Toxoplasma gondii in animals used for human consumption

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Food-borne toxoplasmosis in humans may result from exposure to different stages of Toxoplasma gondii*, in particular from the ingestion of tissue cysts or tachyzoites contained in meat, primary offal (viscera) or meat-derived products of many different animals, or the ingestion of sporulated oocysts that are contained in the environment and may contaminate food and water. Although the potential for transmission of the parasite to humans via food has been known for several decades, it is not known which routes are most important from a public health point of view. It is likely that transmission of the parasite to humans is influenced not only by the potential contamination of various food sources, but also by the individual behaviour of consumers in different ethnic groups and geographical regions. Most current methods for detection of* T. gondii *in meat-producing animals, in products of animal origin, or in the environment are insufficient because they do not allow quantification of infectious stages. Hence, most studies report only qualitative data from which it is difficult to assess the true risk of infection in individual cases. There is a need for quantitative data so that efficient strategies to reduce food-borne transmission of* T. gondii *to humans can be developed.*

Key words: *Toxoplasma gondii* - epidemiology - food-borne transmission

Toxoplasma gondii is a ubiquitous parasite that occurs in most areas of the world. It is capable of infecting an unusually wide range of hosts and many different host cells. The life cycle of *T. gondii* includes asexual multiplication in various tissues of intermediate hosts and sexual reproduction in the intestine of definitive hosts. Intermediate hosts are probably all warm-blooded animals including most livestock and humans. Definitive hosts are members of the family Felidae, for example domestic cats.

T. gondii is facultatively heteroxenous and has developed several potential routes of transmission within and between different host species. There are three infectious stages, i.e. tachyzoites, bradyzoites (contained in tissue cysts) and sporozoites (contained in sporulated oocysts) that are infectious for both intermediate and definitive hosts. The parasite may be transmitted from a definitive to an intermediate host, and *vice versa*, as well as between different definitive hosts, or between different intermediate hosts. It is currently not known which of the various routes of transmission is more important epidemiologically. However, the prevalence of *T. gondii* infection is not confined to the presence of a certain host species. Its life cycle may continue indefinitely by transmission of tissue cysts between intermediate hosts (even in the absence of definitive hosts) and also by transmission of oocysts between definitive hosts.

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Development of *T. gondii* in animals used for human consumption

All mammals and birds that are consumed by humans may serve as intermediate hosts for *T. gondii* and, thus, may be a potential source of infection for humans. In intermediate hosts, *T. gondii* undergoes two phases of asexual development. In the first phase, tachyzoites multiply rapidly by repeated endodyogeny 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. Within the tissue cyst, bradyzoites multiply slowly by endodyogeny. 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 et al. 1998). Tissue cysts are the terminal life-cycle stage in the intermediate host and are immediately infectious.

Tissue cysts of *T. gondii* contained in meat, meat-derived products or offal may be important sources of infection for humans. However, for public health purposes it is important to note that the organotropism of *T. gondii* and the number of tissue cysts produced in a certain organ vary with the intermediate host species. In livestock, *T. gondii* tissue cysts are most frequently observed in various tissues of infected pigs, sheep and goats, and less frequently in infected poultry, rabbits, dogs and horses. By contrast, tissue cysts are found only rarely in skeletal muscles of cattle or buffaloes (Tenter et al. 2000).

Development of *T. gondii* in felids and in the environment

If ingested by a definitive host, the bradyzoites that are contained in the tissue cyst initiate another asexual phase of proliferation in epithelial cells of the small intestine. The terminal stages of this asexual multiplica-

tion initiate the sexual phase of the life cycle (gamogony followed by formation of oocysts). Finally, unsporulated oocysts are released into the intestinal lumen and passed into the environment with the faeces. Sporogony occurs outside the host and leads to the development of infectious oocysts which contain two sporocysts, each containing four sporozoites (Dubey et al. 1998).

Food-borne infections in humans

In the course of evolution, *T. gondii* has developed a broad range of potential routes of transmission to humans. Most human infections with *T. gondii* are acquired postnatally. However, it has not been elucidated which sources of infection are important from a public health point of view. For example, we know little about the major reservoirs of the parasite in nature, the relative importance of horizontal transmission of *T. gondii* between different host species, or the epidemiological impact of different food sources causing infection or disease in humans.

