

TOXOPLASMA GONDII IN PORK & PORK PRODUCTS – TOO MUCH ON OUR PLATE?

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Abstract

Background. The constant growth of the global population has led to increasing food production, which has been particularly evident in the production of pork in recent years. In 2015, the number of pigs surpassed one billion, and in 2018 a record high of 120 million tonnes of pork was produced worldwide. In spite of the expansion and dominance of specialized industrial farming in developed countries, *Toxoplasma gondii* infection in pigs as a source of human infection remains an issue in traditional small-scale farming. The disease burden of toxoplasmosis is estimated to be the highest of all parasitic infections and even higher than that of salmonellosis and campylobacteriosis.

Scope and Approach. This paper reviews the latest research on *T. gondii*-contaminated pork and pork products, published in the past decade. As current methods do not allow for practical and cost-effective detection of *T. gondii* at slaughter, efforts towards safe meat have focused on the detection of the parasite in pork and ready-to-eat pork products and on post-harvest mitigation measures.

Key Findings and Conclusions. In contrast to recommendations for preventing *Trichinella* infection, there are no globally applicable standardised and validated inactivation procedures for rendering *T. gondii* infected pork/pork products safe for consumers. Moreover, there are no EU regulations in place for the prevention of *T. gondii* infection by consumption of pork and pork products. Recommended actions, both at the producer and consumer levels, include post-harvest processing such as cooking, freezing, and proper salting/curing.

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INTRODUCTION

Toxoplasma gondii is one of the most omnipresent zoonotic parasites on Earth. It is a protozoan capable of both sexual and asexual reproduction and has a complex life cycle involving three life forms. These are the rapidly dividing tachyzoites, slowly dividing bradyzoites (within tissue cysts), and sporozoites (in oocysts), all of which are infectious to hosts (Dubey, 1998). Although members of the Felidae family are the only definitive hosts, *T. gondii* can spread asexually among intermediate hosts which comprise a wide range of species, including humans. The main modes of transmission include ingestion of tissue cysts in contaminated meat (from an infected animal),

