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## Pork as a source of transmission of *Toxoplasma gondii* to humans: a parasite burden study in pig tissues after infection with different strains of *Toxoplasma gondii* as a function of time and different parasite stages

Ignacio Gisbert Algaba<sup>a,\*</sup>, Bavo Verhaegen<sup>a</sup>, Malgorzata Jennes<sup>b</sup>, Mizanur Rahman<sup>b</sup>, Wim Coucke<sup>c</sup>, Eric Cox<sup>b</sup>, Pierre Dorny<sup>b,d</sup>, Katelijne Dierick<sup>a</sup>, Stéphane De Craeye<sup>a</sup>

<sup>a</sup> Scientific Institute of Public Health, Communicable and Infectious Diseases, National Reference Center for Toxoplasmosis, 1050 Brussels, Belgium

<sup>b</sup> Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium

<sup>c</sup> Scientific Institute of Public Health, Quality of Medical Laboratories, 1050 Brussels, Belgium

<sup>d</sup> Department of Biomedical Sciences, Institute of Tropical Medicine, 2000 Antwerp, Belgium

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## ABSTRACT

*Toxoplasma gondii* is an ubiquitous apicomplexan parasite which can infect any warm-blooded animal including humans. Humans and carnivores/omnivores can also become infected by consumption of raw or undercooked infected meat containing muscle cysts. This route of transmission is considered to account for at least 30% of human toxoplasmosis cases. To better assess the role of pork as a source of infection for humans, the parasite burden resulting from experimental infection with different parasite stages and different strains of *T. gondii* during the acute and chronic phases was studied. The parasite burden in different tissues was measured with a ISO 17025 validated Magnetic Capture-quantitative PCR. A high burden of infection was found in heart and lungs during the acute phase of infection and heart and brain were identified as the most parasitised tissues during the chronic phase of infection, independent of the parasite stage and the strain used. Remarkably, a higher parasite burden was measured in different tissues following infection with oocysts of a type II strain compared with a tissue cyst infection with three strains of either type II or a type I/II. However, these results could have been affected by the use of different strains and euthanasia time points. The parasite burden resulting from a tissue cyst infection was not significantly different between the two strains.

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## 1. Introduction

*Toxoplasma gondii* is an obligate intracellular cyst-forming coccidian parasite. Being prevalent worldwide and zoonotic, *T. gondii* is considered one of the most successful parasites (Halonen and Weiss, 2013). It has a complex life cycle; it can infect virtually any warm-blooded animal (intermediate host), while the sexual replication only takes place in the gastrointestinal tract of the definitive hosts, which are domestic and wild Felidae (Tenter et al., 2000).

The life cycle in the intermediate host, including humans, is characterised by an acute phase involving fast asexual intracellular replication of tachyzoites in almost all tissues, followed by a

chronic phase involving the development of tissue cysts containing bradyzoites, mainly in the central nervous system and in skeletal muscles, which may persist lifelong (Dubey, 2010). Different infection routes have been demonstrated: (i) consumption of meat from a chronically infected animal; (ii) ingestion of sporulated oocysts (resulting from the sexual replication in felids) via contaminated water, soil or vegetables; (iii) vertical transmission through the placenta (Opsteegh, 2016).

The consumption of raw or undercooked *T. gondii*-infected meat is considered a main route of transmission for humans (Cook et al., 2000); in developed countries 50% of the infections are estimated to be meatborne (Scallan et al., 2011) and pork is considered to account for 41% of foodborne human toxoplasmosis cases in the USA (Batz et al., 2012).

The presence of anti-*Toxoplasma* antibodies and direct detection of the parasite in different tissues have been widely used in different animal species for the demonstration of a *T. gondii* infection (Tenter et al., 2000; Dubey, 2010). The development of a Magnetic

\* Corresponding author at: National Reference Laboratory for Toxoplasma, Scientific Service Food-borne Pathogens, Scientific Institute of Public Health (WIV-ISP), Rue Juliette, Wytsmanstraat 14, 1050 Brussels, Belgium. Fax: +32 02 642 5696.

E-mail address: [igisbertalgaba@wiv-isp.be](mailto:igisbertalgaba@wiv-isp.be) (I. Gisbert Algaba).

Capture-quantitative PCR (MC-qPCR) enables reliable parasite quantification in contrast to the qualitative results obtained with bioassays and classic PCR. As such, the MC-qPCR allows study of the quantitative distribution of the parasite through the carcasses of different animal species (Opsteegh et al., 2010; Juránková et al., 2013; Aroussi et al., 2015; Hosein et al., 2016), and the assessment of the risk of infection for the consumer of the different animal tissues. In pigs, the parasite burden has been studied following experimental infection with either oocysts (Opsteegh et al., 2010; Juránková et al., 2013) or tissue cysts (Verhelst et al., 2015; Jennes et al., 2017), but the potential effect of different parasite stages on the parasite burden is not known. In addition, parasitic load has been shown to be strain-dependent with a more pronounced clearance of the parasite in some tissues when using a hybrid Type I/II strain (*T. gondii* ISP-Gangji) than using a classical type II strain (Verhelst et al., 2015; Jennes et al., 2017).

The aim of this study is to use the recently upgraded and ISO 17025 validated MC-qPCR (Gisbert Algaba et al., 2017) to study the potential effect of different parasite stages (oocysts and tissue cysts) and different strains (Type II and Type I/II strains) on the parasite burden in pigs during acute and chronic infections. By comparing the parasitic loads of different tissues under different conditions, the role of pork as one of the main sources of infection for humans can be better assessed.

