International Journal for Parasitology 48 (2018) 555–560

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

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 [a,*], Bavo Verhaegen [a], Malgorzata Jennes [b], Mizanur Rahman [b], Wim Coucke [c], Eric Cox [b], Pierre Dorny [b,d], Katelijne Dierick [a], Stéphane De Craeye [a]

[a] Scientific Institute of Public Health, Communicable and Infectious Diseases, National Reference Center for Toxoplasmosis, 1050 Brussels, Belgium
[b] Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium
[c] Scientific Institute of Public Health, Quality of Medical Laboratories, 1050 Brussels, Belgium
[d] Department of Biomedical Sciences, Institute of Tropical Medicine, 2000 Antwerp, Belgium

ARTICLE INFO

Article history:
Received 20 October 2017
Received in revised form 14 December 2017
Accepted 19 December 2017
Available online 3 April 2018

Keywords:
Toxoplasma gondii
Meat
Magnetic capture
Quantitative PCR
Pig
Parasitic load

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.

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 (I. Gisbert Algaba).

https://doi.org/10.1016/j.ijpara.2017.12.009
0020-7519/

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-Gangii (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®20, the suspension filtered through gauze and centrifuged. Subsequently, the pellet was resuspended in a sucrose solution (1.15 g/ml) and centrifuged at 800*g* 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_2SO_4 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_2SO_4 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 10^5 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-Gangii 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-Gangii strain; group C4, three animals orally infected with 10^5 oocysts of the *T. gondii Tg-SweF2* strain. One hundred thousand oocysts ($10^5 \times 8$ 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^3.

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).

Table 1
Overview of the different experimental animal groups infected with *Toxoplasma gondii*.

Phase	Group	N° of pigs	Challenge	Parasite stage	Strain type	Euthanasia (days p.i.)
Acute	A1	15	6000 tissue cysts	Bradyzoites	LR (Type II)	2,4,8,14,28
	A2	15	6000 tissue cysts	Bradyzoites	Gangji (Type II/I)	2,4,8,14,28
	A3	6	na	na	Control group	35
Chronic	C1	2	na	na	Control group	130
	C2	3	6000 tissue cysts	Bradyzoites	LR (Type II)	130-182
	C3	4	6000 tissue cysts	Bradyzoites	Gangji (Type II/I)	137-182
	C4	3	10^5 oocysts	Sporozoites	*Tg Swe2* (TypeII)	90

na, not applicable.

The specific biotin labelled probes against the *T. gondii 529 bp RE* Pubmed accession number: AF146527 and cellular 18S rDNA were added to the biotin-free lysates and the samples were first denatured at 95 °C, followed by an incubation at room temperature in order to hybridise the probes with the complementary target sequences. After hybridisation, the biotin-labelled probes bound to the target DNA were captured using streptavidin magnetic beads (binding capacity >2.5 nmol/mg (Solulink)) and after washing and re-suspension in elution buffer, the target DNA was released from the beads by exposure to UV light.

Finally, the qPCR was performed on the final DNA extract to detect the presence of *T. gondii* DNA and the results analysed using the BioRad (USA) CFX manager software to obtain the crossing point (Cp) values.

2.4. Statistical analysis

All the samples with an exponential-amplification curve crossing the threshold (Cp) were considered positive for *T. gondii*, samples with no amplification curve for the *T. gondii* target but amplification of the NCIAC (Not Competitive Internal Amplification Control) were considered negative. As described in Gisbert Algaba et al. (2017), the limit of detection of the method is 65.4 parasites per 100 g of tissue. For each round of samples, a positive control with a known number of parasites (calibrator) was used to correct for possible deviations due to manipulation errors. The number of parasites (n° p) was calculated according to the following formula:

$$log_{10}(n^{\circ}p) = \frac{Cp_{value} - 44.75}{-3.0788}$$

The formula resulted from a standard curve established with known concentrations of parasites ranging from 100 to 10^5 spiked in 100 g of tissue.

A linear model was fitted to look for the differences between the groups to be compared after a log10 transformation of the parasitic load. Restricted maximum likelihood estimates were calculated, taking into account the non-detected, which were modelled as '<log(LOQ)'. Model estimates and their variance–covariance matrix were used to set up contrasts to compare infection types per tissue, tissues per parasite stage types and days. *P* values for each contrast were corrected in accordance with the Šidák statistical method in order to set the global type I error rate at 0.05 (Hsu, Jason, 1996). The normal distribution of the residuals was assessed by means of a normal quantile plot. All data was analysed using S-Plus 8 for Linux (http://doi.org/10.17632/bk47m9hksm.2).