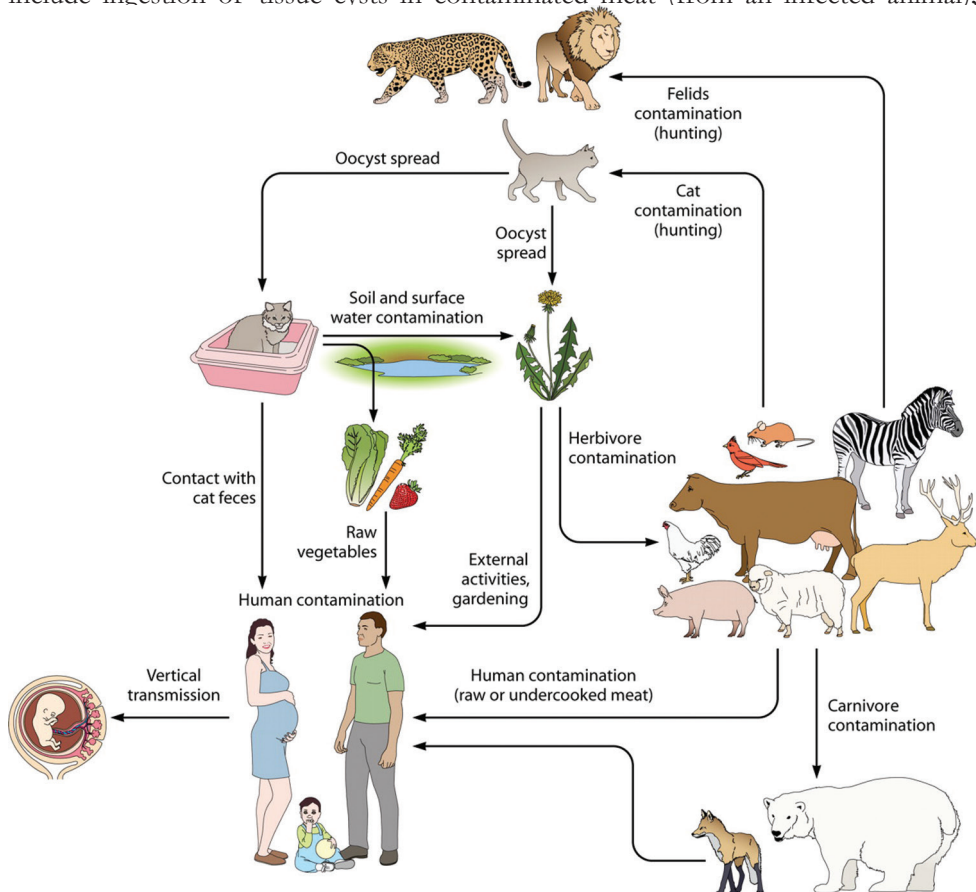


Figure 1. *Toxoplasma gondii* transmission modes.

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ingestion of oocysts excreted by cats via contaminated produce, water or from the environment, and vertical transmission (Figure 1).

Although it is generally mild in immunocompetent individuals, *T. gondii* infection is a great risk for the immunologically compromised or immature. Acute infection in pregnant women can result in foetal damage and subsequent miscarriage, stillbirth, hydrocephalus, blindness and other early or late sequelae of congenital toxoplasmosis during childhood or early adolescence. Patients undergoing transplantation and, therefore, on strong immunosuppressive treatments, as well as HIV/AIDS patients are at great risk, usually developing generalized toxoplasmosis or toxoplasmic encephalitis. In a global WHO and FAO report on food and waterborne parasites with an impact on human health, *T. gondii* ranked 4th (FAO/WHO, 2014), while in Europe, it ranked 1st in a similar report on the prioritisation of foodborne parasites (Bouwknegt et al., 2018). The Netherlands pioneered the determination of the disease burden of toxoplasmosis in disability adjusted life years (DALYs), which in two different studies was estimated at 620, and even at 2,300 DALYs, higher than that of salmonellosis and campylobacteriosis (Kortbeek et al., 2009). In the United States, a report on foodborne illnesses stated that among all deaths attributed to foodborne pathogens, *T. gondii* is responsible for as many as 24% of the total (Scallan et al., 2011).

There is strong epidemiological evidence for the consumption of meat, especially if raw or undercooked, as a major risk factor for *T. gondii* infection (Bobić et al., 2007; Cook et al., 2000; Bobić et al., 1998; Kapperud et al., 1996). Also, most of the *T. gondii* foodborne infections in humans in the U.S. have been attributed to meat (Batz et al., 2012). One type of meat consumed in great quantities in many countries is pork and pork products, which has important public health implications, especially in view of the parasites that can be transmitted by consumption of pork (Djurković-Djaković et al., 2013), and specifically, the lifelong persistence of *T. gondii* tissue cysts in numerous edible tissues of slaughter pigs (Dubey et al., 1986). According to recent food attribution models and economic cost estimates of meat- and poultry-related illnesses, the economic burden of *T. gondii* in pork amounts to \$1,900,000,000 in the U.S. alone (Scharff, 2020). Moreover, in the Netherlands, two different studies using quantitative microbial risk assessment (QMRA) determined that 12% and 11.2% of the predicted *T. gondii* infections in the country could be attributed to pork (Deng et al., 2020; Opsteegh et al., 2011). A QMRA of human toxoplasmosis associated with pork consumption in Italy has shown the bulk of the infection to be associated with the consumption of fresh meat cuts or products, with the cooking temperature and muscle cyst burden having the greatest influence on the risk (Condoleo et al., 2018).

The constant growth of the global population has led to increasing food production, which has been particularly evident in the production of pork in previous years. In 2015, the number of pigs surpassed one billion, and in 2018, a record high of 120 million tonnes of pork was produced worldwide (FAO, 2019). In spite of the expansion and dominance of specialized industrial farming in developed countries, *T. gondii* infection in pigs remains an issue in traditional small-scale farming. However, even a very low

prevalence of infection on large farms presents a risk for pork consumers, since a single market-weight pig translates to more than 600 servings of meat (Dubey et al., 2008).

