

Toxoplasma gondii in Meats — A Matter of Concern?

Toxoplasma gondii is an obligate intracellular protozoan parasite which can infect warm-blooded animals. Toxoplasmosis is probably the most widespread zoonotic disease in the world. It is believed that approximately 50% of the population in the United States have been exposed to *T. gondii* (i.e., they are serologically positive) and at least 500 million people in the world are infected with the organism. In healthy adults and children, toxoplasmosis is generally asymptomatic. However, during pregnancy, an infection can be quite destructive, resulting either in fetal death or in a child with physiological, physical or neurological deficits. In immunocompromised individuals, an infection by the organism or reactivation of the disease resulting from an earlier exposure can be life threatening.

Life Cycle of *T. gondii*

T. gondii has three infectious stages:

1. tachyzoites (trophozoites), rapidly multiplying forms;
2. bradyzoites, present in tissue cysts;
3. and sporozoites, present in oocysts found only in feline feces.

The definitive host is the feline; thus, the sexual cycle of *T. gondii* is completed only in members of the cat family. The disease is acquired by both cats and intermediate hosts through carnivorousness, ingestion of cat feces, and/or by congenital infection. Generally, the cat is infected through ingestion of cysts present in prey or uncooked meats. The cyst wall is then dissolved by enzymes present in stomach and small intestine. The released bradyzoites penetrate the epithelial cells of the small intestine where a few cycles of asexual reproduction of the parasite takes place. Following asexual development, gametes are produced and after fertilization, a wall is formed resulting in the oocyst which are shed by the cat during defecation. Freshly shed oocysts are *non-infectious* but upon exposure to air, they sporulate in 1-5 days and become infectious. Anaerobic conditions, heat (>45°C), or cold (≤4°C) inhibit sporulation. The sporulated oocyst contains two sporocysts each of which consist of 4 sporozoites.

In the intermediate host, infection occurs via ingestion of tissue cysts or oocysts (or congenitally during pregnancy). Bradyzoites released from tissue cysts or sporozoites released from oocysts penetrate intestinal cells and multiply

as tachyzoites and eventually spread via lymph and blood to other parts of the body. Tachyzoites can multiply in most cells and eventually destroy the invaded cells. Cell lysis is followed by invasion of new cells. As immunity develops, replication of tachyzoites decrease and tissue cysts develop which do not normally produce a host reaction. The cysts (each cyst may contain > 1000 bradyzoites) are found in brain, muscle, heart, and visceral organs of both cats and intermediate hosts and persist for life. The encysted organisms are protected from circulating antibodies. Cysts may occasionally rupture but the released bradyzoites are inactivated by the immunocompetent host and as long as the immune system is intact, the encysted bradyzoites are inactive. Oocysts are never shed by intermediate hosts.

Survival of *T. gondii* Oocysts

Cats prefer to defecate in soft soils (gardens, sandboxes), water, animal feeds, hay or animal bedding. The sporulated and infectious oocyst is very hardy and can survive extremes of environmental conditions. Oocysts retain their infectivity in soil or water for 150-400 days at temperatures ranging from 4 to 37°C. They are less resistant to drying, with loss of infectivity occurring within 30 days at relative humidities of 50-80% and within 3 days at 0-37%. Freezing does not eliminate oocysts from soils. Oocysts are killed by ammonia solutions; exposure to 10% ammonia for 10 min or to 5% for 30 min completely eliminates infectivity. Treatment with boiling water for 5 min is also effective in destroying oocysts.

Distribution of *T. gondii* Nature

Oocyst survive well in water and toxoplasmosis can result in animals if they drink water from oocyst-contaminated sources such as streams.

Infectious *T. gondii* oocysts have been isolated from soils from all over the world. Rodents and birds may become infected when they are exposed to oocyst-contaminated soils since they may ingest oocysts during grooming or by eating coprophagous insects or earthworms. Earthworms transfer oocysts from deeper to upper layers of soil and oocysts present in earthworms are infectious. Flies, cockroaches and other coprophagous insects contacting cat feces also contain infectious oocysts in and on their bodies. These insects can contaminate human food or act as food for rodents and birds

and infect them. By eating these infected animals, foraging uninfected cats become infected in turn and the cycle of oocyst production and contamination of the soil with *T. gondii* oocysts is repeated.

