various countries conducted since 1988, and found 3–96% positive results. This author also concluded that in general most sheep acquired infection before 4 years of age. Congenitally-infected lambs that survive the first week after birth usually grow normally and can be a source of infection for humans. In Europe, data available in sheep report seropositive rates of 16–66% (Tenter et al., 2000; Dumètre et al., 2006; Pinheiro et al., 2009; Panadero et al., 2010; Pikka et al., 2010). High seroprevalences were found also in Bangladesh (40%) (Shahiduzzaman et al., 2011), and in New Zealand (61%) (Dempster et al., 2011). Seropositivity is found correlated with age, increasing from lambs (22%) to ewes (65.6%) (Dumetre et al., 2006) and also differences among different geographical areas were found (Panadero et al., 2010).

Prevalence of *T. gondii* in lambs can be high but the role of ingestion of infected lamb in the epidemiology of toxoplasmosis in humans remains to be determined (Dubey & Jones, 2008). Raw or undercooked lamb meat is considered a delicacy in certain countries such as France and is therefore considered an important source of infection in that country (AFSSA, 2005). Adult sheep meat is often well cooked and therefore probably poses a smaller risk of infection to the consumer than lamb meat. All case-control studies have identified the consumption of mutton/lamb meat as a highly significant risk factor for contracting *T. gondii* infection in pregnant women. Symptomatic toxoplasmosis in a family in New York City was circumstantially linked to eating rare lamb (Masur et al., 1978). Recently a large-scale screening of sheep farms has shown that 3.4% of sheep were shedding *T. gondii* in their milk (Fusco et al., 2007).

Although abortion and neonatal mortality are the main clinical signs, adult goats can develop clinical toxoplasmosis involving liver, kidneys and brain (Dubey & Beattie, 1988). Seroprevalence is dependent on the presence of oocysts in the environment, housing and climatic conditions (Tenter et al., 2000). Seropositivities reported in Europe in farmed goats vary from 4% to 77% and data in slaughtered goats in non-European countries range from 0% to 40% (data reviewed by Tenter et al., 2000). Recently, a high seroprevalence was found in goats in Bangladesh (32%) (Shahiduzzaman et al., 2011).

Although studies have reported the isolation of *T. gondii* from caprine tissues, no large-scale prevalence data are available on the presence of parasites in goat meat products (Dubey, 1980, 1981; Sharma et al., 2003). Small ruminants such as goats are an important source of meat (very popular in several ethnic groups, especially from Asia) and milk in many undeveloped countries and may play a role as a source of infection for humans residing in these areas (Shrestha & Fahmy, 2005). As well, consumption of raw goat's milk and milk products has been linked to cases of toxoplasmosis in humans (Riemann et al., 1975; Sacks et al., 1982; Skinner et al., 1990; Meerburg et al., 2006).

Numerous serologic surveys on prevalence of *T. gondii* in pigs from different countries have been performed. The results showed wide variation in the prevalence values among countries and between regions within the same country (Tenter et al., 2000; Dubey, 2009; Dubey & Jones, 2008; Alvarado-Esquivel et al., 2011; Yu et al., 2011). *T. gondii* infection is widespread in pigs raised in Spain and a seroprevalence of 16.6% was obtained in a recent study (Garcia-Bocanegra et al., 2010). Similar seroprevalences were obtained in studies carried out in Italy (Villari et al., 2009; Veronesi et al., 2011), Portugal (De Sousa et al., 2006) and Germany (Damriyasa et al., 2004). Lower seroprevalence levels were found in USA (2.6%) (Hill et al., 2010), Sweden (5.2%) (Lundén et al., 2002), the Netherlands (10.9%) (Kijlstra et al., 2004) and Mexico (12.7%) (Alvarado-Esquivel et al., 2011); while higher values were observed in Serbia (28.9%) (Klun et al., 2006) and Argentina (37.8%) (Venturini et al., 2004). Analysis of swine management practices indicated that rodent control methods and carcass disposal methods were associated with differences in the number of *T. gondii* positive samples on farm (Hill et al., 2010).

Also, prevalence of *T. gondii* varies dramatically among the classes of pigs surveyed (market pigs *versus* sows, indoor pigs with a biosecurity system *versus* free-range) (Dubey & Jones, 2008). Seropositivities in swine reared in Europe (Tenter et al., 2000) vary from null to 64% for fattening/slaughter pigs and from 3 to 31% for sows.