Pork is eaten either freshly cooked or preserved by various methods of salting and curing, which not only allow for an extended shelf life but also provide specific taste and aroma to preserved pork products. Unfortunately, both prior to slaughter and during meat inspection, it is impossible to detect *T. gondii*-infected animals, i.e. those bearing tissue cysts. Serological testing can only detect which animals may have been exposed to *T. gondii*, while direct detection methods, such as molecular tests (detecting only parasite DNA) and cat or mouse bioassays (confirming parasite viability), are expensive and infeasible on an industrial scale. Seroprevalence in pigs intended for human consumption varies greatly according to country and region, with values ranging from 0 to 65.8 and even 92.7% (Foroutan et al., 2019; Guo et al., 2015; Dubey, 2009). However, seropositivity is not necessarily a marker of actual consumer risk, since the correlation between the results of serological tests and parasitological findings (i.e. the presence of viable tissue cysts) is not absolute. A number of studies that have compared the serological findings with parasite (or its DNA) detection in different tissues have shown, on average, 59% detection in tissues of seropositive pigs and 5% detection in tissues of seronegative ones (rev. in Opsteegh et al., 2016; Djokić et al., 2016; Opsteegh et al., 2010).

RAW PORK – RISKY CUTS

The cyst burden in muscles has continuously been shown to be low, which is why sensitive methods such as cat and mouse bioassays have to be used to efficiently detect *T. gondii* (Rani et al., 2019; Dubey, 2010; Dubey, 2001). However, in the past decade, a more sensitive PCR method has been devised, which utilizes magnetic capture (MC) technology for DNA extraction to discriminately bind small amounts of *T. gondii* DNA present in large volumes of meat lysate. This MC-qPCR has been further developed and ISO 17025 validated; now, it promises to substitute the mouse bioassay, showing equal sensitivity in both detecting and quantifying very small numbers of parasites (limit of detection of 65.4 parasites, or, more importantly, of only one tissue cyst per 100 g of tissue) in the meat matrix (Gisbert Algaba et al., 2017). Nevertheless, an important point to remember is that in spite of the increased sensitivity of such PCR methods, an isolation assay in either mice or cats remains the only way of properly assessing the risk for consumers, since the detection of parasite DNA does not always equate to the presence of viable tissue cysts.

The attempt to pinpoint the organs and meat cuts most likely to contain significant numbers of tissue cysts and which present the highest risk for the consumer is important. In fact, *T. gondii* cysts were detected in most examined tissues of pigs intended for human consumption, including the heart, diaphragm, brain, liver, tongue, masseter muscle and raw ham (Paraboni et al., 2020; Miura et al., 2019; Paștiu et al.,

2019; Cubas-Atienzar et al., 2018; Vergara et al., 2018; Kuruca et al., 2017; Franco-Hernandez et al., 2016; Hernández-Cortazar et al., 2016; Herrero et al., 2016; Cademartori et al., 2014; Dubey et al., 2012), and the parasite was even isolated from the blood of market-weight pigs (Klun et al., 2011).

Since it has been shown that predilection organs, such as the heart and brain, usually have significantly higher parasite burdens than muscle meat (Gisbert Algaba et al., 2018), direct data need to be gathered for each of the commercial meat cuts of interest. Commercial meat cuts of experimentally infected pigs have been examined by mouse bioassay in a Brazilian study; *T. gondii* was detected in muscles including the loin (musculus longissimus), coppa (m. longissimus, m. spinalis dorsi, m. rhomboideus), tenderloin (m. psoas major), outside flat (m. biceps femoris) and top sirloin (m. gluteus medius) (Alves et al., 2019). In contrast, *T. gondii* was detected by mouse bioassay in two of 25 fresh pork samples from supermarkets and butcher shops in one city in Spain (Bayarri et al., 2012). Furthermore, in a study of retail fresh pork loin and leg from butcher shops in one city in Mexico, only one (2.1%) of 48 samples contained *T. gondii* (Galván-Ramírez et al., 2010). *T. gondii* (parasites or DNA) was also detected in 18% of fresh pork samples in China (Wang et al., 2012), in 7% of loin muscle samples in Mexico (Hernández-Cortazar et al., 2016), and in 4.2% retail pork samples in Scotland (Plaza et al., 2020). Importantly, it was shown by mouse bioassay of shoulder muscle samples as small as 5 grams that tissue cysts are unevenly distributed in the muscle and that they are formed very early post infection (day 7). Thus, even recently infected pigs can be a source of infection for humans (Rani et al., 2019).

