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Toxoplasmosis as a food-borne infection

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Abstract. *Toxoplasma gondii* is a globally distributed parasite that infects all mammals, including one third of the world population. Long known to cause disease in the developing foetus and in immunosuppressed individuals, a body of data that has emerged in the past decades suggests its role in human pathology may be even more important. The WHO and FAO have recently established toxoplasmosis as a foodborne infection of global concern, with a disease burden the greatest of all parasitic infections. Transmission of toxoplasmosis occurs by ingesting tissue cysts from undercooked meat and meat products, and oocysts from the environment with contaminated fresh produce or water. This review provides an update on the current understanding of toxoplasmosis, focusing on the risk of infection from food of animal origin, with particular reference to the risk in Serbia and the region of South-East Europe.

1. Introduction

Known for over a century, *Toxoplasma gondii* is considered one of the most successful parasites on Earth due to its omnipresence and widest array of hosts, including all mammals.

Domestic cats or other *Felidae* are the definitive host of the *Toxoplasma gondii* parasite, in whose intestines the sexual cycle takes place, and they are the primary source of infection for all animals including humans, by contaminating the environment with oocysts excreted in the faeces. Herbivores are infected by ingestion of food and water contaminated by oocysts, and carnivores by eating tissue cysts present in the flesh of infected animals. Humans (and other omnivores) can be infected both ways, by food contaminated with oocysts (improperly washed vegetables or fruits), contaminated water or hands, and by eating improperly processed or raw meat of infected animals.

Human infection is widespread; it has been estimated that one third of the global population is infected [1]. Infection is generally mild and self-limiting in the immunocompetent host, and according to the classical understanding, of no further concern. Once infected, the parasite is thought to persist for the lifetime of its host, under control by the host immune response. This explains the vulnerability of population categories with an incompetent immune system, including the unborn baby and immunosuppressed individuals, to serious, even life-threatening *Toxoplasma*-induced disease. Congenital toxoplasmosis (CT), for which preventive strategies have long been implemented, is quite rare, but the population of immunosuppressed individuals is increasing, with the AIDS pandemic, and with the advance in transplantation medicine, which depends on immunosuppressive treatment to avoid graft rejection. In this context, it is important that treatment options have not advanced much for decades and there is still no drug able to eliminate encysted parasite.

A body of data has emerged in recent decades, however, which sheds different light on some existing concepts. The concept that *T. gondii* infections are mostly subclinical has been challenged by a current intriguing line of research on the potential role of toxoplasmosis in the pathogenesis of neurologic and psychiatric diseases, including schizophrenia, Parkinson's disease and depression [2]. Others stem from the insight into the molecular epidemiology of *T. gondii* infection.

The WHO and FAO have recently established toxoplasmosis as a foodborne infection of global concern, with a disease burden, similar to that of salmonellosis and campylobacteriosis, the greatest of all parasitic infections [3]. This has naturally renewed the interest in this zoonosis, particularly from the aspect of food safety, and calls for new strategic approaches in both management and prevention.

This paper reviews the novel understanding of *T. gondii* infection, focusing on the risk of infection from food of animal origin, particularly in the region of South East Europe (SEE).

2. *T. gondii* population structure and implications

The *T. gondii* genus comprises a single species infective for all hosts, with limited genetic diversity characterised by clonal lineages designated as types I, II and III predominating in Europe and North America (and a fourth one in the latter), and by a higher frequency of non-clonal, atypical strains in sub-tropical and tropical countries, and a wider genetic diversity characterised by non-clonal, atypical strains in South America and Africa. The diversity encountered in the tropical and subtropical areas has been associated with the presence of diverse *Felidae* in which sexual reproduction, and consequentially, genetic recombinations, occur [4].

Insight into the *T. gondii* population structure may have huge implications on our understanding of this infection. Differences in infecting genotype have been related to the clinical presentation. Atypical strains have been associated with more severe ocular toxoplasmosis, atypical presentations and even life-threatening disease in both immunocompetent and immunosuppressed individuals. The concept of only primary maternal infection during pregnancy being a risk for foetal infection has been challenged by cases of CT in babies born to immunised mothers, re-infected with atypical strains [5].

This, along with the widely varying geographically-dependent prevalence of infection and the geographic differences in the *T. gondii* population structure, determines toxoplasmosis as a travel risk [6]. Moreover, globalisation of food, including importation of meats from areas of a highly divergent population structure, may also present a risk factor for severe infections.

3. Toxoplasmosis as a food-borne infection

3.1. Basic epidemiology of human toxoplasmosis

Transmission of toxoplasmosis occurs by ingesting tissue cysts from undercooked meat and meat products, and oocysts from the environment along with contaminated fresh produce or water.

Notably, there has been a decreasing trend of *T. gondii* infection prevalence over the last 30 years across Europe [7]. The same has been noted in SEE. Continuous monitoring of the prevalence of *T. gondii* infection in women of childbearing age in Slovenia, Serbia and Greece has shown a significant decrease in the infection prevalence since the eighties onwards [rev. In 8]. The largest decrease, from 86% in 1988 to 31% in 2007 and beyond, was noted in Serbia.

