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Toxoplasma-safe meat: close to reality?

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In 2008, the centennial of the discovery of *Toxoplasma gondii* was celebrated. However, toxoplasmosis is still seen as a neglected and underreported disease, despite having a disease burden similar to that of salmonellosis and campylobacteriosis. Human vaccines are not available and current antiparasitic treatment is disappointing. This has led to an urge to focus more on prevention. Food, soil or water contaminated with oocysts from cat faeces and undercooked meat from infected intermediate hosts are important routes of infection. Oocyst contamination is difficult to control, whereas in Western countries, the control of *T. gondii* in meat should be feasible. Here, we discuss strategies aimed at developing a *Toxoplasma*-safe meat chain.

Recent developments in the disease burden of toxoplasmosis

Toxoplasma infection during pregnancy can lead to serious and sometimes fatal disease of the foetus or newborn [1]. Information programmes for the prevention of congenital toxoplasmosis have been dedicated to this specific group. Ocular toxoplasmosis leads to blindness in 25% of cases. Most ocular cases are now attributed to acquired disease, which means that prevention should be directed not only to pregnant women but also towards the general population. The disease burden of toxoplasmosis is quantified via the numbers of still births, handicapped newborns and individuals with chorioretinal eye disease [2] (see also 'Priority setting of foodborne pathogens – disease burden and costs of selected enteric pathogens' at http://www.rivm.nl/bibliotheek/rapporten/330080001.pdf).

In contrast to other foodborne infections, toxoplasmosis is a chronic infection, whereby cysts can remain in various tissues including the eyes and brain of infected individuals. There are data indicating that this could lead to behavioural changes and even psychotic diseases such as schizophrenia [3]. This would make the economic and social impact of toxoplasmosis much higher than even previously anticipated [4]. Reactivation of tissue cysts after immunosuppression (by AIDS or transplantation) can lead to a fatal encephalitis and reactivation of cysts in the retina, which might lead to a blinding intraocular inflammation [5]. The main drugs used for treating toxoplasmosis (sulfonamides, pyrimethamine and spiramycine) have been used for several decades [1]. They are effective against tachyzoites but are unable to eradicate *Toxoplasma* tissue cysts and are ineffective in curing the disease, resulting in frequent recurrences of ocular toxoplasmosis despite treatment [6,7] and minimal effect of antibiotic treatment against congenital toxoplasmosis [8].

Toxoplasmosis can be contracted by the oral ingestion of oocysts present in cat faeces and the environment, or tissue cysts present in the meat of infected animals. In this paper, we discuss the importance of these sources of infection, how to prevent transmission and what strategies can currently be deployed and need to be developed to achieve a *Toxoplasma*-safe meat chain.

Meat as a major source of *T. gondii* infection in humans

Any meat from warm-blooded animals and birds traditionally has been considered a major source of *Toxoplasma gondii* infection in Western countries. This idea stems from small outbreaks associated with the consumption of undercooked meat and several epidemiological studies that showed that in pregnant women, the consumption of undercooked meat (products) is the most likely source of infection [9–12]. Indirect evidence comes from the observation that *T. gondii* seroprevalence is lower in strict vegetarians [13,14]. Many animals used for meat production show evidence of a *T. gondii* infection as measured via serum antibodies, and viable parasites have been isolated from the meat of these animals with the exception of beef [15,16] (Table 1). Meat as a source of infection in humans relates to the observation that the decline in human *T. gondii* seroprevalence [17] parallels the decrease in seroprevalence in animals raised for food [15]. It has been suggested that the introduction of indoor farming has led to a marked drop in the *T. gondii* seroprevalence in pork, but public demand for animal-friendly outdoor production systems has led to a re-emergence of *Toxoplasma* infection in these animals [18–21].