Minced meat has also been investigated and often found to harbour tissue cysts. This is not surprising since it usually comprises several different cuts, thus increasing the odds of harbouring parasites. A study in Canada demonstrated *T. gondii* DNA in 3.2% of 94 retail ground pork samples (Iqbal et al., 2018). In a large study from Poland, *T. gondii* DNA was detected in 4.5% of 756 samples of retailed minced pork and in 5.8% of 1355 samples of raw sausage (Sroka et al., 2019). *T. gondii* DNA in raw pork sausages was also detected in a Brazilian study in almost half of the samples (Costa et al., 2018); however, no positive results were noted in another study but which tested a small number of samples (Paraboni et al., 2020).

Raw home-made pork sausage positive for *T. gondii* DNA has been linked to a case of acute symptomatic toxoplasmosis in Italy (Vitale et al., 2014). Raw sausage as a plausible source of human infection was also examined in a study of experimentally infected pigs, whose meat was prepared as commercial fresh sausages that were fed to mice after different ripening times. *T. gondii* DNA was detected in four of 288 mice, implying that raw sausage products can be infective for consumers (Abdulmawjood et al., 2014).

Pork is increasingly being marketed as dry aged meat, when fresh meat cuts are first vacuum-wrapped in multi-layered polyethylene and polyamide packaging and then refrigerated for a period of usually 14 days. During this maturation process, the meat

softens and its flavour and colour are enhanced (Li et al., 2014; Li et al., 2013), thus increasing its appeal for customers. The impact of the process of dry ageing of vacuum-packed pork loins on the viability of *T. gondii* tissue cysts after 14, 21 and 28 days' storage at 0°C has recently been examined (Alves et al., 2020). Using both cat and mouse bioassays, it was determined that 14 days is not a sufficient period to inactivate the parasites; conversely, no viable parasites were detected after 21 or 28 days' maturation. The authors stated that a 21-day period may be recommended for application in the meat industry.

PIGS IN A PICKLE

There is a whole body of research on the safety of some of the finest Mediterranean dry cured hams, appreciated worldwide, such as the Spanish Serrano ham and the Italian Parma ham. Part of the reason for their particular and exquisite taste and texture lies in the fact that no thermal or smoking treatments are allowed during their production; this naturally limits the preservation procedure to salting and curing and a lengthy maturation process. From the standpoint of *T. gondii* infection control, however, these differing and sometimes un-standardised curing procedures do not guarantee tissue cyst inactivation.

The effects of dry curing have been studied in hams from naturally infected pigs (Bayarri et al., 2010). A commercial mixture of curing salts was used (containing salt, sugar, sodium citrate, sodium ascorbate, potassium nitrate and sodium nitrite) and the hams were cured for up to 14 months under controlled temperature and humidity, when the curing process was considered to be complete. The final water content was 47.8%, with a final concentration of NaCl of 3.9%. Mouse bioassays of ham samples showed the presence of *T. gondii* after 7 months of curing, but none were detected after 14 months, indicating that the particular salt mixture together with proper conditions and length of curing procedure can make the product safe for consumers. The same group also tested 25 samples of retailed dry cured hams, and none were positive by mouse bioassay (Bayarri et al., 2012).