Seropositivity in general is a good indicator of the presence of viable parasites in tissues (Dubey et al., 1995, 2002; Dubey & Jones, 2008) and the level of isolation increased with antibody titre in the pig (Dubey et al., 1995). However, the antibody titre that should be considered indicative of latent infection in pigs is not always certain because viable *T. gondii* has been isolated from seronegative pigs (Hejlíček & Litérak, 1993; Omata et al., 1994; Dubey et al., 1995, 2002; De Sousa et al., 2006). These results indicates that either these pigs were recently infected and had not yet developed *T. gondii* antibodies, or that the antibody titres had declined to undetectable levels (Dubey et al., 1995). In this regard, Hill et al. (2006) suggest that the antibody response may be independent of parasite burden.

Toxoplasma cysts in pork can persist for a long time, and has been considered an important source of infection for humans (EFSA, 2007). A qualitative risk assessment identified

Toxoplasma gondii and *Trichinella* spp., as well as *Salmonella* spp. and *Yersinia enterocolitica*, as the most relevant biological hazards in the context of meat inspection of swine (EFSA, 2011a). However, due to major changes in animal production hygiene, the prevalence of *T. gondii* in pork meat has decreased (Tenter et al., 2000; Dubey & Jones, 2008), and currently, modern production systems, especially those in which intensive management has been adapted, have virtually eliminated *T. gondii* infection in pigs (van Knapen et al., 1995; Davies et al., 1998; Kijlstra et al., 2004; van der Giessen et al., 2007; Kijlstra & Jongert, 2008b).

Several epidemiological studies conducted in The Netherlands show that the prevalence of antibodies specific for *Toxoplasma* antigens in industrially kept fattening pigs is declining over the years. In 1969, a seroprevalence of 54% for *Toxoplasma* antibodies was observed in fattening pigs, while in 1982 this was only 1.8% (van Knapen et al., 1995). In a survey conducted in 1983–1984, seroprevalence was 23% in market pigs and 42% in breeder pigs (sows) (Dubey et al., 1991). When pigs from these same areas were tested in 1992, prevalence had dropped to 20.8% in breeders and 3.1% in finisher pigs (Dubey et al., 1995). In Brazil, the seroepidemiological investigation of *T. gondii* on farms revealed a prevalence of 37.8% in the early 1990s, ten years later this rate had decreased to 15.4% (Vidotto et al., 1990; Tsutsui, 2000). This downward tendency has been observed worldwide and is presumed to be related to the indoor housing system of pigs, where contact with cats is prevented and vermin in the stables is under control (van Knapen et al., 1995). However if bio security standards are low, the prevalence could increase up to 51-55% (Dubey et al., 2005).

Although modern meat production has reduced the prevalence of *T. gondii* in young pigs in Europe and North America (Tenter et al., 2000), a higher seroprevalence of *T. gondii* has been observed in sows compared to fattening pigs (Dubey, 1986a; Weigel et al., 1995; Lundén et al., 2002; Damriyasa, et al., 2004; Villari et al., 2009; García-Bocanegra et al., 2010; Alvarado-Esquivel et al., 2011). The higher seroprevalence in sows compared with market age pigs is epidemiologically relevant with respect to transmission of *T. gondii*. Market age pigs are sold for use in fresh, unprocessed pork products whereas meat from breeding sows is usually processed (such as sausages, salami, etc.) and processing kills or reduces *T. gondii* in pork (Dubey, 2009).

Recent trends in consumer habits indicate a shift towards consumption of "animal-friendly" or "organic" pigs. This animal production system of rearing pigs outdoors increased risk of exposure to *T. gondii* and it is likely to increase *T. gondii* seroprevalence. A study on seroprevalence of *Toxoplasma* antibodies conducted in 2001-2002 in The Netherlands indeed demonstrated a prevalence of 2.9% in pigs kept in animal-friendly housing systems, while 0% of the indoor pigs were seropositive (Kijlstra et al., 2004). Moreover, a seroprevalence study in indoor, organic and free ranging pigs carried out in 2004 in the Netherlands showed an overall prevalence of antibodies specific for *Toxoplasma* of 2.6%. In this study the seroprevalence in intensively raised pigs was close to nil (0.38%), whereas in organic pigs was 2.74%, and in free-range pigs the prevalence was 5.62%. The risk of detecting *Toxoplasma* antibodies in a free-range farm is statistically higher (almost 16 times higher) than in an intensive farm (van der Giessen, 2007). Pigs reared in organic farms and free-ranging pigs have indeed increased opportunities of contact with *Toxoplasma* compared to animals reared in close confinement, as they may be more exposed to contact with soil contaminated with *Toxoplasma* oocysts or to ingestion of infected preys, like rodents harbouring tissue cysts. In addition, farm management

practices in organic farms such as feeding goat whey to pigs and allowing contact between pigs and cats may influence the prevalence of *Toxoplasma* infection in the herd (Meerburg et al., 2006). In fact, increasingly popular animal friendly production systems may cause a re-emergence of pork meat as an infectious meat source (Kijlstra et al., 2004; Schulzig & Fehlhaber, 2006; van der Giessen et al., 2007).

