The meat of goat kids and lambs as a possible source of *Toxoplasma gondii* for consumers

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Abstract

Toxoplasmosis is a worldwide zoonosis and the consumption of undercooked meat is a major risk factor for human infection. Antibodies to *Toxoplasma gondii* were found in 28.2% (11/39) and 27.8% (5/18) of home-slaughtered goat kids (n = 39) and lambs (n = 18) from four farms in the Czech Republic. The DNA of the parasite was detected in the tissues (diaphragm and/or lung) of 10.3% (4/39) of kids and 16.7% (3/18) of lambs. Although the consumption of kid and lamb meat is low in the Czech Republic, its consumption may become a source of *Toxoplasma* infection in humans.

Lamb and kid meat, qPCR, serology, Toxoplasma gondii, zoonosis

Introduction

Toxoplasmosis, caused by the ubiquitous intracellular parasite *Toxoplasma gondii*, is one of the most common parasitic zoonoses. Its definitive host are felids, in whose intestinal mucosa the sexual reproduction of *T. gondii* occurs. The oocysts are shed in the faeces into the external environment where they sporulate and become infectious (Plate I, Fig. 1). The majority of warm-blooded vertebrates, including humans, are potential intermediate hosts of the parasite with the formation of tissue cysts (Plate I, Fig. 2), particularly in the muscle and nervous tissue (Tenter et al. 2000).

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, and hepatitis). Parasitaemia in a primarily infected pregnant woman may result in congenital toxoplasmosis with abortion, neonatal death and/ or fetal damage, such as encephalomyelitis, retinochoroiditis, intracranial calcifications, hydrocephalus or mental retardation in survivors. The infection in animals is also generally asymptomatic and cannot be recognized at either *ante*- or *post*-mortem inspection. (Tenter et al. 2000 and Cenci-Goga et al. 2011). The consumption of undercooked meat is considered the most significant cause of infection in humans, even though other pathways exist, such as faecal-oral transmission of infection via oocysts in contaminated soil, water or food and transplacental infection (Cook et al. 2000; Tenter et al. 2000 and Cenci-Goga et al. 2011).

Sheep and goats are sensitive to *T. gondii* infection which is a significant cause of abortions and neonatal mortality in these animals. The infection also causes clinical symptoms (fever, apathy, anorexia, diarrhoea, etc.) in adult animals and consequential economic losses on farms (Dubey 2009; Juránková et al. 2013 and EFSA 2013). The typical method of rearing sheep and goats increases the likelihood of contact with environments contaminated by oocysts (soil, water, pastures - in Plate I, Fig. 3) and thereby increases the risk of infection in animals and, subsequently, in humans (Cenci-Goga et al. 2011; EFSA 2013 and Guo et al. 2015).

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Global seroprevalence reaches as much as 99.2% in sheep and 77% in goats depending on locality, method of rearing, the age of the animals and the testing methods used (Tenter et al. 2000 and Guo et al. 2015). The European Food Safety Authority (EFSA) emphasises the necessity of modernising the existing procedures in veterinary inspection with the aim of preventing the entry of infected meat of not only domestic pigs, farm-raised deer and wild boars but also of sheep and goats into the human food chain (EFSA 2013). The consumption of undercooked mutton and lamb has been adjudged a significant risk factor in human infection in studies performed in Europe and the USA (Cook et al. 2000; Jones et al. 2009 and Lopes et al. 2014).

Although the consumption of mutton and goat meat is low in the Czech Republic - 0.4 kg per person in 2015, which includes the consumption of horse meat (Czech Statistical Office 2017), the number of animals reared and production of mutton, lamb and goat meat increased in the years 2009 – 2013 (Anonymous 2014). These kinds of meat are more popular in other European countries, e.g. France, Greece, Italy, or Spain and among many ethnic groups.

The seroprevalence in adult sheep: 54.6 – 59% (Hejlíček and Literák 1994; Bártová et al. 2009) and goats: 20.2 – 66% (Hejlíček and Literák 1994; Literák et al. 1995; Bártová and Sedlák 2012) reared in the Czech Republic has already been studied. On the whole, however, there is little available data on the presence of *T. gondii* in the tissues of these animals destined for human consumption (Guo et al. 2015).

The aim of our pilot study was to assess the occurrence of *T. gondii* in goat kids and lambs whose meat was destined for human consumption.

Materials and Methods

Samples of the diaphragm and lungs (25 – 50 g) were taken during the home-slaughter of kids (n = 39, age 3 – 6 months) and lambs (n = 18, age 4 – 8 months) from four small farms in the Czech Republic whose meat and organs were destined for consumption in the households of the breeders. The samples of tissues were stored at -70 °C until processed. Samples of meat juice were collected following thawing of the muscle of the diaphragm and stored at -20 °C until testing. A commercial ID Screen® Toxoplasmosis Indirect Multi-species ELISA kit (IDVET, France) was used to detect specific antibodies to the *T. gondii* P30 protein. Samples of the diaphragm and lungs (25 g) were processed by mechanical homogenisation and subsequent enzymatic lysis with the use of Proteinase K (Juránková et al. 2013). The DNA was isolated using the DNeasy Blood & Tissue kit (QIAGEN, Germany) according to a published procedure (Slana et al. 2010). The presence of *T. gondii* in a sample was determined by means of species-specific triplex real-time PCR (qPCR) detecting the genes *B1* and *549rep* (Slaný and Lorencová 2014).

