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# *TOXOPLASMA GONDII* IN WILD RUMINANTS BRED IN GAME PRESERVES AND FARMS WITH PRODUCTION DESTINED FOR HUMAN CONSUMPTION IN THE CZECH REPUBLIC

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## ABSTRACT

*Toxoplasma gondii* is the causative agent of the most common parasitic infection in humans. Almost all warm-blooded animals, as well as humans, can act as intermediate hosts that harbour infective cysts in their tissues. Felids act as definitive hosts excreting oocysts in faeces. In humans, *T. gondii* can cause subclinical infection but also severe clinical disease with a wide range of symptoms, especially in immunocompromised individuals. The infection is usually asymptomatic in animals and is not recognized at either *ante-* or *post-mortem* inspection. The consumption of undercooked meat from infected animals is one of the most important routes by which the infection can be transmitted to humans. Handling of the organs and other tissues of game animals and eating their undercooked meat have been described as a risk of *T. gondii* infection. For diagnosis of toxoplasmosis, the combination of serological and molecular methods has been described as a suitable approach. Antibodies against *T. gondii* were detected in 20.8%, 50.0%, 23.1%, and 24.4% of red deer, sika deer, fallow deer and mouflons, respectively, coming from game preserves and farms in the Czech Republic. *T. gondii* DNA was found in the muscle tissue of red deer (8.3%) and mouflons (14.6%). The lower prevalence rates based on molecular screening could be due to the random distribution and low density of cysts in tissues of infected animals. Bearing in mind the increase in the number of hunted animals and the growing trend in game consumption, it is important to educate hunters and game meat consumers about the risk of exposure to this zoonotic infection during handling and consumption of the meat.

**Keywords**: zoonosis; food safety; meat; tissue cyst; antibodies

## INTRODUCTION

*Toxoplasma gondii* is a ubiquitous zoonotic parasite of significant concern to human health **(EFSA, 2007)**. *Toxoplasma* only rarely causes severe clinical symptoms, the infection is mostly asymptomatic or only mild symptoms occur (self-limiting lymphadenopathy, fever or intraocular inflammation). However, it can cause life-threatening infections in immunocompromised individuals (disseminated disease with encephalitis, meningoencephalitis, myocarditis or hepatitis**).** Parasitaemia in a primarily infected pregnant woman may result in congenital toxoplasmosis with abortion, neonatal death, or fetal damage as encephalomyelitis, retinochoroiditis, intracranial calcifications, hydrocephalus or mental retardation in survivors **(Tenter et al., 2000; EFSA, 2007; Kijlstra and Jongert, 2008)**.

Almost all warm-blooded animals, as well as humans, can serve as intermediate hosts of *Toxoplasma* with the formation of infective tissue cysts having a high affinity for neural and muscular tissues. Tissue cysts may persist for the life of the hosts. Wild and domestic cats and other felids act as definitive hosts excreting oocysts in their faeces. Infection in hosts, including humans, can be acquired by the consumption of raw or undercooked meat containing tissue cysts, from soil, water or food contaminated by oocysts, or congenitally. Also, contact with infected carcasses during evisceration, dressing and processing presents a risk of infection to humans **(Cook et al, 2000; Tenter et al., 2000; EFSA, 2007; Jones et al., 2009; EFSA 2013b)**.

The infection usually does not cause clinical signs in animals or visible lesions in carcasses and is not recognized at either *ante-* or *post-mortem* inspection. Although toxoplasmosis is the most reported parasitic zoonosis in humans in EU, there is inadequate system for routine monitoring and therefore the incidence of toxoplasmosis in humans and animals and the presence of *T. gondii* in food is underestimate **(EFSA, 2007)**. According to the European Food Safety Authority (EFSA), *T. gondii* was identified as a relevant biological hazard to be addressed in revised meat inspection regulations for pigs, sheep, goats, farmed deer and farmed wild boar **(EFSA, 2011; 2013a; 2013b)**. Infected game can serve as a source of infection for other animals, especially carnivores, and humans **(Ross et al., 2001; EFSA, 2013b)**. Wildlife may also be a good indicator of environmental contamination with *T. gondii* oocysts **(Olamendi-Portugal et al., 2012; Ferroglio et al., 2014)**.

One of the suitable diagnostic methods is the detection of specific antibodies against *T. gondii* in serum or meat juice and seropositivity has been correlated with the presence of cysts in muscles and other animal tissues **(Dubey, 1995)**.

Molecular methods, which can detect the genome of the parasite in tissues but without confirmation of its viability, are less sensitive because the density of these parasites is low in meat **(Dubey, 1988; Dubey et al., 2014)**.