Any part of infected pork can be a source of infection because the parasite has been found in most edible tissues or cuts of meat, both in experimentally and naturally-infected pigs (Kijlstra & Jongert, 2008a). However, Dubey et al. (1996) have estimated that less than 1 cyst per 50g of tissue is likely to be found in *Toxoplasma*-infected pigs. Tissue cysts have a high affinity for neural and muscular tissues. They are located predominantly in the central nervous system, the eye as well as skeletal and cardiac muscles, and to a lesser extent they may also be found in visceral organs, such as lungs, liver, and kidneys (Dubey, 1993; Dubey et al., 1998).

Examinations were conducted on the presence of *Toxoplasma* cysts in fresh pork sausages, produced in factories in Londrina (Parana State, Brazil). After bioassay in mice, 13 (8.7%) sausage samples were positive, in one of them *Toxoplasma* was isolated and in the other 12 the mice seroconverted (Dias et al., 2005). Lower detection was obtained by Galván-Ramírez et al. (2010) who analyzed meat samples of pork meat from butcher shops in Ocotlán (Jalisco, Mexico), detecting *T. gondii* in 1 of the 48 samples analyzed (2.1% positivity).

Bayarri et al. (in press), carried out a study on the prevalence of viable *T. gondii* in retail fresh pork meat collected in the city of Zaragoza (Northeast Spain). To ensure that samples were not from the same animal, sampling was carried out in different weeks and in different shops (supermarkets and butchers) distributed in different quarters of the city. More in detail, 25 pieces of fresh pork meat were sampled, corresponding to tongue, rib, loin, and shoulder loin. A mouse concentration bioassay technique was used, and the presence of the parasite in mice was determined by indirect immunofluorescence assay (IFA). *T. gondii* were detected in two samples of rib, reflecting a frequency of 8% positive fresh pork meat. Brains of seropositive mice were analyzed by histology and PCR, although the parasite was not isolated in the seroconverted mice. No viable forms were detected either in other type of fresh meat.

Aspinall et al. (2002) analyzed 58 pork meat product samples obtained from United Kingdom retail outlets, and obtained that 20 were *T. gondii* positive by PCR detection (34.5%). The higher positivity obtained may be because unlike the previously mentioned studies, detection of *Toxoplasma* in this work was purely by PCR, and they only demonstrated the presence of *T. gondii* and not the presence of viable parasites capable of initiating a human infection.

Birds can serve as a potential source of infection for humans. In chickens, *T. gondii* was found in skeletal muscles, heart, brain, ovary, oviduct, kidney, spleen, liver, lung, pancreas, gizzard, proventriculus, intestine and retina, and even in eggs (Jacobs & Melton, 1966; Kaneto et al., 1997). Several surveys in different non-European countries have shown that seropositivities to *Toxoplasma* in poultry can be moderate to high. Seroprevalence of up to 65% in free ranging chickens has been reported and the presence of the parasite in meat could be shown in 81% of seropositive animals (Da Silva et al., 2003; Lehmann et al., 2006). Recently, Bártová et al. (2009) found very low antibody prevalence in gallinaceous birds.

Seropositivity to *Toxoplasma* and isolation of the parasite has been reported in free-range chickens reared in Austria (Dubey et al., 2005) and in Portugal (Dubey et al., 2006). Viable *T. gondii* from ovaries, oviducts and leg muscle were isolated by Jacobs & Melton (1966) and viable *T. gondii* was isolated from 27% to 100% of chickens from backyard operations on small farms in USA (Dubey et al., 2003, 2004). Tissue cysts of *T. gondii* were found in breast and leg muscles, heart, brain, liver and stomach of experimentally infected domestic ducks (Bártová et al., 2004).

Free ranging chickens, especially in developing countries, may be considered as an important source of *T. gondii* infection in humans. In the Western world, commercially produced free ranging chickens intended for meat consumption (broilers) have a limited life span and to date no recent data are available concerning *T. gondii* seroprevalence in these chickens, however it can be expected that poultry kept outside has a higher chance of being infected with the parasite (Dubey et al., 2004). The recent trend of consumers demanding meat from organically grown free-range poultry will increase the prevalence of *T. gondii* in chickens consumed by humans and it will be necessary to cook the meat properly to protect consumers from infection. However, chicken meat is mostly well cooked for consumption (Dubey & Jones 2008).

Due to their habit of feeding close to the ground, poultry is considered a good indicator of environmental contamination by *Toxoplasma* oocysts and to identify *Toxoplasma* strains throughout the world (Lehman et al., 2006).