3. Results

3.1. Acute phase

All the animals in groups A1 and A2 seroconverted and showed some mild clinical signs such as fever, apathy and anorexia during the first week after infection.

Regarding the comparison between the two strains used (LR and Ganji) at the level of parasitic loads during the acute phase of infection, statistical analyses could not be performed due the number of negative values. The results of the acute phase are presented in Figs. 1 and 2, for the lungs and hearts, respectively.

3.1.1. Lungs

During the acute phase of infection, at day 2 p.i., *T. gondii* was already present in the lungs of two out of three animals of both

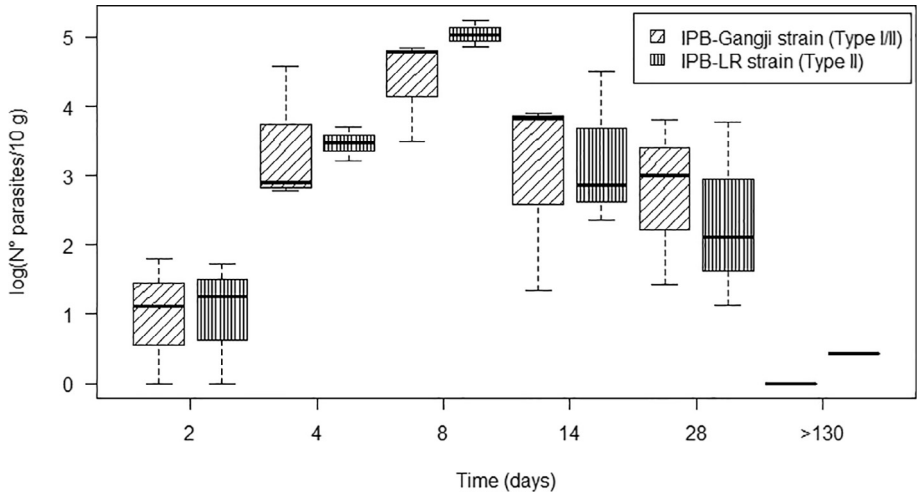

Fig. 1. Box and whisker plots for log (N° parasites/10 g) in lungs as a function of days p.i. Plots with a vertical pattern represent the results of animals infected with the IPB-LR *Toxoplasma gondii* strain (Type II) and plots with a diagonal pattern the results of animals infected with the IPB-Gangji *T. gondii* strain (Type I/II).

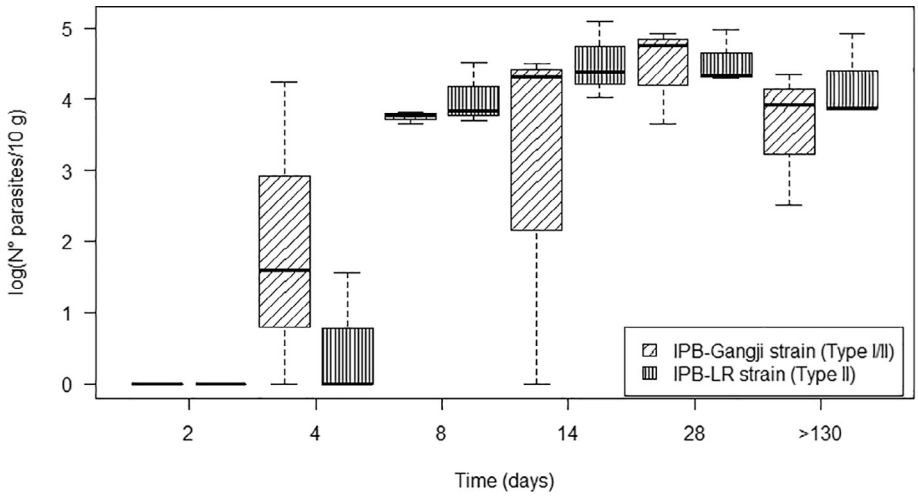

Fig. 2. Box and whisker plots for log (N° parasites/10 g) in hearts as a function of days p.i. Plots with a vertical pattern represent the results of animals infected with the IPB-LR *Toxoplasma gondii* strain (Type II) and plots with a diagonal pattern the results of animals infected with the IPB-Gangji *T. gondii* strain (Type I/II).