Children, playing in sand or dirt where cats have defecated are at risk for exposure. Small children are not very sanitary-conscious and may put their hands in their mouths in an indiscriminate manner while playing. Children or adults with geophagy (pica) can readily be infected by eating soils containing oocysts.

Among food animals, *T. gondii* infection is widely prevalent in pigs. In pregnant sows, an infection may induce abortion or congenital toxoplasmosis. While most pigs acquire a subclinical infection upon exposure to the parasite, clinical toxoplasmosis can occur in neonates, often leading to death. Thus, toxoplasmosis in swine can represent an important economic loss to farmers. Swine can become infected through cannibalism, ingestion of tissue cysts when eating rodents or birds, eating uncooked garbage, and probably through ingestion of soil oocysts due to the rooting nature of pigs. Studies on the seropositivity of swine to *T. gondii* indicate that the extent of infected pigs on farms in the U.S. can range from < 1 to 69% with a national prevalence of approximately 23%.

Infection of sheep with *T. gondii* is usually subclinical; however, goats are much more susceptible to toxoplasmosis and clinical symptoms may be observed. Infection with *T. gondii* is an important cause of abortion in both goats and sheep and the results can be economically devastating to the farmer. Seropositive females can be expected to deliver normal young even if they have aborted once; therefore, seropositive ewes and does should be kept as breeding animals. A recent survey indicated that approximately 37% of sheep and 20% of goats are seropositive for *T. gondii*. Most sheep and goats acquire the parasite from eating oocyst-contaminated hay, ground feeds and drinking contaminated water.

Under natural conditions, *T. gondii* is not an important cause of abortion or clinical disease in cattle. While *T. gondii* can multiply in bovine tissue, it is quickly eliminated. The role of beef in the epidemiology of human toxoplasmosis is uncertain but it appears to be a minor source of infection to humans. However, more studies are needed to determine the extent of *T. gondii* infections in cattle and the role of beef as a source of human toxoplasmosis.

Clinical cases of toxoplasmosis in horses and other equids are rare. Limited surveys indicate that approximately 15% of the horses in the U.S. are positive for *T. gondii*. Edible American wild game animals show seropositivity for *T. gondii*, also. Both wild and domestic birds are susceptible to *T. gondii* infections; however, the prevalence of toxoplasmosis in chickens is not known because chickens do not develop antibodies against *T. gondii* that can be detected by the commonly used methods.

Mice, rats, raccoons, squirrels, opossums, and other small wild animals that live close to the human environment show *T. gondii* seropositivity. Zoo animals such as felines, marsupials, canines, ungulates (hoofed mammals), and birds show seropositivity, also. Zoos are easily accessed by feral domestic cats and oocyst-shedding cats can contaminate

feed and bedding of zoo animals. Newly infected zoo felines can shed oocysts which may be spread throughout the zoo population by keepers moving from cage to cage. Feeding of raw meats to zoo carnivores may also lead to infected animals if the meat contains tissue cysts. Only thoroughly cooked meats should be fed to zoo animals. If cooking is not practical, all meats should be frozen before feeding to animals.

Generally, dogs do not show clinical signs when infected by *T. gondii*. Fatal toxoplasmosis has been observed in dogs concurrently infected with the immunosuppressive distemper virus. In the U.S., approximately 17% of dogs are seropositive for the parasite and 29% are positive worldwide. Other canines (fox, coyote, wolf) are susceptible to toxoplasmosis, also. There is no direct route of *T. gondii* infection from dogs to humans; therefore, playing with the family pet will not lead to toxoplasmosis.

Members of the cat family are the only animals known to shed *T. gondii* oocysts. In terms of human infection, the common house cat, both pet and feral, is the most important source of oocyst contamination of foods and the environment. Limited studies indicate that approximately 30% of the house cats in the U.S. are seropositive; world-wide, the figure is approximately 37%. Kittens become infected soon after weaning, usually by sharing prey with its mother. Infections generally are subclinical. Oocysts are shed for 1-2 weeks and in general, cats that have excreted oocysts do not do so again. The house cat should not be allowed to hunt and should never be fed uncooked meats.