In a comprehensive study, the prevalence of *Toxoplasma* was determined in 2,094 meat samples of different species from 698 retail meat stores of the United States (Dubey et al., 2005). None of cats fed chicken samples became positive. There are several reasons why the results of this study do not negate the possibility that infected chickens may be important sources of infection for humans. In this study, chicken breasts were selected for sampling because of the experimental design that required testing of 1 kg of boneless meat for each sample, although the authors were aware that the prevalence of *T. gondii* in chicken breast is lower than in other tissues. Further, many of the chicken breasts had been injected with enhancing solutions that have a deleterious effect on *T. gondii*. Finally, some of the samples collected might have been frozen or hard chilled, although the labels indicated otherwise. Standards of hard chill are vague and *T. gondii* is highly susceptible to freezing. In contrast to the bioassay results, antibodies to *T. gondii* were found in 1.3% of the juice extracted from the breast meat using an ELISA, with values six times higher than in control chicken sera. These data suggest that *T. gondii* does occur in commercially marketed chickens in the USA but processing and handling procedures inactivate the organisms prior to sale to consumers.

Several surveys have also reported the finding of antibodies to *Toxoplasma* in horses. Seropositivities reviewed by Tenter et al. (2000) ranged from <1% to 8% in EU, up to 32% in non-EU countries, even in certain regions of the world, up to 90% of the animals were shown to be seropositive (Tassi, 2007). Presence of cysts has been shown in edible tissues from horses (Alkhalidi & Dubey, 1979). The role of horses as a source of *T. gondii* infection depends on regional preferences for horse meat, the preparation method and the seroprevalence of horses used for consumption (Gill, 2005; Tassi, 2007). Raw or undercooked horse meat is frequently consumed in countries such as Belgium, Italy, France and Japan (Gill, 2005).

Cattle are considered a poor host for *T. gondii*. Although cattle can be successfully infected with *T. gondii* oocysts, the parasite is eliminated or reduced to undetectable levels within a few weeks (Dubey, 1983, 1986b), perhaps due to innate resistance. For cattle, resistance to *Toxoplasma* infection and the ability to clear the infection has been suggested (Munday & Corbould, 1979).

Seroprevalence can be high in bovine (up to 92% has been reported) (van Knapen et al., 1995; Tenter et al., 2000; Sroka, 2001; More et al., 2008; Santos et al., 2009). Recent surveys show prevalences of 7.3% in Spain (Panadero et al., 2010) and 12% in Bangladesh (Shahiduzzaman et al., 2011). However, despite the high seropositivity reported in some studies, this may not correlate with presence of parasites in the meat. Isolation of infective tissue cysts from beef is rarely reported (Hellmann & Tauscher, 1967; Canada et al., 2002). However, we cannot be sure that beef does not play a role in *T. gondii* transmission as only relatively small amounts of beef have been tested for viable *T. gondii* parasites (Dubey & Jones, 2008).

The relationship between a seropositive calf or cow and the presence of infective tissue cysts needs to be clarified, because although food habits differ between European countries, eating raw beef or beef products is common in many regions of Europe. Epidemiological studies have shown that the consumption of raw or undercooked beef is considered a risk for *T. gondii* infection in humans (Baril et al., 1999; Cook et al., 2000). Outbreaks have been reported following consumption of raw beef, although doubt was raised whether the meat was unadulterated (Dubey & Jones, 2008). Drinking unpasteurized cow milk was not associated with *T. gondii* infections (Kapperud et al., 1996).

Few surveys have been carried out in farmed rabbits, reporting seropositivities between 6% and 53% in Europe (Hejlíček & Literák, 1994; Sroka et al., 2003).

3. Detection methods in meats

T. gondii cannot be macroscopically detected during current meat inspection of livestock either *ante-* or *post-mortem*. The hazard can be detected only through laboratory testing. The testing methods are based on direct detection of *T. gondii* in tissues or on the indirect detection of specific antibodies in serum.

It is difficult to find *T. gondii* tissue cysts in large animal species for several reasons, including sampling bias and preferred parasite sites. Dubey et al. (1996) have estimated that less than 1 tissue cyst/50 g of tissue is likely to be found in *T. gondii*-infected pigs. Thus, it is possible that when performing any test for tissue cyst detection, false-negatives can result from insufficient sample size or improper sample acquisition (Esteban-Redondo et al., 1999).