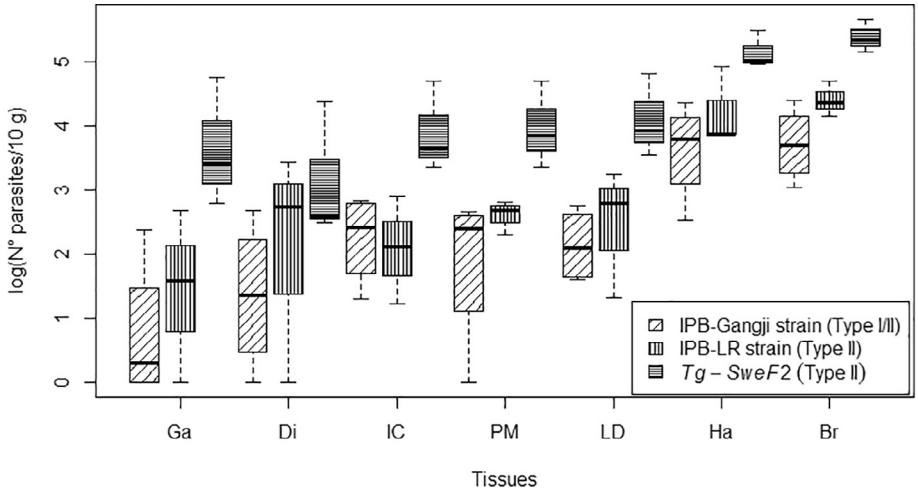

Fig. 3. Box and whisker plots for log (N° parasites/10 g) in different tissues. The vertical line pattern in plots is used for the results of animals infected with tissue cysts of the IPB-LR *Toxoplasma gondii* strain (Type II), the diagonal line pattern in plots is used for the results of animals infected with tissue cysts of the IPB-Gangji *T. gondii* strain (Type I/II) and the horizontal line pattern in plots is used for the results of animals infected with oocysts of the *Tg-SweF2 T. gondii* strain (Type II). Ga, muscle (m.) gastrocnemius; Di, diaphragm; IC, intercostal muscles; PM, m. psoas major; LD, m. longissimus dorsi; Ha, heart; Br, brain.

infected groups (A1 and A2). At 8 days p.i. the concentration of parasites reached a maximum and the parasite was found in all the tested animals for both groups (A1 and A2) while the parasite burden decreased slowly during the following days (14 and 28) (Fig. 1).

3.1.2. Heart

In the heart, which has been previously described as the predilection tissue of the parasite in pigs (Opsteegh et al., 2010), the parasite was not found in any of the animals of the experimental groups (A1 and A2) at 2 days p.i. and only in one animal of the A1 group (A1:1/3) and two of the A2 group (A2: 2/3) at 4 days p.i. In contrast to what was observed in the lungs, in hearts the median parasite burden remained stable after 8 days p.i. ($P > 0.1$) (Fig. 2).

3.2. Chronic phase

All the animals in groups C2, C3 and C4 seroconverted and showed some mild clinical signs such as fever, apathy and anorexia during the first week after infection.

3.2.1. Infection with tissue cysts

3.2.1.1. Type II strain (T. gondii IPB-LR). During the chronic phase, in the animals infected with tissue cysts of a classical Type II strain (group C2), the parasite was found in all the tested tissues in only one of the animals. In the other two pigs of this group (pig 1 and pig 3), the parasite was found in all the tissues of the animals except for the m. gastrocnemius in pig 3 and the diaphragm in pig 1 (Fig. 3, Supplementary Table S1).

The parasite burden was significantly higher in the heart compared with the M. gastrocnemius diaphragm and intercostal muscles ($P < 0.05$), but not compared with the M. psoas major and M. longissimus dorsi. The highest *T. gondii* concentration was found in the brain, being significantly higher ($P < 0.05$) compared to m. gastrocnemius, diaphragm, intercostal muscles and m. longissimus dorsi (Fig. 3, Supplementary Table S1). When comparing heart and brain, no significant difference was found between these two tissues.

3.2.1.2. Type I/II strain (T. gondii IPB-Gangii). The median parasite burden in the animals infected with a hybrid Type I/II strain (group

C3) was not significantly different from those infected with the type II strain, although they were consistently lower in all the tissues except for the Ic muscle. When comparing the different tissues of the Type I/II strain-infected animals, the parasite burden was significantly higher in the brain and the heart ($P < 0.05$) compared with PM, Ga and Di. The highest parasite burden was found in the heart and not in the brain as it was found in the *T. gondii* IPB-LR experimentally infected group (Fig. 3, Supplementary Table S1).