Many factors may have contributed to such a change, including increased public awareness as a result of health education, better hygiene on livestock farms, and more frequent use of frozen meat (freezers now present in most households).

Importantly, consumption of undercooked or insufficiently cured meat has repeatedly been shown to be a major risk factor for human infection with *T. gondii* [9].

3.2. Toxoplasmosis in meat animals in SEE

A major reason for the control of *T. gondii* infection in meat animals is the reduction of the reservoir of human infection. Accordingly, the meat route has been intensively explored, in parallel with the development of serological and molecular tools that allowed for the analysis of meat/meat products.

Earlier serological investigations of various animal species in SEE countries were generally carried out in limited samples sizes and using different and non-standardised methodologies. Thus, only studies in the last 20 years are referred to here.

In Serbia, the first nation-wide cross-sectional survey on the seroprevalence of *T. gondii* infection in cattle, sheep and pigs carried out in 2002/2003 showed a seroprevalence of 76.3% in cattle, 84.5% in sheep and 28.9% in pigs [10]. The antibody levels ranged from 1:25 to 1:400 in cattle, and up to 1:25,600 in sheep and to 1:12,800 in pigs. Among the seropositive animals, the proportion of high antibody levels (≥1:1600), suggestive of acute infection, and indicating continuous presence of infection reservoirs in the environment, was 10% in sheep, and 4% in pigs.

Risk factors for cattle were small herd size and farm location in Western Serbia, while housing in stables with access to outside pens was protective. In sheep, an increased risk of infection was found in ewes from state-owned flocks vs. Private flocks, and, interestingly, also in those from Western Serbia. In pigs, the risk of infection was highly increased in adult animals, as well as in those from finishing type farms.

Despite the high prevalence of 85% in sheep of which 10% had high antibody levels suggestive of acute infection, correlation with ovine abortions could not be established, since aetiological laboratory diagnosis of ovine abortions in Serbia does not include diagnosis of *T. gondii*.

An outbreak of toxoplasmosis in sheep has recently been reported; massive abortions (60%) occurred in a flock of 500 dairy sheep in Northern Greece at 110-130 days of pregnancy, diagnosed upon observation of tissue cysts in brain smears of aborted foetuses, and by serological (ELISA) examinations of mother and foetal serum samples. The abortion rate declined immediately upon instituting sulphadimidine therapy [11].

Another study showed a high prevalence of 74.7% in goats in Serbia as well [12], higher than in Greece (62.9%), Bulgaria (59.8%) and very markedly so than in Croatia – 8.4% [13].

A later study on *T. gondii* infection in slaughter pigs in Serbia [14], showed, however, a three-fold lower prevalence of 9.2% in a total of 488 swine from abattoirs in the vicinity of Belgrade. This difference was largely attributed to the difference in the studied samples since the latter research consisted of a large majority (96%) of market-weight pigs, which generally have a much lower prevalence than adult pigs. Similarly to the 2006 study, risk factors for infection in slaughter pigs included age and farm type, with a 41-fold higher likelihood of infection in adult *vs.* Market-weight pigs, and a 15-fold higher likelihood of infection in pigs of all ages from smallholders' finishing type farms *vs.* Those from farrow-to-finish intensive farms. It was proposed that a national strategy to reduce the level of *T. gondii* infection in pigs should include a shift towards the development of more farrow-to-finish farms, as well as vigilance in farm management and implementation of zoo-hygienic measures at finishing farms. Damriyasa et al. [15] stated that *T. gondii* seropositivity is an indicator of the hygienic status of the pig farm. In the 2011 study [14], the demonstration of viable *Toxoplasma* in blood samples of seropositive swine indicated that slaughtering took place during parasitaemia, which suggests frequent swine reinfections. The study concluded that swine meat is a significant potential risk for human *Toxoplasma* infection, but that the risk cannot be predicted on the basis of specific antibody level in the pigs.

On the other hand, the modern approach in farm management to provide for the welfare of the animals as well as organic food for human consumption is to develop animal-friendly (organic) farms. According to experience from the Netherlands [16], development of such farms can result in an increase in *T. gondii* infection. Nevertheless, a report from organic sheep and goat farms in Greece [17] showed similar prevalence rates to those in animals from conventionally managed farms [18].

Cattle are generally thought not to be significant in this context [19]. However, beef is often consumed undercooked (rare beef steaks, roast beef, steak tartar), and at least one outbreak of toxoplasmosis whose source was raw beef has been documented [20]. In addition, one out of four beef samples randomly chosen from UK retail outlets tested positive for *T. gondii* by PCR [21]. These facts, along with the circumstantial evidence provided by the high prevalence of cattle infection of 92% in Italy and 69% in France [22], and in Serbia, countries in which human infection is highly

prevalent as well, all suggest a role for cattle as a *T. gondii* reservoir for human infection. In addition, Bobić et al. [9] have demonstrated that among all the meat consumed, undercooked beef presents the highest risk for human infection in Serbia. Similarly, although Opsteegh et al. [23] did not establish a correlation between seropositivity and the detection of parasites in cattle, a study in which the relative contribution of sheep, beef and pork products to human *T. gondii* infection in the Netherlands was quantified (by Quantitative Microbial Risk Assessment), showed that beef is indeed an important source even if the seroprevalence in cattle is low [24].