The established reference method for the isolation of *Toxoplasma* from foodstuffs is gavage or inoculation into animals. These tests (bioassays) are carried out in laboratory mice or in cats. Mice are either inoculated by the intraperitoneal or subcutaneous route or fed with a homogenate of tissues and maintained in observation for 6-8 weeks, when they are tested for antibodies to *Toxoplasma* and their brain is examined for the detection of tissue cysts. Cats are fed with muscle tissue and their faeces are examined for oocysts 3 days after inoculation. The sensitivity of bioassay is good, since it allows the detection of 1 cyst in 100

grams of tissue (Dubey et al., 1995). Bioassay in cats is more likely to detect *Toxoplasma* in meat than bioassay in mice, because cats are more susceptible than mice to the infection, and more tissue can be fed (Dubey et al., 2005). However, mouse and cat bioassays require use of live animals, which are not desirable for screening large numbers of samples from an animal ethics point of view, are time-consuming and not suitable for slaughterhouse testing (Warnekulasuriya et al., 1998; Opsteegh et al., 2010).

Therefore, PCR-based methods to detect *T. gondii* in meat samples have been developed. However, although the PCR itself is usually sensitive in detecting *T. gondii* DNA, when used on meat samples, these methods lack sensitivity in comparison to the bioassay (Da Silva & Langoni, 2001; Garcia et al., 2006). This lack of sensitivity of PCR-based methods is likely due to the inhomogeneous distribution of *T. gondii* tissue cysts, in combination with the small size of the sample. For PCR, DNA is usually isolated from 50 mg of sample at maximum, while in the bioassay either up to 500 g of meat is fed to a cat, or digestion extract from 50-100 g of meat is inoculated into mice. As a consequence, taking fifty milligrams of the homogenate of a large sample, instead of taking a fifty milligram sample randomly, will increase the probability of isolating *T. gondii* DNA. However, it will be present at a low concentration in a high background of host DNA, which might lead to inhibition of the PCR (Bellete et al., 2003).

Opsteegh et al. (2010) have developed a method for detection and quantification of *T. gondii*. The method involved preparation of crude DNA extract from hundred gram samples of meat, magnetic capture of *T. gondii* DNA and, quantitative real-time PCR targeting the *T. gondii* 529-bp repeat element. The detection limit of this assay was approximately 230 tachyzoites per 100 g of meat sample. Results obtained with the PCR method were comparable to bioassay results for experimentally infected pigs, and to serological findings for sheep. The authors state that the PCR method can be used as an alternative to bioassay for detection and genotyping of *T. gondii*, and to quantify the organism in meat samples of various sources.

In a study on detection of *Toxoplasma* in ready-to-eat cured meat samples by amplification of the parasite's P30 gene, PCR was able to detect parasite contamination down to a level of 5×10^3 trophozoites/g while viable *Toxoplasma* could be detected in tissue culture at a level of 10^3 trophozoites/g cured meat. The high salt content of some cured meats limited sensitivity of the PCR assay by inhibition of the polymerase enzyme and reduced the sensitivity of tissue culture due to osmotic pressure causing cytopathic effect (Warnekulasuriya et al., 1998).

The presence of DNA shows that the meat originates from a *Toxoplasma*-infected animal but does not necessarily mean that the product contains infectious organisms (Aspinall et al., 2002). To address this problem, Zintl et al. (2009) have developed a robust method for the assessment of *Toxoplasma* tissue cyst viability that combines an in vitro culture approach with quantitative PCR.

Hill et al. (2006) compared the efficacy of serum serology, tissue extract serology, real time PCR, nested PCR, and direct PCR for the detection of *T. gondii* in pork using samples from 25 naturally infected pigs from a farm, 10 experimentally infected pigs, and 34 retail meat samples, and then ranked detection methods in the following descending order of sensitivity: serum ELISA (test sensitivity 100%), serum MAT

(80.6%), tissue fluid ELISA (76.9%), real time PCR (20.5%), semi-nested PCR (12.8%), and direct PCR (0%). Neither ELISA nor MAT reliably detected antibodies in frozen and thawed muscle samples (Hill et al., 2006). Garcia et al. (2006) compared PCR, bioassay, and histopathology in 10 pigs fed *T. gondii* oocysts and killed 60 days later; *T. gondii* was detected in 55.1% of 98 muscle samples by bioassay in mice, in 16.6% of 150 muscle samples by PCR and in 0 samples by histopathology. Tsutsui et al. (2007) compared distribution of *T. gondii* in commercial cuts of pork by bioassay and PCR in 10 pigs 59 days after feeding them oocysts; *T. gondii* was found in 67.5% by bioassays in mice and 22.5% of samples by PCR.

The European Food Safety Authority (EFSA) states that the analytical methods to be used to detect and identify *Toxoplasma* in food and animals need to be characterised in terms of sensitivity, specificity and other performance parameters associated with the reliability and consistency of such methodologies. In order for such characteristics to be attained, there is an absolute requirement for reference materials and reagents (EFSA, 2007).