Not all research has shown such favourable results. For instance, among the 475 samples of commercially cured Serrano ham from seven producers, 8.8% were found positive by qPCR (range 0-32.3%), about half of which (4.8%) harboured viable tissue cysts as evidenced by mouse bioassay (Gomez-Samblas et al., 2015). The observed differences among the different products were attributed to the length of curing time. Uneven salting could be another reason for the variation in parasite burdens. Namely, in dry salting, the salt concentration required for the destruction of *T. gondii* cysts might not be achieved uniformly through the thickness of the ham, especially in very thick areas or near the bone.

However, different curing treatments can also influence the speed of *T. gondii* inactivation. In a study where pigs were experimentally infected with *T. gondii* and

their ham legs and shoulders prepared and cured as Serrano ham using four different treatments (Gomez-Samblas et al., 2016), no viable tissue cysts were detected after 7 months of curing. The procedures included freezing prior to or after the curing process or no freezing at all, and the use of marine salt with or without nitrites. The swiftest effect (at 3 months) was noticed for treatments which used freezing, whether before or after curing; interestingly, the addition of nitrites seemed to delay the destruction of *T. gondii*, probably by interfering with lipid degradation and hydroperoxide formation. The efficiency of the curing process was examined in a study which looked at naturally infected pigs, split into groups of pigs with a low titre (1:20-1:80) and a higher titre (\geq 1:80) of specific antibody (Herrero et al., 2017). A total of 41 pigs were included, three of which were negative controls. First the meat from raw hams was tested, in which tissue cysts were detected by bioassay in 68.4% of samples from the seropositive pigs, and the cysts were found more often in hams from pigs with higher serological titres. After a curing period of 9 or 12 months, however, there was a marked loss of *T. gondii* viability in cured as opposed to fresh hams ($p < 0.001$); the loss was also noted in those with a lower fat content ($p = 0.039$). Interestingly, moisture content, water activity, and nitrate, nitrite and salt content had no influence on the presence of viable *T. gondii* in cured ham. It was concluded that while the curing process reduced the risk of infection for consumers, it did not afford its complete elimination.

On the other hand, examination of traditional Parma ham originating from 12 pigs experimentally infected with a type II *T. gondii* strain (Genchi et al., 2017) and cured for either 12, 14 or 16 months, showed absolutely no parasite viability for all curing times. Cured products have also been examined in Poland, and parasite DNA was detected in 5.7% of 856 retail samples of smoked meat, and 5.5% of 256 samples of fermented ham (Sroka et al., 2019).

Fredericks et al. (2020) conducted a study on the inactivation of *T. gondii* in dry cured whole ham (originating from experimentally infected pigs), prepared according to the U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) guidances for commercial production. The ham samples were tested by mouse bioassay while raw, after the initial salting and curing period of 33 days, and during drying at three month intervals, up to 12 months when they were considered a finished product. No viable parasites were detected starting from the first post-salting sample at 33 days until the end of the drying period. The finished hams conformed to the FSIS final composition requirements of salt concentration of no less than 4% and water activity of no more than 0.92. The results of this study are encouraging in that they showed that the approved protocols for dry cured ham production in the U.S. are effective in inactivating *T. gondii*, thus lowering the risk for consumers.

The safety of dry cured pork sausages (e.g. pepperoni) was also studied, and it was shown that at endpoint pH values in the range of 4.6 to 5.2, inactivation of *T. gondii* bradyzoites in tissue cysts occurs rapidly, during the first four hours of fermentation, even at salt concentrations much lower than usually used in the industry (Fredericks

et al., 2019; Hill et al., 2018). These studies confirm that ready-to-eat pork products such as dry sausages are safe considering *T. gondii* if they are prepared using NaCl concentrations of 1.3% or higher and then fermented and dried according to industry standards.

