Molecular investigation of sow abortions for the detection of
Toxoplasma gondii and Hammondia hammondi

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Abstract. Toxoplasma gondii and Hammondia hammondi are two related coccidian parasites, with cats as definitive hosts and warm-blooded animals as intermediate hosts. Pigs infected with T. gondii are considered an important source of infection for humans in several countries. The purpose of this study was to determine the frequency of T. gondii and H. hammondi in sow abortions. Between November 2008 and May 2010, tissue samples (heart and thoracic and abdominal fluid) were collected from 32 sow abortions. Samples were tested in the direction of detecting genomic DNA of T. gondii and H. hammondi, using primers that specifically amplify the ITS-1 region. No genomic DNA of T. gondii was detected by PCR in the tissue samples of the 32 sow abortion. Hammondia hammondi DNA was detected in one abortion tissue (prevalence 3.2%). According to this study, T. gondii isn’t an important pathogen in sow abortions from Romania.

Keywords: Toxoplasma gondii; Hammondia hammondi; PCR; Tissues; Sow abortions.

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Introduction

Toxoplasma gondii and Hammondia hammondi are closely related protozoan parasites which infect a broad spectrum of animals as intermediate hosts. Felids, including domestic cats, are the definitive hosts. T. gondii and H. hammondi complete the sexual part of their life cycle in the small intestine of felids, whereby oocysts are produced and passed in the feces. Oocysts shed into the environment are relatively resistant to harsh conditions (Dubey and Sreekumar, 2003). The distinction of T. gondii from H. hammondi oocysts is epidemiologically important, because T. gondii is a significant pathogen of humans and animals, whereas no disease has yet been associated with H. hammondi infection (Dubey and Sreekumar, 2003). Usually, T. gondii does not produce clinical signs, but the primary infection during pregnancy in women and in other animal species may result in abortions, fetal abnormalities or stillbirth (Cook et al., 2000).

Infected pork is a source of T. gondii infection for humans and animals in many countries. This parasite also causes mortality in pigs, especially in neonates. Most pigs acquire T. gondii infection after birth, by ingestion of
oocysts from contaminated environment or ingestion of infected tissues of infected animals. Few pigs become infected before birth, via transplacental transmission of the parasite. Raising swine indoors in confinement has greatly reduced *T. gondii* infection in pigs, but the recent trend of organic farming is likely to increase *T. gondii* infection in this species (Dubey, 2009). However, the importance of congenital *T. gondii* infection as a cause of reproductive disorders is better known in sheep (Hartley and Marshall, 1957) and goats (Munday and Mason, 1979) than in pigs.

*H. hammondi* has low virulence to mice; it is nonpathogenic for other hosts that have been tested (Christie and Dubey, 1977). While *T. gondii* is an important pathogen of humans and a broad range of other vertebrates, disease or abortions has not yet been associated with *H. hammondi* infection.

The aims of this study were to determine the prevalence of genomic DNA of *T. gondii* and *H. hammondi* in the tissues samples of sow abortions from different counties of Romania.

**Materials and methods**

**Samples**

We collected samples from 32 sow abortions from intensive farming system (8 from Mureş county and 11 from Bihor county) and from backyard pigs (13 from Cluj county). The foetuses were examined by necropsy and samples of heart (32 samples) and thoracic and abdominal fluid (11 samples), were collected. These samples were stored at -20°C until use.

**DNA extraction**

DNA extraction was performed with a Metabion, my-Tissue genomic DNA Isolation Kit. After thawing, 100 mg of each tissue were homogenized and suspended in 300 μl Cell Lysis Buffer and 20 μl protease K, mixed by vortexing followed by incubation at 55°C for 2 hours. After incubation, 3 μl of RNase A (10 mg/ml) were added, followed by incubation at 37°C for 30 minutes. After the second incubation 100 μl PPT Buffer was added, mixed by vortexing and centrifuged at 13,000 rpm for 10 minutes. Supernatant was transferred to a new tube and 600 μl of Column Binding Buffer were added, mixed by vortexing and transferred into spin column. The column was centrifuged at 13,000 rpm for 2 minutes. The spin column was transferred in a new collection tube and 750 μl Column Wash Buffer were added, followed by a new centrifugation and addition of another 250 μl Column Wash Buffer. Finally, the DNA was eluted from column by centrifugation with 100 μl of elution buffer TE. The DNA was stored at -20°C till using.