Occurrence of *T. gondii* In Foods

In industrialized countries, raw and undercooked meats containing *Toxoplasma* cysts probably serve as the major source of human toxoplasmosis. While some people prefer raw or undercooked meats, changes in eating habits, i.e., foods eaten away from home more often may expose consumers to undercooked meats which might not occur if meals were prepared at home. Modern cookery methods such as microwave ovens may not expose meats to proper time-temperature relationship to ensure destruction of cysts. As the U.S. population becomes more sophisticated in their food preferences, there will be a willingness to try new food combinations which may include raw or undercooked meats.

Beef and veal seem to be less contaminated with *T. gondii* than other meats but tissue cysts have been found in edible tissue of beef and consequently, the eating of raw or rare beef could expose the individual to infection. Even if the presence of *T. gondii* in beef and veal is low, these meats could be contaminated in another way. *T. gondii* cysts present in pork, mutton or lamb may be ruptured during grinding or cutting with ensuing contamination of equipment with bradyzoites. If thorough cleaning is not performed, these bradyzoites can cross-contaminate other meats that are processed using the equipment.

In the U.S., eating undercooked or raw pork containing *T. gondii* cysts is believed to be the major meat source of toxoplasmosis in humans. Tissue cysts persist in swine for several months and cysts have been demonstrated in edible tissue of swine. During butchering, wounds, particularly on the hands, should be covered to prevent entry of bradyzoites

from ruptured cysts. Tasting raw pork (or other meats) during the preparation of sausage should be avoided.

The prevalence of *T. gondii* in U.S. market lamb is unknown but is estimated to be more than 5%. Viable *T. gondii* has been isolated from edible tissues of lamb, goat and sheep; there are reports of transmission of *T. gondii* by drinking goat milk. Lamb and goat meat are not important meat foods in the U.S. and mutton is rarely consumed but it is used in pet foods and should be thoroughly cooked before use. Lamb, mutton or goat meat may be major meat items in certain ethnic groups in the U.S. and for people in other countries. These individuals are at risk for toxoplasmosis if the meats are not thoroughly cooked.

Horse meat is not an important meat item in the U.S. but since tissue cysts persist in horses for several months, people who reside in areas where horse meat is consumed may contact toxoplasmosis if the meat is not well-cooked. Horse meat is a common ingredient of pet foods in the U.S. and pets should be only fed thoroughly cooked horse meat.

Small game animals such as squirrel and rabbit may be sources of *T. gondii*. The parasite has been found in tissues of large wild game animals such as deer and toxoplasmosis has been associated with eating undercooked venison. Caution should always be exercised in the preparation of wild game to ensure that bradyzoites from ruptured cysts do not enter a wound or contaminate butchering equipment.

T. gondii has been isolated from tissues and organs of chickens and pigeons but not from eggs. Poultry is probably not an important source of toxoplasmosis to humans since poultry is normally served well-done.

T. gondii oocysts may be present on vegetables, particularly root vegetables that are not cooked. Cats are known to defecate in garden soil and it is very possible that vegetables could be contaminated with infectious oocysts and pose a health risk if they are not well washed. Leafy vegetables may be contaminated by insects that have come in contact with cat feces.

While few studies have been performed concerning the incidence of *T. gondii* in market meats, studies have shown that *T. gondii* is present in the tissues of warm-blooded food animals. Except perhaps in the case of cattle, the parasite appears to persist in animals as tissue cyst for long periods if not for life. It may be possible that a significant part of the raw meats appearing in markets today contain *T. gondii* and there is obviously a need for market surveys to determine the incidence of the parasite in raw meats. One of the reasons for the lack of knowledge concerning the presence of *Toxoplasma* in market meats is due to the difficulty of the assay: tissue from suspect meat is fed to cats and observing for oocyst excretion or by intraperitoneal inoculation of tissue digests into mice and then searching for tissue cysts. Some of the newer techniques such as enzyme linked immunosorbent assay (ELISA) or DNA probes linked to the polymerase chain reaction (PCR) may be useful tools in future market surveys.