On the other hand, according to official statistical reports (RZS, 2006–2010), pork comprises approximately 50% of all meat consumed in Serbia. Thus, although pigs were the least infected of the examined species, and given the findings that the prevalence increases with age and reaches 41% in sows [10], pork consumption could significantly contribute to human infection. When used for cooking, pork is generally properly thermally processed, but in most of the SEE countries' traditions, mature pork is also highly valued for making delicatessen meat products. Raw or improperly cured sausages and ham are the source of small (family) epidemics of trichinellosis which, in spite of mandatory meat examination for *Trichinella spiralis*, occasionally occur in Serbia [25], and thus are a quite plausible source of human *T. gondii* infection too.

Horsemeat, on the other hand, is typically consumed rare or undercooked. In horses, which generally have lower seroprevalence values than small ruminants [26], a prevalence of only 1.7% has been determined in Greece [27]. However, a study which included 105 horses from all regions of Serbia slaughtered at two abattoirs between 2013 and 2015 showed a seropositivity of 48.6% at the 1:6 MAT cut-off, but of only 12.4% at the standard 1:25 cut-off. Importantly, viable parasites were isolated from two grade type mares; according to microsatellite genotyping, both isolates were type III, of which one was similar to a strain isolated from a duck in Iran, and the other one was identical in all markers to three strains isolated from a goat from Gabon, a sheep from France and a pig from Portugal. Interestingly, one of the source horses was seronegative, the other weakly positive. The isolation of viable *T. gondii* parasites from slaughter horses points to horsemeat as a potential source of human infection, but the fact that viable parasites were isolated from horses with only a serological trace of *T. gondii* infection presents further evidence that serology may not be adequate to assess the risk of toxoplasmosis from horsemeat consumption.

For most meat animals, there is no visible reduction in *T. gondii* prevalence, as opposed to the decreasing trend in humans (explained, among other reasons, by increased frozen meat use and better farm management). Farming practices and level of environmental contamination have not changed much, and except for the intensive pig farms in which a major reduction in *T. gondii* prevalence has occurred, a decline in *T. gondii* prevalence in meat animals is yet to be achieved. Moreover, for strictly herbivorous species that require outdoor access, this is probably impossible [28].

Ubiquitous contamination of the environment is also shown by the presence of *T. gondii* in both farm and urban rodents, pigeons and dogs [rev. In 13]. As long as there is such widespread environmental contamination in SEE, a decrease of *T. gondii* prevalence in meat animals may hardly be expected in the absence of energetic and systematic prevention measures throughout the region.

3.3. Food-borne is not only meat-borne: other food sources
In contrast to the availability of various serological and molecular tools which have allowed study of the *T. gondii* transmission meat route, it is precisely the lack of such tools that has left the oocyst stage much less studied, and until recently, it was virtually impossible to distinguish if a case of toxoplasmosis was caused by the tissue cyst or the oocyst. Several lines of evidence have linked oocyst infection to cases of acute toxoplasmosis, including hydric epidemics, case-control studies which showed consumption of fresh produce as an infection risk factor, and experimental studies showing the adherence of oocysts to fruits such as berries that produced infection in mice, (although the latter has yet to be confirmed in natural settings). A recently developed sporocyst-specific antibody test enabled clinical studies to be carried out, which showed that 43% of infections in pregnant women in Chile and 78% in mothers giving birth to congenitally infected children in the USA were caused by

oocysts [29], but this has not been studied extensively, nor is such an antibody commercially available. Importantly, oocyst-induced infections appear to be clinically more serious than those caused by tissue cysts, and in fact, most often induce clinical infection.

Oocyst sources for human infection include soil and water, directly or indirectly through contamination of produce. Additional sources increasingly gaining importance include marine mammals, as well as filter-feeding invertebrates such as mussels and oysters, that do not get infected but can concentrate viable oocysts and serve as an infection reservoir for marine predators and humans. Continuous climate and man-made environmental changes favour an increase in oocyst-induced infections in both humans and animals, calling for the development of commercial technologies to detect oocysts in produce as well as for strategies for large-scale detection of oocysts in terrestrial and aquatic environments.

4. Conclusions

Strategies for the prevention of toxoplasmosis generally target only CT, and even their implementation, although clearly diminishing the incidence, has not eradicated CT. In addition, in the light of the new understanding of *T. gondii* infection, there may be additional risks calling for prevention of toxoplasmosis in other population categories. This clearly shows that new, comprehensive prevention strategies are needed, based on accurate and validated data on: (1) the routes and risk factors for human infection at the local level, which would allow for more efficient health education; (2) routes and risk factors for meat animal infection to diminish infection reservoirs, and; (3) environmental contamination. Such a complex task may best be accomplished within the One Health concept, with physicians, veterinarians, food technologists and ecologists acting in concert to prevent this important zoonosis.

Acknowledgement

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