CONCLUSION

Although the goal of *T. gondii*-free meat has not yet been achieved, mounting research evidence in recent years seems promising. *T. gondii* tissue cysts can be inactivated by various treatments of fresh pork and pork products. Some of them are long known, such as adequate cooking temperature, freezing duration and salt concentration (Hill et al., 2004; Dubey, 1988; Dubey et al., 1990; Dubey, 1974). Appropriate procedures and maturing times for particular preserved pork products need to be validated for effective inactivation of *T. gondii* cysts. Products guaranteed to have been made according to proper procedures gain an added value, as well as provide safety and peace of mind for consumers. Moreover, the adherence to proper procedures could be certified and displayed on the packages in addition to origin certification, again for the benefit of consumers.

Some of the suggested methods for efficient inactivation of *T. gondii* in the meat used for making ready-to eat products include freezing at below -20 °C for 3 days, followed by a slow thawing and then salting and curing (Gomez-Samblas et al., 2016). Also, combined effects of ham dehydration and the accumulation of free fatty acids during the traditional curing process seem destructive to cysts (Gomez-Samblas et al., 2015). Whereas effective *T. gondii* inactivation in pork products has been attributed to salt concentration, salting and curing equalization, water activity, drying/maturation duration, storage temperature and HACCP guidelines, research data differ in the attribution of the relative significance of these individual factors or their combinations (Fredericks et al., 2020; Genchi et al., 2017; Bayarri et al., 2012). This remains to be clarified in further work. Also, no curing procedures have been validated for *T. gondii* inactivation in hams (Fredericks et al., 2020). All these unresolved issues are fruitful topics for future research.

For the time being, given the obstacles to efficiently prevent *T. gondii* infection at the farm and slaughter levels, the application of post-harvest methods including freezing, proper salting and curing procedures of adequate duration, and thorough cooking at the consumer level, remain the optimal approach to rendering pork and pork products safe for human consumption.

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Authors' contributions

IK conceived the mini review and wrote the original draft. ODjDj reviewed and edited the draft. Both authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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TOXOPLASMA GONDII U SVINJSKOM MESU I PRERAĐEVINAMA – IMA LI EFIKASNE PREVENCIJE?

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Kratak sadržaj

Uvod. Stalan rast svetske populacije zahteva i stalno povećanje proizvodnje hrane, što je posebno očigledno u proizvodnji svinjskog mesa poslednjih godina. Broj svinja je 2015. godine premašio milijardu, a 2018. proizvedeno je rekordnih 120 miliona tona svinjskog mesa. Uprkos ekspanziji i dominaciji specijalizovanog industrijskog uzgoja u razvijenim zemljama, infekcija svinja protozomom *Toxoplasma gondii* i potencijalni rizik za potrošače i dalje predstavljaju problem; procenjuje se da je opterećenje bolešću kod toksoplazmoze najveće od svih parazitskih infekcija, pa čak i veće od salmoneloze i kampilobakterioze, te je neophodno preduzimanje preventivnih mera.

Cilj i pristup. Ovaj rad daje pregled najnovijih istraživanja problema svinjskog mesa i prerađevina kontaminiranih *T. gondii*, objavljenih u poslednjih deset godina. Budući da je *T. gondii* prilikom klanja gotovo nemoguće otkriti, barem ne na praktičan i ekonomski

isplativ način, brojna istraživanja su usredsređena na otkrivanje parazita u svežem mesu i prerađevinama kao i na mere prevencije nakon klanja.

Ključni nalazi i zaključak. Za razliku od preporuka za suzbijanje trihineloze, ne postoje postupci inaktivacije *T. gondii* standardizovani i validirani na globalnom nivou koji bi kontaminirano svinjsko meso ili prerađevine učinili bezbednim za potrošače. Takođe, ne postoje ni propisi na nivou Evropske Unije čijom primenom bi se mogao sprečiti prenos *T. gondii* putem konzumiranja svinjskog mesa i prerađevina. Određene mere koje bi se mogle preporučiti, i na nivou proizvođača i potrošača, spadaju u obradu i preradu nakon klanja, kao što su termička obrada, zamrzavanje i pravilno soljenje i sušenje mesa.

Ključne reči: mere prevencije, soljenje i sušenje, svinjetina, svinjske prerađevine, *Toxoplasma gondii*, zoonoza prenošena hranom