Destruction of *T. gondii* in Foods

When tissue from pigs infected with *T. gondii* was ground and mixed with ground brain tissue from infected mice (mixing pork with mice brain increases the number of

tissue cysts several fold) and heated to various internal temperatures, D-values obtained for the inactivation of bradyzoites were 53.5 min at 49°C, 5.8 min at 55°C, 3.8 min at 61 °C and 3.6 min at 67°C. A similar mixture of porcine and mouse tissue were frozen and stored for various intervals to determine the effect of freezing temperatures on *T. gondii* viability. There was no survival of the parasite at -8.0°C stored ≥ 3 days, nor was there survival at -6.7°C stored ≥ 17 days. At freezing temperatures ranging from -1.0 to -8.0 C, storage for ≥ 34 days rendered the tissue cysts non-infectious. *T. gondii* cysts are more sensitive to heating and freezing than *Trichinella spiralis*; therefore, heating and freezing temperatures that inactivate *T. spiralis* will inactivate *T. gondii* also. It is believed that *T. gondii* is more sensitive to present day meat curing processes than is *T. spiralis* and since cured meat products are processed to ensure destruction of *T. spiralis* cysts, it is assumed that *T. gondii* cysts are destroyed also. However, no real information is available concerning the effect of commercial cured meat processing on *T. gondii*. Research is needed to determine the effect of cured meat processes (including curing salts, drying, fermentation, smoking, etc.) on the lethality of *Toxoplasma* cysts present in meats.

When pork tissue containing approximately two *T. gondii* cysts/100 g or mouse brain tissue containing at least 10,000 cysts/100 g were irradiated with a Cs-137 or Co-60 source, 20 krad (200 Grays) inactivated the cysts present in pork tissue whereas 50 krad (500 Grays) were necessary to inactivate those in mouse brain. Therefore, a dose of at least 100 krad (1000 Grays) would ensure that pork tissue is free of infectious *T. gondii*. Unlike heating and freezing, it appears that *T. gondii* cysts are more resistant to radiation than are the cysts of *T. spiralis*.

Limited studies indicate that, in general, conditions that rid pork of infectious *T. spiralis* larvae also frees pork (and presumably other meats) from infectious *T. gondii*. However, more information is needed to determine if commercial cured meat processing conditions actually eliminate the parasite from meats.

Human Toxoplasmosis

Individuals can develop toxoplasmosis through the ingestion or inhalation of oocysts, by ingestion of tissue cysts present in meats or wound infection by bradyzoites from cysts ruptured during butchering. However, the importance and extent of oocyst ingestion or inhalation in causing disease is not known. Congenital infection occurs also.

When a "normal" individual is initially infected with *T. gondii*, there is parasitemia followed by dissemination and encystment of the parasite in various parts of the body. There are usually no clinical symptoms and the presence of infection can be detected only by serology. If there are clinical symptoms, the disease is usually mild and self-limiting and fatalities are rare. The symptoms may present as fever, malaise, skin rash, pneumonia, myocarditis, hepatitis, involvement of the lymph glands, encephalitis or a combination of these.

The disease in immunocompromised individuals is usually recrudescence, i.e., reactivation of a latent *T. gondii* infection. The immune system of the seropositive

immunocompromised individual can no longer maintain the parasite as benign cysts and bradyzoites are released into the system leading to an active disease. In seronegative immunocompromised individuals, a newly acquired infection is possible if the individual eats raw or undercooked meats or comes in contact with oocysts. Acquired immune deficiency syndrome (AIDS) or other immunocompromised patients generally present with central nervous system (CNS) infections but sometimes myocarditis or pneumonitis among other symptoms may be seen. Recipients of organ transplants are necessarily immunocompromised and it is important to determine prior exposure to *T. gondii*. The seropositive recipient is susceptible to his own *Toxoplasma* cysts and the seronegative recipient may be infected by receiving an organ from a seropositive donor. It is even possible that blood transfusions may lead to toxoplasmosis.

Congenital toxoplasmosis may occur if the placenta is invaded by *T. gondii*. Organisms may be shed in the fetal circulation and cause a fetal infection. Women who are seropositive for the parasite before pregnancy (approximately 30% of the women of child-bearing age in the U.S. are seropositive) do not transmit *T. gondii* to the unborn child but this may not be true of the immunocompromised seropositive pregnant woman.

Since the immune system of the fetus is poorly developed, a large number of fetal infections result in clinical symptoms. If the infection occurs in the first trimester and spreads to the CNS of the fetus, abortion and stillbirth may result. Living babies may demonstrate microcephaly, hydrocephaly, cerebral calcifications, convulsions, and psychomotor retardation with an estimated mortality rate of 12%. A milder disease results if the fetus is infected during the second or third trimester. Many of the babies infected later *in utero* are born asymptomatic but may develop epilepsy, retardation, or vision problems later. The most common delayed manifestation of congenital toxoplasmosis is retinochoroiditis, leading to loss of visual acuity or blindness and may show up when the child is a teenager or even when an adult.