Results and Discussion

In the Decree No. 326/2001, as amended, goat kid and lamb meat is limited to animals less than 12 months old, in the Czech Republic. It is an extremely tender and delicate meat with high biological value and a low fat content, and is easily digested. It can be considered as dietary food. The consumption of these kinds of meat is, however, much less common than, e.g., that of pork or poultry in the Czech Republic (Czech Statistical Office 2017). An increased demand here is seen only at Easter and Christmas time. Sheep and goats are reared primarily on small farms (up to 10 animals) in the Czech Republic, and home slaughter predominates. By comparison, the proportion of animals slaughtered at slaughterhouses is low (Anonymous 2014).

In our study, antibodies to *T. gondii* were detected in 28.2% of goat kids (11/39) and 27.8% of lambs (5/18). Although the detection of antibodies testifies only indirectly to the presence of the parasite, there is a positive correlation between the detection of antibodies and the presence of cysts in the tissues of animals (Dubey et al. 1995). The presence of specific antibodies in young animals may be either the result of transplacental transmission

of infection (in the case of acute infection or the reactivation of a chronic infection of the mother during gestation) or colostral antibodies received passively, which may persist at detectable levels for 1 – 3 months after birth). However, the production of antibodies is usually the result of infection acquired after birth from oocysts in a contaminated environment, in particular feed or water (Dubey 2009; Guo et al. 2015 and Rahman et al. 2015).

The diaphragm muscle and the lung tissue of slaughtered animals were selected for the detection of the parasite´s DNA in the present study. The diaphragm muscle, as well as the heart and the skeletal muscle, were frequently found sites of occurrence of *T. gondii* cysts in naturally and artificially infected goats in the studies by Dubey (1980) and Dubey et al. (1980). Furthermore, in addition to the liver, kidneys, brain, spleen, lymph nodes and intestines of goats, the lungs were another tissue with frequent occurrence of *T. gondii* in the above mentioned studies. The lungs were the site of the highest concentration of the parasite also in experimentally infected goat kids in the study by Juránková et al. (2013).

The parasite's DNA in the lung tissue (in three animals) and/or the diaphragm muscle (in six animals) was detected in 10.3% of goat kids (4/39) and 16.7% of lambs (3/18). The lower positivity rate achieved by the use of qPCR as compared with the serological method may have been caused by random distribution and the low concentration of cysts in the tissues (one cyst per 50 – 100 g of tissue) and by the small size of samples that could be examined in this way. The detection of the parasite's DNA without an antibody response (in three animals) may be the result of an acute stage of infection before an antibody response is induced, a decline in the antibody titre below the detection limit, or a congenital infection without an antibody response (Dubey et al. 1995 and EFSA 2013).

Our results are comparable with other previously published data. In the USA, antibodies were detected in 53.4% of goat kids (Dubey et al. 2011) and 27.1% of lambs (Dubey et al. 2008) under 12 months old whose meat was destined for human consumption. *T. gondii* has also been isolated from the heart muscle of animals in mouse bioassay. In the USA, the consumption of the meat of goat kids and lambs plays an important role in the diet of numerous ethnic minorities. The risk inherent in consuming goat meat has also been confirmed by a study in Brazil, in which antibodies were detected in 32.2% of goats largely under 12 months old, and virulent isolates of *T. gondii* were obtained from the tissues of seropositive animals (the brain, heart, diaphragm and masseter) (Ragozo et al. 2009). Lopes et al. (2013) detected antibodies to *T. gondii* in 16.0% of lambs and 9.3% of goat kids under 6 months old slaughtered for human consumption in Portugal. They recorded a higher level of seropositivity in older animals. An extensive study in France with traditional consumption of lamb meat estimates the prevalence of *T. gondii* at 17.7% in lamb meat and as much as 89% in sheep meat (Halos et al. 2010). The consumption of undercooked lamb meat was the cause of three cases of human toxoplasmosis in France (Ginsbourger et al. 2012). In the Czech Republic, Hejlíček and Literák (1994) isolated *T. gondii* from the tissues (brain, diaphragm) of 4.8% of sheep slaughtered at slaughterhouses.

Conclusions

Although the number of tested animals was not large, the results show that the prevalence of *T. gondii* in lambs and goat kids destined for human consumption may be relatively high. In spite of the fact that consumption of these types of meat is minimal in the Czech Republic, their consumption, particularly if undercooked, and the handling of raw meat may be a cause of *T. gondii* infection in humans.

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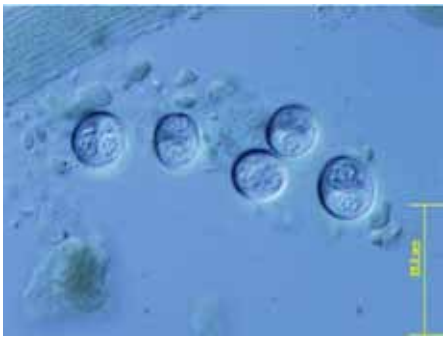

Fig. 1. Sporulated oocysts of *Toxoplasma gondii* in cat faeces (Koudela B)

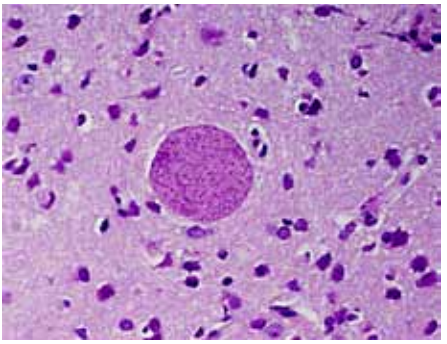

Fig. 2. A tissue cyst of *Toxoplasma gondii* in the brain of a mouse (Koudela B)

Fig. 3. A typical method of rearing sheep and goats in contact with the external environment increases the risk of *Toxoplasma* infection (Hanáková D)