4. Influence of processing of meat products on the viability of *Toxoplasma gondii*

Approaches to meat safety assurance in respect to tissue cysts of *T. gondii* have to be considered, particularly for high risk populations. They are primarily based on meat treatments with aim to inactivate the cysts. Studies have indicated that *T. gondii* tissue cysts in meat are susceptible to various physical procedures (thermal and non-thermal) such as heat treatment, freezing, irradiation, high-pressure, among others. All these technologies try to be mild, guarantee natural appearance, energy saving and environmentally friendly while knocking the parasites (Aymerich et al., 2008). Their combination, as in the hurdle theory proposed by Leistner (2000) may improve their effectiveness.

It seems that currently, the most reliable cyst inactivation treatments are based on application of either adequate meat heating or meat freezing treatments, and some temperature-time regimes of these treatments have been assayed. According to the opinion adopted in 2007 by the Scientific Panel on Biological Hazards of the EFSA, to prevent foodborne transmission of *Toxoplasma* to humans, meat and other edible parts of animals should not be consumed raw or undercooked, *i.e.* they should be cooked thoroughly (at 67°C or higher) before consumption. Although freezing alone is not a reliable means of rendering all tissue cysts non-infective, deep-freezing meat (-12°C or lower) before cooking can reduce the risk of infection. In addition, meat should not be tasted during preparation or cooking (Paul, 1998; Cook et al., 2000).

It is also essential that preventive measures to reduce the risk of horizontal transmission of *Toxoplasma* to humans via tissue cysts include a high standard of kitchen hygiene. Thus, in a case-control study in Norway, washing kitchen knives infrequently after preparation of raw meat was independently associated with an increased risk of primary infection during pregnancy (Kapperud et al., 1996). Both tissue cysts and tachyzoites are killed by detergents and, thus, hands and all kitchen utensils used for the preparation of uncooked meat or other food from animals should be cleaned thoroughly with hot water and soap (Dubey, 2000).

The first experiments describing the inactivation of tissue cysts of *T. gondii* examined the effects of storage conditions on parasite survival and showed that parasite tissue cysts could be lysed in distilled water (Jacobs et al., 1960), but survived for several weeks in the presence of physiological saline (0.85%) and storage at 4 °C (Jacobs et al., 1960; Dubey et al., 1990). Raising the salt concentration or the temperature led to inactivation of the parasite (Kijlstra & Jongert, 2008a).

4.1 Thermal technologies

4.1.1 Heating and microwave cooking

The primary control factor for prevention of *T. gondii* infection via meat consumption is adequate cooking and prevention of cross-contamination (McCurdy et al., 2006). Limited data are available concerning consumer cooking habits and it is certainly possible that parts of meat being grilled or barbecued do not reach sufficiently high-temperatures to kill the parasite.

Jacobs et al. (1960) were the first to show that heating could inactivate tissue cysts: at 50 °C it takes 1 h to inactivate tissue cysts. Studies on killing of tissue cysts in meat by cooking (49-67°C for 0.01-96 min) were conducted by Dubey (2000) and found that *T. gondii* was rendered nonviable when internal temperatures had reached at least 67 °C.

Survival of tissue cysts at lower temperatures depends on the duration of cooking. For example, under laboratory conditions tissue cysts remained viable at 60 °C for about 4 min and at 50 °C for about 10 min (Dubey et al., 1990). It is important to note that cooking for a prolonged period of time may be necessary under household conditions to achieve the temperatures that are required to kill all tissue cysts of *Toxoplasma* in all parts of the meat. For this reason, cooking infected meat in a microwave does not guarantee killing some tissue cyst, which can remain infective, most probably due to uneven heating (Lundén & Uggla, 1992).

4.1.2 Freezing

Freezing of meat by consumers is widely applied in westernized countries. The loss of sensory quality may be an important factor in consumers' attitudes towards freezing of meat. However, it is very valuable for food safety as, in general, freezing can inactivate the *T. gondii* tissue cysts, although proper timing and temperature are necessary for a 100% parasite killing efficiency.

The effect of freezing on *T. gondii* cyst viability was first described in 1965 (Sommer et al., 1965). It was observed that freezing for 2 days at -20 °C was sufficient to inactivate the parasite.

Experiments with meat from pigs that were fed with *T. gondii*-infected mice, showed that all meat samples were rendered non-infectious by freezing 6-35 days at -25 °C (Grossklaus & Baumgarten, 1968).

Freezing meat for 1 day in a household freezer rendered tissue cysts nonviable (Dubey, 1988). Kotula et al. (1991) carried out experiments using different freezing temperatures

(from -1 °C to -171 °C for 1 second to 67 days) and found that an internal temperature of -12 °C was sufficient to render the parasite non-viable. In addition, *Toxoplasma gondii* tissue cysts remained viable up to 22 days at -1 and -3.9 °C and 11 days at -6.7 °C.