3.2.2. Infection with oocysts

In the pigs infected with oocysts (group C4), the parasite was found in all the tissues of all the animals after 90 days p.i. Additionally, the higher parasitic loads were found in heart and brain as in the other two experimental groups (Groups C2 and C3) (Fig. 3, Supplementary Table S1).

3.3. Evolution of the parasite burden in heart and lungs during the acute and chronic phases of infection

When comparing the results during the acute and the chronic phases of infection (A1 and A2 compared with C2 and C3), in the lungs the parasite burden at day 182 p.i. (chronic phase of infection, (C2 and C3)), was significantly lower ($P < 0.05$) compared with day 8 (acute phase of infection (A1 and A2)), confirming the decreasing trend of the parasite burden in lungs in function of time observed during the acute phase. In contrast, in the hearts, the parasite burden measured during the acute phase remained stable after 182 days p.i. in the chronic phase of infection.

4. Discussion

Pork is considered among the most important sources of *T. gondii* infection in humans (Batz et al., 2012). Although several studies have demonstrated the presence of the parasites in different tissues (Dubey, 2010; Opsteegh et al., 2010; Jurànková et al., 2013), only a few studies have quantified the parasitic burden in different tissues (Opsteegh et al., 2010; Jurànková et al., 2013).

In this study, to our knowledge for the first time, the parasitic load in the heart and the lungs was studied during the acute phase of infection following infection with tissue cysts. The heart and the lungs were selected for this study as a high parasite burden has been consistently described in the mouse and the pig models for these two tissues (Dubey, 2010; Dubey et al., 2012). The parasite was found earlier in the lungs than in the heart (2 days p.i.), but the parasitic load in this tissue decreased over time after reaching a maximum at day 8 p.i. Moreover, the lungs tested during the chronic phase of infection (182 days p.i.) showed very low or even no parasite presence in contrast to what was described by Jurànková et al. (2013) where lungs were found as the tissue with the second highest parasite burden. This difference can be explained by the earlier euthanasia of the infected animals at day 76 after infection.

On the other hand, in the heart tissue the presence of the parasite was demonstrated only from day 4 after inoculation. Remarkably, no significant variation in parasite burden was found from day 8 till day 28 p.i. When compared with the chronic phase, no significant difference was found and the parasite burden remained stable in the heart until day 182 p.i. These results confirm the importance of the heart in demonstrating the actual presence of the parasite in seropositive pigs. Although there was a trend of lower parasite burdens in pigs infected with the type I/II strain compared with the type II strain, this difference was not significant.

Considering the chronic phase of infection, the results of the present study are in agreement with previous studies identifying brain as the most infected tissue when the pigs are infected with oocysts (Opsteegh et al., 2010; Jurànková et al., 2013). Overall, from all the tested tissues, heart and brain showed the highest parasitic loads and no significant difference was found between both tissues in any of the experimental groups. These results contrast with those obtained by Jurànková et al. (2013) where the parasitic load in heart tissues was significantly lower than in the brain. The difference could be explained by the lower dose used in their experimental infections (5000 oocysts). A dose effect has been previously described in mice and pigs, showing a sigmoidal relationship of the probability of infection as a function of the infective dose (AFSSA, 2005). However, those studies do not give much information of the quantitative parasite burden in tissues after infection with different infective doses. A recent study used a quantitative analysis of the parasite burden in pig tissues as a function of the infective dose employed. Strikingly, a lower parasite burden was shown in tissues after infection with a high dose of tissue cysts from the Gangji strain than with a lower dose, while no effect was observed between high and low doses of tissue cysts from the LR strain (Jennes et al., 2017). However, these studies were performed using less sensitive and less accurate quantitative techniques.

When comparing the parasitic load resulting from infection with tissue cysts, no significant difference was observed between the two strains used *T. gondii* IPB-LR strain (type II) and *T. gondii* IPB-Gangji (type I/II). However, although not significant, a higher parasitic load was observed in all the tissues except for intercostal muscles after infection with the classic type II strain compared with the hybrid type I/II strain. These results are in agreement with previous studies using less sensitive techniques (Verhelst et al., 2015; Jennes et al., 2017).