According to EFSA, there are limited data on the prevalence of toxoplasmosis predominantly in farmed cervids **(EFSA, 2013b)**. By means of both serological and PCR testing, the aim of this study was to determine the prevalence of *T. gondii* in wild ruminants from game preserves and farms in the Czech Republic, destined for human consumption.

## MATERIAL AND METHODOLOGY

During the period 2012-2014, muscle samples (musculus gluteus, diaphragma and/or masseter) were collected from 82 carcasses of wild ruminants (red deer, sika deer, fallow deer, and mouflons) immediately following hunting and killing. Animals came from game preserves (n=5) and farms (n=6) in the Czech Republic (regions Kralovehradecky, Pardubicky, Stredocesky and Vysocina) and their meat was intended for human consumption. The ages of the majority of the animals were not available for the purposes of this study.

Meat juices were obtained by subsequent freezing and thawing of the muscle tissue and stored at -20 °C until tested for antibodies to *T. gondii* by the ID Screen® Toxoplasmosis Indirect Multi-species ELISA kit (IDVET, Montpellier, France) according to the manufacturer's instructions. This ELISA kit was evaluated as a screening test for toxoplasmosis in sera or meat juice of ruminants, cats, dogs and pigs and was also used in serological surveys in wild ruminants **(Roqueplo et al., 2011)**.

In addition, 25 g of muscle tissue were analyzed with real time PCR specific for *T. gondii*. Tissue samples were processed according to **Opsteeght et al. (2010)** prior to DNA isolation based on the manufacturer's protocol (QIAGEN) slightly modified to include mechanical homogenization with zirconia/silica beads (0.2 mm) in a MagNALyser instrument (Roche, Mannheim, Germany). The isolated DNA was used as a template for the triplex real time PCR assays. The detection of *T. gondii* via primers and probes specific for *B1* and *529rep* was adopted from **Lass et al. (2012)** and **Opsteeght et al. (2010)**. The previously published internal amplification control was introduced to eliminate false negative samples **(Slana et al., 2008)**.

## RESULTS AND DISCUSSION

It has been suggested that the handling of the organs and other tissues of game animals and eating their undercooked meat carries a risk to humans of potential *T. gondii* infection **(Cook et al., 2000; EFSA, 2007)**. **Ross et al. (2001)** described acquired ocular toxoplasmosis with flu-like symptoms in deer hunters after ingestion of venison. Three cases of acute toxoplasmosis in deer hunters were reported by **Sacks et al. (1983)**. Ingestion of raw or rare venison was considered to be the most likely route of infection.

The consumption of game in the Czech Republic is relatively low and traditionally confined to the hunting fraternity. However, there is an increasing trend towards a wider appreciation amongst those who seek occasional dietary diversification (0.5 kg of game per capita per year in 2006 in comparison with 0.9 kg in 2013; **Anonymous, 2015a**). This increase is linked to the growth of wild populations and number of hunted animals in recent years **(Anonymous, 2015b)**.

We collected muscle samples from a total of 82 wild ruminants of four species whose meat was intended for human consumption. *T. gondii* antibodies were detected in 24.4% of animals (Table 1). Using real time PCR, the presence of *T. gondii* DNA was detected in tissues of eight (9.8%) animals. The lower prevalence rates, based on molecular screening as compared with serological testing, could be due to the random distribution and low density of cysts in tissues of chronically infected and asymptomatic animals (one cyst per 50-100 g of tissue; **Dubey et al., 1988; Dubey et al., 2014**). Nevertheless, real time PCR is a useful method for demonstrating the presence of parasites in muscle samples of food-producing animals. *T. gondii* DNA was detected in four out of eight cases in

**Table 1** Prevalence of *Toxoplasma gondii* infection in wild ruminants from game preserves and farms in the Czech Republic.

| | | Prevalence of *T. gondii* antibodies | | Detection of *T. gondii* DNA in muscles | |
|---|---|---|---|---|---|
| Species | No. examined | No. positive | Prevalence (%) | No. positive | Prevalence (%) |
| Red deer (*Cervus elaphus*) | 24 | 5 | 20.8 | 2 | 8.3 |
| Sika deer (*Cervus nippon dybowskii*) | 2 | 1 | 50.0 | 0 | 0 |
| Fallow deer (*Dama dama*) | 13 | 3 | 23.1 | 0 | 0 |
| Mouflon (*Ovis musimon*) | 41 | 10 | 24.4 | 6 | 14.6 |
| **Total** | **82** | **20** | **24.4** | **8** | **9.8** |

seronegative animals (two red deer and two mouflons). It indicates either recent infection without the development of antibodies or that the antibody titers had declined to undetectable levels **(Dubey et al., 1995)**. The combination of serological and molecular methods should be used for accurate diagnosis of toxoplasmosis **(EFSA, 2007; Halová et al., 2012)**.