Tachyzoites, which play the major role in vertical transmission of *T. gondii*, are sensitive to environmental conditions and are usually killed rapidly outside the host. Hence, food-borne transmission of *T. gondii* via tachyzoites is probably not important epidemiologically, but may occur infrequently. In general, it is believed that the majority of transmissions from animals to humans are caused either by ingestion of tissue cysts in infected meat, meat-derived products or offal (viscera) or by ingestion of food or water contaminated with sporulated oocysts derived from the environment or (less frequently) directly from feline faeces. However, the relative importance and frequency of horizontal transmissions via tissue cysts versus oocysts in a given population is unknown. While many studies have been carried out on the asexual stages of *T. gondii*, in particular on the tachyzoite, far fewer studies have considered the sexual stages or their infectious product, i.e. the sporozoites within the oocyst.

Only a few studies have been aimed at identifying risk factors that may be associated with acquiring an infection with *T. gondii* postnatally. Not all possible routes of infection are important from a public health point of view and sources of infection may vary greatly among different ethnic groups and geographical locations. However, little is known about the relative importance of different food sources in the transmission of *T. gondii* from animals to humans.

The potential role of tachyzoites in food-borne transmission to humans

Tachyzoites of *T. gondii* have been detected in body fluids, including saliva, sputum, urine, tears, semen and milk of several intermediate hosts, including sheep, goats, cows and camels (Tenter et al. 2000). An early study reported that *T. gondii* tachyzoites may be isolated from raw chicken eggs laid by hens with experimentally induced infection (Jacobs & Melton 1966).

Tachyzoites are sensitive to proteolytic enzymes and are usually destroyed by gastric digestion. However, infants who are more susceptible to toxoplasmosis than adults have a lower concentration of proteolytic enzymes in their gastrointestinal tract. This may explain one report of toxoplasmosis in a breast-fed infant whose mother acquired a primary infection with *T. gondii* (Bonametti et al. 1997b). In addition, tachyzoites may occasionally survive for a short period of time (up to 2 h) in acid pepsin solutions (Dubey 1998). In adult humans, solid meals may raise the stomach pH up to five for several hours so that tachyzoites may be deposited into the small intestine. It has also been suggested that, on rare occasions, tachyzoites may enter the host by penetration of mucosal tissue and thereby gain access to the host's circulation or lymphatic system before reaching the stomach (Riemann et al. 1975, Sacks et al. 1982, Johnson 1997).

Thus far, clinical toxoplasmosis in humans has been associated only with consumption of unpasteurised goat's milk (Riemann et al. 1975, Sacks et al. 1982, De Andrade et al. 1984, Skinner et al. 1990). By contrast, it has been thought that the risk of acquiring an infection with *T. gondii* by drinking cow's milk, if any, is minimal (Jackson & Hutchison 1989, Dubey 1991). However, it cannot be excluded that any type of milk is a potential source of infection, in particular if consumed raw. For example, a study assessing risk factors associated with primary *T. gondii* infections in women of childbearing age suggested that in Poland, drinking milk may be a potential risk factor for horizontal transmission to humans (Paul 1998). Likewise, it has been suggested that the high seropositivity for *T. gondii* (67%) of pastoral camels in Sudan may be of public health significance for nomads who consume cameline milk raw (Elamin et al. 1992).

Thus, for the reasons explained above it can not be excluded that at least some postnatally acquired human infections are caused by ingestion of tachyzoites. Consequently, it is advisable that eggs and milk should be boiled or pasteurised before human consumption, as these procedures will inevitably kill any potentially present tachyzoites.

The potential role of tissue cysts in food-borne transmission to humans

The consumption of *T. gondii* tissue cysts contained in meat, meat-derived products, or offal can be an important source of infection for humans. Professional groups such as slaughterhouse workers, butchers and hunters may also become infected during evisceration and handling of meat.