In 2005, a nationwide survey of *T. gondii* in meat (chicken, beef and pork) obtained from retail stores in the United States was performed and a prevalence of only 0.38% in pork was found [22]. It is estimated that one pig is consumed by 200–400 individuals, and meat products are often made by combining the meat from different animals, thereby enhancing the risk of transfer of infection. Although the chances of infection via pork have declined over the years, infection risk from other meat sources such as sheep, goats and horses has not changed over time because the source of infection for these herbivorous animals has remained unchanged. This source is the *Toxoplasma* oocyst, which might be present in pastures and surface water. *T. gondii* oocysts are the product of an

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Table 1. Animal Toxoplasma seroprevalence in Europe

Meat animal	Seroprevalence with range	Isolation of viable parasite from meat cuts from naturally infected animals
Game[a]	55.8% (0–100%) [16]	Yes [40]
Sheep	35.9% (4–92%) [16]	Yes [41–43]
Cow	33.6% (2–92%) [16]	No [22,44]
Goat	33.4% (0–77%) [16]	Yes [43,45]
Horse	25.8% (0–80%) [46]	Yes [47]
Chicken	10.4% (5–36%) [16]	Yes [43,48]
Pig	6.0% (0–64%) [16]	Yes [22,49]
Companion animal	**Cats actively shedding *T. gondii*-like oocysts**	**Oocysts per square meter**
Cat	0.28–0.9% [23,24]	94 to 4,671 [23]

[a]Includes pigeons, ducks, geese, rabbits, wild boars, wild turkey, deer, bears, reindeer and moose.

enterosexual cycle that only occurs in the *felidae*, and millions are secreted, probably only once in the lifetime of the cat, for a period of 2–3 weeks after infection. A cross-sectional survey indicates that <1% of all cats are actively shedding *T. gondii*-like oocysts [23,24] and that oocyst contamination is concentrated in cat defecation sites [25]. Oocysts can remain infectious for up to two years in a moderate climate and are practically impossible to remove from the environment once it has been contaminated. A continuous input of sporulated oocysts, originating from young infected cats, must be present to sustain the oocyst reservoir in the environment. It is estimated that the number of cats in Western countries has not decreased in recent years [17]. Animals with outdoor access can become infected via oral uptake of these oocysts and the parasite will remain present in their tissues for life. Therefore, animals such as horse, sheep and game are at a high risk of infection and act as a transmission route to humans. Seroprevalencies in these animals are high and range from 26% to 56% in European countries (Table 1). How animals used for meat are infected and contribute to human infection has been reviewed recently [26]. Game is gaining in popularity and, depending on the region, very high *T. gondii* seroprevalence rates in these meats have been observed [16]. An outbreak in Australia was reported to be caused by consumption of undercooked kangaroo meat [27].

Pre-harvest prevention of *T. gondii* infection in meat-producing animals

Several epidemiologic studies have identified the risks of *T. gondii* infection in farm animals. Pre-harvest prevention should be aimed at controlling these risks (Table 2). Removing cats from the farm surroundings, or vaccinating cats, could theoretically lead to a reduction of the oocyst load in the neighbourhood of the farm and could be useful in preventing *T. gondii* infection of farm animals, but effects might only be apparent a few years later. Experimental vaccination of farm cats against *Toxoplasma* oocyst shedding only led to a small drop in pork *T. gondii* seroprevalence [28]. These disappointing findings can be explained by the long half-life of environmental oocysts and that other routes of infection, such as consumption of rodent remnants, could be a direct source of infection to pigs [29]. Pre-harvest prevention programmes would require strict rodent control and certification that the farm and surroundings are free of oocyst-shedding cats.

The risk of environmental oocyst contamination can be addressed by using sterilized feed and bedding, and not allowing animals outdoor access. This approach has led to a *Toxoplasma*-free production system in modern pork and broiler farms [30].