## 2. Materials and methods

### 2.1. *Toxoplasma gondii* strains

In order to study the most prevalent *T. gondii* genotype in Europe and North America (Type II), the following strains were used: *T. gondii* IPB-LR (tissue cysts) (type II, low virulence in mice), *T. gondii* IPB-Gangji (tissue cysts) (Type II/I, a rare hybrid strain, virulent in mice) and *T. gondii* Tg-SweF2 (oocysts) (type II, low virulence in mice). The strains were genotyped by the Center of Biological Resources (Limoges, France) as described by Ajzenberg et al. (2010).

#### 2.1.1. Preparation of tissue cysts

Swiss white outbred mice (Janvier Labs, Rennes, France) were inoculated i.p. with *T. gondii* IPB-LR (type II) or IPB-Gangji (Type II/I) strains. After 6 weeks of incubation, the mice were euthanised and their brains were collected and homogenised in PBS supplemented with penicillin and streptomycin using a Potter homogeniser. The concentration of tissue cysts was determined three times by counting a volume of 8 µl with a phase contrast microscope.

#### 2.1.2. Isolation, purification and preparation of oocysts

In order to perform an experimental infection with oocysts, feces from a cat naturally infected with the *T. gondii* Tg-SweF2 strain were obtained from the National Veterinary Institute of Sweden (SVA, Uppsala, Sweden). The oocysts were first isolated with sucrose flotation followed by a Caesium Chlorine gradient as described by Staggs et al. (2009). Briefly, the feces were homogenised with MilliQ water and 0.2% Tween<sup>®</sup>20, the suspension filtered through gauze and centrifuged. Subsequently, the pellet was resuspended in a sucrose solution (1.15 g/ml) and centrifuged at 800g for 10 min. The supernatant containing the oocysts was carefully transferred to a new 50 ml polypropylene tube and the sucrose washed away. Subsequently, the oocysts were aerated in 2% H<sub>2</sub>SO<sub>4</sub> at 22 °C for 7 days to allow sporulation. Once sporulated, a Caesium Chlorine gradient was applied and the oocyst suspension was stored in 2% H<sub>2</sub>SO<sub>4</sub> at 4 °C until further use.

The purified oocyst suspension was quantified using a Bürker counting chamber and a phase contrast microscope, and diluted

accordingly to obtain a final concentration of 10<sup>5</sup> sporulated oocysts per 5 ml in PBS, supplemented with penicillin/streptomycin.

### 2.2. Experimental infections in pigs

#### 2.2.1. Acute phase

To study parasite distribution and load in the acute phase, 36 three-week old *T. gondii* seronegative piglets (confirmed by the modified agglutination test (MAT, ToxoScreen DA, Biomérieux, Capronne, France) and an in-house immunofluorescence test (IFT, based on Toxo-Spot IF, Biomérieux) (Verhelst et al., 2015)) were divided into three groups according to the following experimental setup: group A1, 15 animals orally infected with 6000 tissue cysts of the *T. gondii* IPB-LR strain; group A2, 15 animals orally infected with 6000 tissue cysts of the *T. gondii* IPB-Gangji strain; group A3, six negative control animals.

Three animals from each experimental group (groups 1 and 2) were euthanased after 2, 4, 8, 14 and 28 days p.i.. The piglets were serologically monitored and the heart (Ha) and lungs (Lu) collected and tested with MC-qPCR. The animals were observed daily and a humane endpoint was defined to limit the suffering of the animals in case of disease in accordance with European and Belgian legislation (Ethics Committee licence no 2015/102, Ghent university, Belgium).

#### 2.2.2. Chronic phase

To study the chronic phase, 13 three-week-old *T. gondii* seronegative piglets (confirmed by two different serological methods: MAT and IFT (Verhelst et al., 2015)) were divided into four groups according to the following experimental setup: group C1, two negative control animals; group C2, three animals orally infected with 6000 tissue cysts of the *T. gondii* IPB-LR strain; group C3, four animals orally infected with 6000 tissue cysts of the *T. gondii* IPB-Gangji strain; group C4, three animals orally infected with 10<sup>5</sup> oocysts of the *T. gondii* Tg-SweF2 strain. One hundred thousand oocysts (10<sup>5</sup> × 8 sporozoites) and 6000 tissue cysts (6000 × >100 bradyzoites) were inoculated in order to infect pigs with a similar number of parasites.

The piglets were serologically monitored weekly from day 0 until euthanasia to confirm an established infection in the positive animals and to monitor the absence of infection in group C1. The pigs were euthanised 90 days p.i. in group C4 and between 130 and 182 days p.i. in groups C2 and C3. The following tissues were collected: Ha, Lu, muscle (m.) gastrocnemius (Ga), m. psoas major (PM), m. longissimus dorsi (LD), diaphragm (DI), m. intercostales (IC) and brain (BR).

The collected tissue samples (except the brain tissue) were first cleaned by removing fats and connective tissues, and then cut into small pieces of 1 cm<sup>3</sup>.

An overview of all the *Toxoplasma gondii* experimental infections is shown in Table 1.

### 2.3. MC-qPCR

In order to determine the parasitic load, the MC-qPCR was performed as described by Gisbert Algaba et al. (2017). In brief, the meat samples were homogenised in the presence of lysis buffer (100 mM Tris HCl, 5 mM EDTA, 0.2% SDS, 200 mM NaCl, 40 mg/l of proteinase k (30 mAnson-U/mg; Amresco, Ohio, USA), pH = 8.0 ± 0.2) with a pedal homogenator (Labconsult, Brussels, Belgium) and lysed overnight at 55 °C. Fats and cell debris were then removed by centrifugation and the free biotin possibly present in the crude extract was removed by adding streptavidin-coated agarose beads (binding capacity >330 nmol/ml; Solulink, San Diego, USA).

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