Bradyzoites of *T. gondii* are more resistant to digestive enzymes (i.e. pepsin and trypsin) than tachyzoites (Jacobs et al. 1960, Dubey 1998). Therefore, ingestion of viable tissue cysts by a non-immune host will usually result in an infection with *T. gondii*. Tissue cysts of *T. gondii* are relatively resistant to changes in temperature and remain infectious in refrigerated (1-4°C) carcasses or minced meat for up to three weeks (Dubey & Kirkbride 1989, Dubey et al. 1990) this is usually longer than the meat remains suitable for human consumption. Although most tissue cysts are killed at temperatures of -12°C or lower, occasionally some tissue cysts may survive deep-freezing and it has even been suggested that some strains of *T. gondii* may be resistant to freezing (Grosklaus & Baumgarten 1967, Kotula et al. 1991, Kuticic & Wikerhauser 1996, Dubey 2000).

Some studies have suggested that tissue cysts are killed by commercial procedures of curing with salt, sucrose or low temperature smoking (Lundén & Uggla 1992, Dubey 1997, Hill et al. 2004). However, the survival time of tissue cysts varies greatly with the concentration of the salt solution and the temperature of storage. Under laboratory conditions, tissue cysts were killed in 6% NaCl solution at all temperatures examined (4-20°C), but survived in aqueous solutions at lower concentrations of salt for several weeks (Dubey 1997). It has also been shown that salting does not necessarily kill tissue cysts in home-made pork sausages (Jamra et al. 1991, Navarro et al. 1992). In one study, *T. gondii* tissue cysts were killed by 3% table salt after three-seven days (Jamra et al. 1991). This is much longer than the usual storage time for pork sausages and, thus, salting alone is probably not sufficient to prevent transmission to humans via tissue cysts.

Heating to 67°C or higher is a safe way to kill tissue cysts (Dubey 2000). 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 *T. gondii* in all parts of the meat. Some tissue cysts will remain infectious if cooking procedures are used in which the meat is heated unevenly, for example, microwave cooking (Lundén & Uggla 1992).

Tissue cysts are also killed by gamma irradiation at a dose of 1.0 kGy (Dubey 2000). However, irradiation of meat has only been approved in a few countries, including the USA, it is only feasible in industrialised countries and is opposed by consumers in many regions of the world, such as the European Union.

Recently, high pressure processing at 300 MPa or higher has been shown to inactivate tissue cysts of *T. gondii* under laboratory conditions (Lindsay et al. 2006). Although the equipment required for high pressure processing of large quantities of meat at a competitive cost is not yet commercially available and although problems concerning effects on meat colour and texture still have to be solved, this is a promising method and should be further investigated with respect to its ability to efficiently kill *T. gondii* in meat and other products of animal origin.

To prevent food-borne horizontal transmission of *T. gondii* to humans, meat and other edible parts of animals should not be consumed raw or undercooked, i.e. they should be cooked thoroughly (67°C) before consumption. Although freezing alone is not a reliable means of rendering all tissue cysts non-infectious, deep-freezing meat (-12°C or lower) before cooking can reduce the risk of infection. In addition, meat should not be tasted during seasoning or cooking (Paul 1998, Cook et al. 2000).

To avoid cross-contamination of other food sources with *T. gondii* tissue cysts, it is essential that a high standard of hygiene is maintained at slaughterhouses and in kitchens. 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 water (Jacobs et al. 1960) and, thus, hands and all kitchen utensils used for the preparation of meat or products of animal origin should be cleaned thoroughly with hot water and detergents immediately after use.

The potential role of oocysts in food-borne transmission to humans

Sporulated oocysts of *T. gondii* contained in the environment are a potential source of infection for humans and animals used for human consumption. Contamination of the environment with oocysts may be due to infected domestic cats or wild felines. After primary infection with tissue cysts or oocysts of *T. gondii*, a single cat may shed more than 100 million oocysts into the environment. Under environmental conditions with sufficient aeration, humidity and warm temperature, oocysts can sporulate and become infectious within one day, while sporulation may be delayed under microaerophilic conditions.