**Polymerase Chain Reaction**

We used the same PCR technique for both *T. gondii* and *H. hammondi* DNA identification. PCR was performed with *T. gondii* specific primers Tox4/Tox5 from the 18s gene from ribosome DNA, the internal space transcription 1 (ITS1) and Hham 34F/Hham 3R (Sreekum et al., 2005; Schares et al., 2008). Each reaction mixture of 25 μl contained 25 pM of each primer and 4 μl DNA. The amplification was performed in BIO DOC-It™ Imagine System. The cycling parameters for the amplification of *T. gondii* DNA consisted of an initial denaturation at 7 minutes at 94°C, followed by 35 cycles for 1 minute at 95°C for denaturation, 1 minute at 60°C for annealing and 1 minute at 72°C for extension; cycle 37 was followed by a final extension of 10 minutes at 72°C. The cycling parameters for the amplification of *H. hammondi* DNA consisted of 7 minutes at 94°C for denaturation in cycle one, followed by 35 cycles for 1 minute at 95°C for denaturation, 1 minute at 60°C for annealing, and 1 minute at 72°C for extension; cycle 37 was followed by a final extension of 10 minutes at 72°C. Aliquots of each PCR products were electrophoresed on 2% agarose gel and observed for the presence of the specific fragment (529 bp for *T. gondii* and 283 for *H. hammondi*) under UV light.

**Results**

The abortion appeared in the first (21 sow abortions) and second (11 sow abortions) gestation periods. All tissue samples (heart, thoracic and abdominal fluid) of 32 sow abortions were negative for *T. gondii*. One foetus was positive for *H. hammondi*, and only
the heart tissue (figure 1). Unexpectedly the foetus came from an intensive raising farm (Bihor county). The prevalence of infection with *H. hammondii* was 3.2% (1/32).

**Figure 1.** PCR products amplified with primer pair Hham 34F/Hham 3R migration into agarose gel 2%, from heart tissue of sow abortions. Lane 1, 1 kb DNA ladder; lanes 2-13, PCR negative; lane 13, PCR positive

**Discussions**

We didn't identify *T. gondii* DNA in tissues from sow abortions, only *H. hammondii* DNA in one sample 3.2% (1/32). Abortions were probably caused by mechanical factors as well as viral or bacterial pathogens.

Most *T. gondii* infections in pigs are subclinical (Dubey, 1986), and transplacental infections are less common than post-natal infections (Dubey, 1986). Although abortions related to *T. gondii* are uncommon, they may occur in sows infected during pregnancy (Dubey, 1986). Pigs infected transplacentally may be born premature, dead, or weak, or they may die soon after birth (Lindsay et al., 1999). In a study from Korea, higher abortion rates, up to 44%, were observed and unusually high sow mortality rates, up to 19%, were primarily associated with toxoplasmosis (Christie and Dubey, 1977).

Although clinical toxoplasmosis has been reported in young piglets, little was known of abortions associated with *T. gondii* and rates of congenital infection in pigs (Dubey, 1986). Epidemiologically, porcine toxoplasmosis has been classified into sporadic neonatal, postnatal, and epizootic infection (Dubey, 1986). Sporadic fatal toxoplasmosis in piglets has been reported in several countries, including the USA, Japan, and Korea (Kim et al., 2009). Although many infected piglets were born dead or sick, or became sick within 3 months of birth, others remained clinically normal.

Studies aiming to clarify the sources of pig infection for *T. gondii* have suggested that ingestion of oocysts in contaminated feed, water, soil, and living animals were the main sources of infection (Dubey, 1986; Lehmann et al., 2003). In some countries, eating infected rodents has been regarded as a source of infection (Lubroth et al., 1983). Cannibalism has been experimentally shown to be another possible route of infection (Dubey, 1986). However, most studies have suggested that oocysts shed by cats are the most common source. Cats may excrete millions of oocysts after ingesting only one bradyzoite or one tissue cyst, and many tissue cysts may be present in one infected mouse (Dubey, 2001; Hill and Dubey, 2002). Although oocysts are shed only for a short period (1-2 weeks) in the life of a cat, the enormous numbers shed assure widespread contamination of the environment (Hill and Dubey, 2002).

*H. hammondii* share many hosts. *H. hammondii* has not been associated with any disease manifestation in any of these hosts. From 7 to 10 days after oocysts of *H. hammondii* are ingested by intermediate hosts, tachyzoites multiply in the intestinal lamina propria, muscles, and Peyer’s patches, as well as mesenteric lymph nodes, causing necrosis of the infected cells. During the second week of infection, cysts appear in other tissues, primarily in skeletal muscle (Dubey and Streitel, 1976; Frenkel and Dubey, 1975).

**References**