However, for meat animals such as sheep, goats, horses and game, some of the approaches for pre-harvest prevention could be difficult, if not impossible, to implement because these animals require outdoor access. Therefore, vaccination of these meat animals seems a promising prevention strategy, but it is still in the experimental phase and needs further development [31]. A live attenuated *T. gondii* vaccine is available for sheep to prevent abortions, but whether this vaccine decreases the presence of *T. gondii* tissue cysts in sheep meat has not been validated [32]. Farm management procedures for the control of *Toxoplasma* infections in animals have been reviewed recently [26].

Monitoring programmes

Many Western countries have introduced monitoring programmes in slaughterhouses to screen animals for *Salmonella* or *Campylobacter* spp. Results have a direct impact on the income of the farmer and, thus, are an incentive for the farmers to change their management system. As yet, *T. gondii* screening is only performed incidentally and is mostly related to research projects [33]. In the European

Table 2. Pre-harvest measures for prevention of *T. gondii* infection

Risk	Prevention measure	Measure outcome
Active oocyst shedding	Remove cat from farm[a] Vaccinate cat[a,b,][50]	No oocyst source on farm Reduce or prevent oocyst shedding
Environmental oocyst contamination	Sterilize feed and bedding, no outdoor access[a]	Reduction of exposure to oocysts
Toxoplasma-infected rodents	Rodent control programme[a,][29]	Reduction of transmission of *Toxoplasma* to omnivorous meat animals
Tissue cysts in meat	Vaccinate farm animals[a,b,c,][31]	Prevent tissue cyst formation
Prevention programme	Inform people at risk[d,][38]	Awareness of exposure risks

[a]Measures to be included in an on-farm prevention programme.
[b]Experimental procedure.
[c]Can be used as a single procedure to prevent *Toxoplasma* infection in meat animals.
[d]Can be used as a single procedure to prevent *Toxoplasma* transmission to humans.

Table 3. Post-harvest measures for killing *T. gondii* cysts in meat

Prevention measure	Measure outcome
Monitoring programme at slaughter[a] [16]	*Toxoplasma*-positive meat selected for pre-heated or frozen meat products. Grant a *Toxoplasma*-free meat label or status to product and farm.
Cook meat well at >56 °C for 10 min[b,][34]	Tissue cysts are killed.
Cure meat with >2% salt or >1.4% lactate salts for at least 1 day[b,][51]	
Freeze meat for at least two days at temperatures below −12 °C[b,][35]	
Improved kitchen hygiene[b]	Reduction of cross-contamination.

[a]Government-imposed procedure could be used as a single measure to prevent *Toxoplasma* transmission via meat. Validated monitoring techniques are absent but are often used in experimental diagnosis of *T. gondii* infection.
[b]Measures to be taken by meat-product-producing companies and by people at risk.

Union (EU), zoonotic diseases have to be reported according to their epidemiological situation (Directive 2003/99/EC), but despite this regulation, no surveillance data are available for *Toxoplasma* in the EU for humans or for animals or food. To improve data collection and to better evaluate the disease burden of toxoplasmosis, the Biological Hazard Panel of the European Food Safety Authority has recently recommended that *Toxoplasma*-monitoring programmes should be initiated in the pre-harvest sector on sheep, goats, pigs and game [16].

A major restriction on the implementation of such programmes is that currently, no standardized validated serologic tests, such as ELISA or agglutination tests, are available that correlate seropositivity to the presence of infectious parasites in the meat of the animal. This is hampered by the absence of generally available reference sera and tissues. Overcoming these difficulties, a pre-harvest monitoring programme could aim to select *T. gondii*-seronegative animals for sale as *Toxoplasma*-free meat. By contrast, meat from seropositive animals could be processed in pre-heated meat products or selected for freezing, to be sold as *Toxoplasma*-safe meat. Such a programme could be successful if it is government-imposed and accompanied by the introduction of validated tests and certification programmes. This could lead to the introduction of a '*Toxoplasma*-free meat' label for farms without *Toxoplasma* infection. Alternatively, existing quality labels for processed meat could incorporate the requirements needed for the inactivation of tissue cysts to guarantee it is a *Toxoplasma*-safe meat product.