Sporulated oocysts of *T. gondii* are very resistant to environmental conditions. They survive short periods of cold and dehydration and remain infectious in moist soil or sand for up to 18 months. They are highly impermeable and, therefore, are also very resistant to disinfectants (Kuticic & Wikerhauser 1996, Frenkel 2000). In the environment, oocysts are distributed through wind, rain and surface water, or harvested feeds. They may also be spread via earthworms, coprophagous invertebrates or manure. Hay, straw and grain that are contaminated with cat faeces have been identified as sources of infection for livestock (Buxton 1990, Hiepe & Buchwalder 1991, Chinchilla et al. 1994).

The public health significance of oocysts in the transmission of *T. gondii* to humans is highlighted by the high rate of seropositivity (24-47%) in some populations of vegetarians (Hall et al. 1999, Roghmann et al. 1999). Humans may become infected via contact with contaminated soil, for example through gardening (Cook et al. 2000). Oocysts of *T. gondii* have been isolated from samples of soil in various areas of the world (Frenkel 2000). In a case-control study on pregnant women in Norway, eating unwashed raw vegetables or fruits was associated with an increased risk of acquiring an infection with *T. gondii* (Kapperud et al. 1996). Thus, it is advisable to wash or cook vegetables and fruits, which may be contaminated with soil or cat faeces, before consumption.

The relative importance of meat-producing animals in food-borne transmission to humans

Due to the varying organotropism of *T. gondii* in different host species, the number of tissue cysts produced in a certain organ, in particular in skeletal muscles, varies greatly among different animals. Therefore, not all animals used for human consumption are of the same public health significance, even if infected with *T. gondii*. It is important to note that seropositivity of meat-producing animals does not necessarily reflect the risk that

those animals pose for their consumers. For example, the meat of cattle and buffaloes rarely contains tissue cysts, although in some areas more than 90% of these animals are seropositive for *T. gondii*. By contrast, seropositive pigs, sheep and goats can be assumed to harbour large numbers of tissue cysts in their meat (Tenter et al. 2000, Dubey & Jones 2008).

Usually, the consumption of raw or undercooked pork or mutton is regarded as a major factor in food-borne transmission to humans. However, it is possible to significantly reduce the risk of *T. gondii* infection in livestock using intensive farm management with adequate measures of hygiene, confinement and prevention. These measures include: keeping meat-producing animals indoors throughout their life-time; keeping the sheds free of rodents, birds and insects; feeding meat-producing animals on sterilised food and controlling access to sheds and feed stores, i.e., no pet animals should be allowed inside them. Using such preventive measures, it is economically possible to produce pigs and poultry free of *T. gondii* infection (Tenter et al. 2000).

By contrast, production of free-ranging livestock will inevitably be associated with *T. gondii* infection. Animals such as sheep and goats kept on pastures have an increased risk of infection due to contamination of the environment with sporulated oocysts. Such animals show high levels of seropositivity in many areas of the world, i.e. up to 92% and 75%, respectively (Tenter et al. 2000). This is of particular importance because tissue cysts have been found in many edible parts of sheep (Dubey & Kirkbride 1989, Lundén & Uggla 1992) and small ruminants are important in both milk and meat production throughout the world.

Seropositivity is distinctly lower and more varying in horses, rabbits and poultry. This may reflect epidemiological factors such as different types of confinement, hygiene of stables and different types of feed. By contrast, seropositivity is usually high in dogs, indicating their continuous exposure to a natural environment and the cumulative effect of age. All of these animals may harbour a considerable number of tissue cysts in their organs, including skeletal muscles, and thus have importance in food-borne transmission to humans who consume their meat (Tenter et al. 2000, Tassi 2007).

Tissue cysts of *T. gondii* in venison and other meat of wild animals, including hares, wild boars, deer and other cervids, kangaroos and bears are other potential sources of infection for humans (Tenter et al. 2000, Dubey & Jones 2008). In addition to higher environmental pressure of infection, there is a cumulative effect of age in many wild animals that results in a very high prevalence of infection. Some wild animals, such as Australian native marsupials, have evolved in the absence of *T. gondii* until cats were introduced to their environment only a few hundred years ago. As a consequence, these animals are highly susceptible to the parasite. Although seropositivity of *T. gondii* infection in marsupials is usually lower than in placental mammals, kangaroo meat in particular has been recognised as a potential source of infection for humans, because it is very lean with little fat and, thus, is usually consumed rare or undercooked (Robson et al. 1995).