Kuticic and Wikerhauser (1996) reported that parasites in meat from experimentally infected pigs did not survive when frozen for 4 days at -7 °C to -12 °C. Other studies showed that at least 3 days at -20 °C were required to inactivate isolated tissue cysts (Djurkovic-Djakovic & Milenkovic, 2000).

4.2 Non-thermal technologies

4.2.1 Gamma irradiation

Several studies have demonstrated that *Toxoplasma gondii* was rendered nonviable by irradiation at doses of 0.4-1kGy (Song et al., 1993; Dubey & Thayer, 1994; Kuticic & Wikerhauser, 1996; Dubey, 2000), and the strain of *T. gondii* did not affect the killing of tissue cysts by irradiation under defined conditions (Dubey & Thayer, 1994).

However, the adverse effects of irradiation on colour have a major impact on the use of this technology and in certain countries large-scale implementation is restricted due to poor consumer acceptance (Brewer, 2004; Aymerich et al., 2008), in addition, irradiation of meat has not been approved in the EU.

4.2.2 High Hydrostatic Pressure (HHP)

Studies on the effectiveness of HHP in eliminating foodborne parasites show the sensitivity of parasites and their destruction is achieved with relatively low pressures.

High-pressure treatment using 300 MPa or higher can inactivate *T. gondii* tissue cysts under laboratory conditions (Lindsay et al., 2006) but negative effects on meat colour and texture have to be addressed before this method can be developed for routine decontamination (Cheftel & Culioli, 1997).

4.2.3 Curing

Curing treatments are used to preserve meat by the addition of a combination of salt, nitrates, nitrite or sugar. Many curing processes also involve smoking. Some researchers have suggested that tissue cysts are killed during commercial curing procedures with salt, but relatively few studies have been conducted to examine the efficiency of the curing process for the inactivation of *T. gondii*. One of the first experiments describing the inactivation of *T. gondii* tissue cysts was conducted by Sommer et al. in 1965. These authors found that encysted *T. gondii* survived for 4 days in 8% NaCl, but neither these researchers nor Work (1968) could find viable parasites in *T. gondii*-infected pork meat subjected to various curing processes.

Lundén & Uggla (1992) reported the absence of viable *Toxoplasma* in mutton meat after curing and smoking. Curing of lamb meat with salt and sugar for 64 h at 4°C or smoking salt-injected meat at temperatures not exceeding 50°C for 24 to 28 h was effective for killing *T. gondii*. However, these experiments do not reflect conditions under which commercial cured pork products are produced.

The survival time of tissue cysts is highly dependent on the concentration of the salt solution and the temperature of storage. Isolated tissue cysts can survive for 56 days in a solution of 0.85% salt, 49 days in 2% salt, and 21 days in 3.3% salt (Dubey, 1997). Under laboratory conditions, Dubey (1997) found that tissue cysts were killed in 6% NaCl solution at all temperatures examined (4 to 20°C) but survived for several weeks in aqueous solutions with lower salt concentrations. More recent data have indicated that injection of 2% NaCl and/or 1.4% lactate salt solutions into experimentally infected pig meat could kill the parasite. However, 1% NaCl solution provided variable results, and the addition of tripolyphosphate salts had no effect on parasite viability (Hill et al., 2004, 2006). Navarro et al. (1992), studying sausages manufactured from pigs experimentally inoculated with *T. gondii*, concluded that salt in concentration equal to 2.0% and 2.5% inactivated the parasite in 48 hours of the beginning of the curing process.

In order to evaluate the importance of swine sausages in toxoplasmosis epidemiology, Mendonça et al. (2004) investigated the presence of *T. gondii* in 70 samples of the meat product. Samples were analyzed by bioassay in mice and DNA amplification by PCR. Although the parasite was not isolated from any sample in the bioassay, 33 (47.14%) samples were positive in the PCR. The authors concluded that these swine sausages probably had low importance as a source of infection for human toxoplasmosis. Nevertheless, the great number of PCR positive samples showed that the protozoan may be present, but may be inactivated by salt added in sausage manufacture.