We did not find significant differences in seroprevalence amongst the investigated species. The highest prevalence of *T. gondii* antibodies was observed in sika deer (50.0%), but the number of available samples was too low (only two animals, Table 1). *T. gondii* DNA was found in tissues of red deer and a higher prevalence in mouflons (8.3% and 14.6%, respectively). Feeding habits are different in these species - deer feed mainly on grass, leaves and berries, but also on young shoots and twigs, whereas mouflons graze on short grasses and thus may be more frequently infected by oocysts shed by felids. However, the higher prevalence of infection in mouflons than in deer in our study was not confirmed by other authors **(Hejlíček et al., 1997; Gauss et al., 2006; Bartova et al., 2007)**.

In the study of **Hejlíček et al. (1997)** carried out in the Czech Republic (south Bohemia) during the period 1981-1990 which targeted mainly free-ranging wild ruminants, antibodies were detected in 15% (46/303), 100% (3/3) and 10% (2/20) of red deer, fallow deer and mouflons, respectively, but tissue cysts were not isolated from these animals. However, we found a higher occurrence of antibodies, especially in red deer (20.8%) and mouflons (24.4%). It could be influenced by the origin of the investigated animals in our study coming from game preserves and farms. In these smaller, fenced areas with more cats having access and with higher animal densities, the risk of infection increases. The association between positive ELISA results for *T. gondii* and high animal density with the presence of domestic cats being the main sources of toxoplasmosis in wild animals, has already been described **(Hejlíček et al., 1997; Gauss et al., 2006; Olamendi-Portugal et al., 2012)**. **Gauss et al. (2006)**, however, did not find a statistically significant difference in seroprevalence in red deer from open versus fenced areas in Spain (20.9% versus 14.0%).

**Bartova et al. (2007)** surveyed the prevalence of *T. gondii* antibodies in wild ruminants from the countryside and captivity in the Czech Republic. They detected a higher seroprevalence in red deer (45%) which also came primarily from farms and game preserves than in our study (20.8%). On the other hand, we found *T. gondii* antibodies more frequently in fallow deer (23.1% vs. 17%) and mouflons (24.4% vs. 9%) in comparison with the aforementioned study carried out in 1998-2006.

Previous European studies reported seroprevalence of 26.7% amongst white-tailed deer in Finland **(Jokelainen et al., 2010)**, 14.8% of mouflons and 22.8% of fallow deer in Spain **(Gauss et al., 2006)**, 7.7% of red deer in Norway **(Vikøren et al., 2004)**, 6.6% of deer in Ireland **(Halová et al., 2013)** and 0% of red deer in Northwestern Italy **(Ferroglio et al., 2014)**. In France, similarly as in our study, **Aubert et al. (2010)** detected *T. gondii* antibodies in 23%, 17% and 25% of mouflons, red deer and fallow deer, respectively, and viable parasites were recovered by bioassay from one mouflon and one red deer. The identified prevalence of toxoplasmosis in deer hunted in Poland was 8.8% using direct agglutination and 11.6% using PCR, according to the report of EFSA and ECDC **(EFSA, 2014)**.

However, it is difficult to compare results from various studies because different diagnostic methods (serological and molecular) were used and the animal´s age, geographical location and climatic conditions can also influence the prevalence of infection **(Vikøren et al., 2004; Gauss et al., 2006; Jokelainen et al., 2010; Olamendi-Portugal et al., 2012; Dubey et al., 2014)**.

## CONCLUSION

Although toxoplasmosis is the most commonly reported parasitic zoonosis in humans, the pathogen is not routinely monitored in food-producing animals. It is obvious from our study, and previous ones, that wild cervids and mouflons are commonly exposed to *T. gondii* and may serve as a reservoir of infection for humans. When game animals are killed and butchered in the field, discarded viscera left unattended can infect other animals, especially carnivores and omnivores.

To prevent the transmission of infection by ingestion, meat should be cooked well at a minimum temperature of 70 °C. It is also common to freeze game meat for later culinary use. Freezing at a temperature of at least -12 °C for 3 days should be sufficient to inactivate the parasites **(Dubey et al., 1988; Tenter et al., 2000; Kijlstra and Jongert, 2008)**. Hunters and consumers of game should be educated about the risk of exposure to this zoonotic disease during evisceration, handling and consumption.

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