Outbreaks of acute toxoplasmosis in humans in various regions of the world have been associated with a broad range of foods of animal origin, including dried seal meat, seal liver and raw caribou meat (McDonald et al. 1990, Pekeles et al. 1991), rare kangaroo meat and undercooked lamb satay (Robson et al. 1995), raw mutton (Bonametti et al. 1997a) as well as raw spleen and liver of domestic pigs and wild boars (Choi et al. 1997). However, while these reports highlight that the risk of acquiring an infection with *T. gondii* via meat or other edible animal parts varies greatly with cultural tendencies and human eating habits, data derived from outbreaks of acute toxoplasmosis are usually linked to an occasional point source of infection and, thus, do not necessarily reflect the major, epidemiologically important sources of infection for the whole population.

Over the last two decades, comprehensive case-control studies have been aimed at identifying the different sources of infection with *T. gondii* in different human populations. Several of these studies have identified frequent consumption of (undercooked) meat or cured meat products as principle risk factors for food-borne transmission to humans (Buffolano et al. 1996, Kapperud et al. 1996, Bobic et al. 1998, Paul 1998, Baril et al. 1999, Roghmann et al. 1999, Cook et al. 2000, Boyer et al. 2005). However, the relative importance of the risk factor and the type of meat associated with it varied among different countries. For example, in Norway, consumption of undercooked lamb was a stronger risk factor than consumption of undercooked pork (Kapperud et al. 1996), whereas in Poland consumption of undercooked pork was the principle risk factor identified in the study (Paul 1998). These findings may reflect differences in eating habits of consumers, or different prevalence of infection in the meat-producing animals of these regions. Thus, in Norway up to 18% of sheep, but only 3% of slaughter pigs were infected with *T. gondii* (Skjerve et al. 1996, 1998), whereas 36% of slaughter pigs were infected in Poland (Bartoszcze et al. 1991) at the time that these respective case-control studies were carried out in humans.

The next decades

Because of the zoonotic importance of *T. gondii*, public health organisations, such as the European Food Safety Authority, have advised the introduction of monitoring and surveillance programmes for *T. gondii* infections in animals used for human consumption (EFSA 2007). Such data are essential to elucidate the relative importance of the various sources of human infections, to control disease and to prevent reduction in quality of human life caused by *T. gondii*. However, only a few countries regularly monitor toxoplasmosis in humans, and thus far, no country monitors *T. gondii* infection in animals.

Because of the versatility of *T. gondii* and its complex epidemiology, it is not an easy task to develop strategies for control or prevention of human infections that are effective world-wide, or are effective for all ethnic groups in one location. There are many factors that have an impact on food-borne transmission of *T. gondii* to hu-

mans, such as the type of management and production of livestock, hygienic standards of slaughterhouses, food processing and technology, the density of cats or wild felines in the environment and climate change that may influence the sporulation of oocysts in the environment (i.e. temperature, humidity, wind), to name just a few.

Epidemiology is in a state of flux. In case-control studies on pregnant women in Europe, travel outside Europe was identified as a risk factor for acquiring a primary infection with *T. gondii* (Kapperud et al. 1996, Cook et al. 2000). This finding points to the fact that humans change their habits when entering different environments and thus may become temporarily at risk from sources of infection that are not important epidemiologically in their home situation. In addition, there is a tendency for consumers to change their eating habits and develop preferences for new food types, which may have an impact on the sources of food-borne infections with *T. gondii*. For example, meat from camels and kangaroos, which are highly susceptible to *T. gondii*, has recently become available to consumers outside Australia and Africa, like in Europe. Because this meat is very lean, it is usually served undercooked in restaurants and thus is a potential new source of infection for European consumers.

In the past, most epidemiological studies reported only qualitative data on *T. gondii* infections in animals used for human consumption. However, in the future it will be important to obtain quantitative data to assess the relative importance of the various sources of human infections in a given population or a given ethnic group. Moreover, new food processing technologies should be investigated with respect to their ability to kill infectious stages of *T. gondii*. This will be essential to develop efficient strategies to reduce food-borne transmission of *T. gondii* to humans.

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