In contrast, other studies have indicated the potential failure of curing to inactivate *T. gondii* (Warnekulasuriya et al., 1998), and in epidemiological studies of risk factors for recent *Toxoplasma* infection in pregnant women, a strong association was found between infection and eating cured pork or raw meat (Buffolano et al., 1996; Cook et al., 2000; Kapperud et al., 1996). In addition, curing of meat products often involves the mixing of meat from various animals from different farms and sometimes from different farming systems (organic and regular), and thus a few infected animals may lead to the contamination of a whole batch of cured meat products. Warnekulasuriya et al. (1998) investigated the presence of *T. gondii* in cured meat samples by PCR, and using tissue culture in order to isolate viable parasites. The high salt content of some cured meats limited the sensitivity of PCR assay and reduced the sensitivity of tissue culture due to osmotic pressure causing cytopathic effect, but viable *T. gondii* was detected in one out of 67 ready-to-eat cured meat samples (1.5% contamination). However, these authors did not provide information about the time of curing and final salt concentrations, which could affect the viability of the parasite.

From all different meat products made with pork meat, dry-cured ham stands out among them as a high-quality product of increasing economic relevance. It is a non-smoked product manufactured by curing with salt and nitrites and stabilized through decreasing water activity. It is greatly appreciated by consumers because of its flavor and texture and for its nutritional properties. The whole process takes several months (from 6 to 36 months) and it is consumed without heat treatment. In the market, it is possible to find several presentations of dry-cured ham. Consumers can buy unpackaged entire or sliced ham (sold on request), and we also can find refrigerated vacuum-packaged dry-cured hams cuts which may be either distributed to specialized butchery, or directly sold in supermarket displays. Due to the lack of information regarding the efficacy of meat curing for inactivating *T. gondii*

and the inconsistency of results of epidemiological studies in which ingestion of cured meat was identified as a risk factor for acquiring acute *Toxoplasma* infection during pregnancy, Bayarri et al. (2010, in press) carried out some studies with the aim to provide data that could be used to estimate the risk of *T. gondii* infection from eating cured ham.

In a first study, the influence of processing of cured ham on the viability of *T. gondii* was evaluated using bioassay to assess the risk of infection from eating this meat product (Bayarri et al., 2010). Naturally infected pigs were selected for the study, and a mouse concentration bioassay technique was used to demonstrate viable bradyzoites of *T. gondii* in porcine tissues and hams. The selected pigs were slaughtered in a commercial abattoir. Both haunches were obtained for subsequent curing as is normal industry practice. At day 0 (before the curing process began), samples were obtained from the external surface of six haunches (one from each pig) to avoid quality loss of the final product. After 7 months of curing, the whole ham was analyzed for viable forms of *Toxoplasma*. The six remaining hams continued the curing process until 14 months, when samples were collected. They isolated viable parasites from hams after 7 months of curing, while no viable parasites were found in the final product (14 months of curing) based on results of IFA, histological, and PCR analyses. These authors evidenced that curing time is a major factor to ensure that the consumption of this meat product does not pose a risk of contracting toxoplasmosis.

However, both salt composition of the hams and curing time can vary according to the manufacturer or producer countries, and we can find in the market hams with different composition and curing times. Bayarri et al. (in press), carried out an investigation about the prevalence of viable *T. gondii* in commercially available cured ham. Twenty five samples of cured ham were randomly collected for analysis, corresponding to *paleta* and ham, white and Iberian, package sliced of different trade-mark and cut on request. The authors noted that not always information on the length of curing process was provided in the label. A mouse concentration bioassay technique was used, and the presence of the parasite in mice was determined by IFA. *T. gondii* was not detected in any of the samples of cured ham studied. Results reported in this paper are optimistic concerning food safety. However, in order to achieve a complete risk assessment on the viability of *T. gondii*, it is necessary to analyse a much larger number of samples, particularly those from organic farms, as well as cured ham considering different curing times and salt composition.

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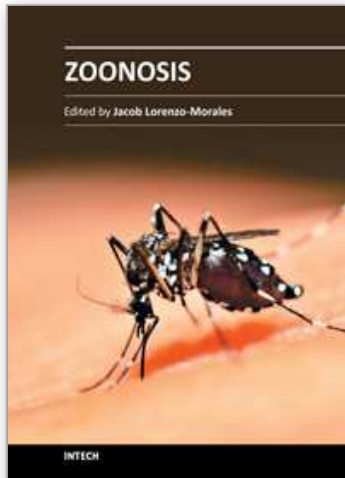
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Zoonosis

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Zoonotic diseases are mainly caused by bacterial, viral or parasitic agents although "unconventional agents" such as prions could also be involved in causing zoonotic diseases. Many of the zoonotic diseases are a public health concern but also affect the production of food of animal origin thus they could cause problems in international trade of animal-origin goods. A major factor contributing to the emergence of new zoonotic pathogens in human populations is increased contact between humans and animals. This book provides an insight on zoonosis and both authors and the editor hope that the work compiled in it would help to raise awareness and interest in this field. It should also help researchers, clinicians and other readers in their research and clinical usage.

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Unit 405, Office Block, Hotel Equatorial Shanghai
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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