A combination of sulfonamides and pyrimethamine is used in the treatment of human toxoplasmosis. These compounds react synergistically against multiplying forms, the tachyzoites. These compounds are effective because they block metabolic pathways involved in the synthesis of p-aminobenzoic acid and folic-folinic acid cycles. Both pyrimethamine and sulfonamides have toxic side effects and are teratogenic. Therefore, they must not be administered during the first trimester of pregnancy. Spiramycin, not available in the U.S., is effective against tachyzoites and is often used during pregnancy. In cases of ocular toxoplasmosis, clindamycin has shown effectiveness. AZT (azidothymidine), commonly used in treatment of AIDS, inhibits the anti-toxoplastic effect of pyrimethamine and complicates the treatment of AIDS patients infected by *T. gondii*. A number of macrolides, purine analogues, and immunomodulators (interferons, interleukins) are currently being investigated for their effects against *T. gondii*. The combination of an antimicrobial agent with an immunomodulator may hold promise in future treatment of toxoplasmosis. At the present time, there is no treatment or drug available on the market

that will attack and destroy bradyzoites in cysts. Therefore, new drugs must be found that can combat toxoplasmosis by eliminating both tachyzoites and bradyzoites. In the absence of a drug that can destroy bradyzoites in cysts, there can be no elimination of *T. gondii* from the host.

There is no vaccine against *T. gondii*. Ideally, a vaccine should be effective in both animals and humans. Such a vaccine would protect kittens against infection and eliminate the excretion of oocysts. Vaccination of food animals would prevent infection and meats would be free of *T. gondii* cysts. There will be no complications due to toxoplasmosis during pregnancy or in immunocompromised individuals if humans could be vaccinated routinely. But until the public sector learns more about toxoplasmosis and become interested in its control, there will be no economic incentive for developing a vaccine.

Economics of Toxoplasmosis

The actual number of congenital toxoplasmosis cases that occur each year in the U.S. is unknown but it has been estimated to range from 400 to 9,500 with a death rate of approximately 2%. Cost (income loss, special education, institutional care, medical treatment) of congenital toxoplasmosis has been estimated at \$270 to 9,000 million each year. For the U.S., the number of clinical cases of non-congenital toxoplasmosis has been estimated at 1-2 million/year with a death rate of at least 0.0001%. The dollar cost has been estimated at 75 million/year.

Nothing is known about the cost of toxoplasmosis to the food animal industry in the U.S. *T. gondii* infections induce abortions or lead to neonatal deaths in swine, sheep and goats. Such losses contribute to the cost of meat. Toxoplasmosis is an expensive disease whether it is apparent or not. There is incalculable fetal wastage in congenital toxoplasmosis as well as deaths in early infancy due to the effects of severe toxoplasmosis. The number of deaths from toxoplasmosis occurring in immunocompromised individuals is unknown but it will escalate since the number of immunocompromised individuals increase each year due to the increasing age of the population and due to advances in medical technology. Aside from death, the cost of medical care for infants, children and adults with clinical toxoplasmosis must be considerable.

At the present state of technology, it is impossible to produce *Toxoplasma*-free livestock and meat. Drugs capable of destroying bradyzoites in cysts combined with an effective vaccine would eliminate *T. gondii* from both animals and man but these approaches appear to be far in the future. For now, the best prevention against *T. gondii* infection would appear to be education. How many physicians talk to their pregnant patients about the danger that toxoplasmosis poses to their unborn babies? It makes good sense that physicians should routinely discuss toxoplasmosis with their pregnant and immunocompromised patients. Veterinarians should talk about *T. gondii* and oocyst excretion by cats with pet owners and give them information on how they can protect the cat as well as themselves from infection. Farm organizations could play an important role in the dissemination of information to farmers and other individuals involved in raising livestock concerning the dangers of cat

defecation in animals feeds and bedding. Commodity trade organizations and public health and regulatory agencies could provide useful information to the public about the potential presence of *T. gondii* in meats and how to prepare meats that are safe for consumption. Consumers knowledgeable about the dangers of *T. gondii* infection (to themselves if they become immunocompromised or to their babies if they become pregnant) will be able to protect themselves.

SUGGESTED READING

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