Post-harvest procedures for killing *T. gondii* cysts in meat

Heating is the most efficient way to kill *T. gondii* tissue cysts. Meat should reach internal temperatures of 56 °C for at least ten minutes [34] (Table 3). Freezing meat for at least two days at temperatures below −12 °C can also kill tissue cysts [35]. Curing and treatment of meat with enhancing solutions, such as potassium or sodium lactate, can also kill *T. gondii* tissue cysts. Inactivation of tissue cysts depends on the interaction between salt concentration, maturation time and temperature. A summary on current inactivation procedures is provided in Table 3. Methods to inactivate *T. gondii* in meat have been reviewed recently [26]. A recent approach that incorporates all these variables has led to a qualitative risk-based assessment of the processing of ready-to-eat smallgoods [36].

Modern food processing technologies such as high pressure or gamma irradiation might also be used to inactivate the parasite [37]. Meat products should be labelled to show that the procedures used to prepare these products, such as heating to >56 °C or freezing for two days, have undergone a validated decontamination procedure for sale as *Toxoplasma*-safe meat.

Currently, the only test that can demonstrate the presence of infective *T. gondii* in meat cuts (raw or processed) is the bioassay in cats and mice, and it is used for experimental procedures. In addition to ethical restrictions, use of this test for routine implementation is hampered by the time it takes before a result can be obtained. By contrast, genetic tests such as PCR can detect the presence of the parasite genome but not whether these parasites are still infectious. There is an urgent need to develop efficient tests that demonstrate the presence of infectious *T. gondii* parasites in meat.

People consume meat at home or in restaurants. It is utopian to enforce recommendations on meat handling to restaurants. Thus, it remains the responsibility of the consumer to request well-cooked meat or choose a dish, such as a stew, that contains well-cooked meat. Alternatively, the consumer might ask whether the meat has been frozen before preparation.

Finally, consumers should be educated to freeze their meat for a couple of days and/or properly cook their meat, and to prevent cross-contamination while handling meat in the kitchen. Long-term prevention programmes aiming to inform pregnant women how to prevent infection with *T. gondii* have been successful in reducing congenital infection [38]. However, a recent survey has questioned whether education of the consumer in general would be as successful as intensive counselling of pregnant women about toxoplasmosis [39]. The guarantee of *Toxoplasma*-free meat, however, should not change any of the current recommendations about cooking and the preparation of meat because other food-borne diseases associated with raw meat, such as campylobacteriosis and salmonellosis, are also prevented by proper handling.

Concluding remarks

Interventions to prevent the introduction of *Toxoplasma*-infected meat into the food chain would be technically feasible in countries where the meat food chain is well organized. Monitoring of farms and adjustment of farm management can play an important part in the control of *Toxoplasma* infections. However, the development and implementation of monitoring programmes pre- or post-harvest, and subsequent post-harvest decontamination, is currently hindered by the absence of validated tests that can certify the *Toxoplasma* status of a meat product, animal or farm. Efforts should be undertaken to make

these tests a reality. Until then, *Toxoplasma* meat labels will remain a utopian idea. Prevention programmes should be aimed at the general public, who should be aware of the fact that certain animal production systems and animals are associated with a higher chance of the meat being infected with *T. gondii*. The risk of *Toxoplasma* transmission by meat is restricted to consumption of fresh meat as raw or incompletely cooked meat preparations.

Toxoplasma-safe meat exists; it is frozen. People at risk must be encouraged to buy frozen meat or freeze their fresh meat for a couple of days because the preparation methods of the meat might be influenced by local traditions and cooking procedures. As such, the information that is provided to the consumer or patient might have a considerable impact on public health and lead to a reduction of congenital and ocular toxoplasmosis.

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