Dose–response studies performed in different animal models have been recently adapted to human *T. gondii* infections, showing a clear effect of the parasite burden present in the tissues on the probability of consumers acquiring the infection (AFSSA, 2005; Guo et al., 2016). These results highlight the importance of accurate *T. gondii* quantitative data in the different tissues, since animals carrying a higher parasite burden in their tissues will represent a higher risk for the consumer than those with lower parasite loads. In this study, we showed a higher parasite load in all the tissues examined compared with previous studies (Opsteegh et al., 2010; Jurànková et al., 2013), which can be explained by the higher sensitivity of the MC-qPCR and the infective dose used. Additionally, the pigs infected with oocysts (Group C4) had a significantly higher parasitic load in m. psoas major, m. gastrocnemius and intercostal muscles compared with the pigs infected with tissue cysts (Groups C2 and C3). In general, the overall parasite load in all the tissues was higher for animals infected with oocysts. These findings suggest a potential effect of the parasite form transmitted in the parasite burden at slaughter age. However, special attention needs to be paid when analysing these results since the use of a different strain (*Tg-SweF2*) and the earlier euthanasia of the pigs infected with oocysts might have also influenced the obtained parasite loads. If confirmed, these findings indicate a higher parasite burden in pigs infected with oocysts than with tissue cysts, in contrast to what happens in the definitive host (Dubey, 2010, Dubey et al., 2012).

In conclusion, our results demonstrate the presence of *T. gondii* in the different tissues of pigs (intermediate hosts) and confirm that heart and brain are the predilection tissues for the direct detection of the parasite during the chronic phase of infection, independent of the transmission form and strain. During the acute phase of infection, the lungs are highly infected and they may therefore be used to demonstrate a recent infection. Overall, all the studied tissues showed a potential presence of the parasite at slaughter age, posing a risk of infection for humans and other inter-

mediate hosts consuming these tissues. Additionally, the potential role of the parasite stage on the parasite burden is suggested. However, in order to confirm these findings further experimental infections are needed using the different parasite stages originating from the same *T. gondii* strain. Also, studies on the parasite burden of naturally infected animals need to be addressed to confirm the parasite burden in pigs exposed to different doses of infection.

Acknowledgements

This study was funded by a grant from the Belgian Federal Public Service for Health, Food Chain Safety and Environment (grant RF 13/6274). The authors wish to thank colleagues of the Scientific Institute of Public Health (WIV-ISP), Belgium for their technical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijpara.2017.12.009.

References

AFSSA, 2005. Toxoplasmose : état des connaissances et évaluation du risque lié à l'alimentation. Agence Française de Sécurité Sanitaire des Aliments. https://www.anses.fr/fr/system/files/MIC-Ra-Toxoplasmose.pdf

Ajzenberg, D., Collinet, F., Mercier, A., Vignoles, P., Dardé, M.-L., 2010. Genotyping of *Toxoplasma gondii* isolates with 15 microsatellite markers in a single multiplex PCR assay. J. Clin. Microbiol. 48, 4641–4645.

Aroussi, A., Vignoles, P., Dalmay, F., Wimel, L., Dardé, M.L., Mercier, A., Ajzenberg, D., 2015. Detection of *Toxoplasma gondii* DNA in horse meat from supermarkets in France and performance evaluation of two serological tests. Parasite 22, 14.

Batz, M.B., Hoffmann, S., Morris Jr., J.G., 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. J. Food Prot. 75, 1278–1291.

Cook, A.J.C., Gilbert, R.E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P.A., Dunn, D.T., 2000. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. BMJ 321, 142–147.

Dubey, J.P., 2010. Toxoplasmosis of Animals and Humans. CRC Press. Taylor & Francis Group, US.

Dubey, J.P., Ferreira, L.R., Martins, J., Mcleod, R., 2012. Oral oocyst-induced mouse model of toxoplasmosis: effect of infection with *Toxoplasma gondii* strains of different genotypes, dose, and mouse strains (transgenic, out-bred, in-bred) on pathogenesis and mortality. Parasitology 139, 1–13.

Gisbert, Algaba I., Geerts, M., Jennes, M., Coucke, W., Opsteegh, M., Cox, E., Dorny, P., Dierick, K., De Craeye, S., 2017. A more sensitive, efficient and ISO 17025 validated Magnetic Capture real time PCR method for the detection of archetypal *Toxoplasma gondii* strains in meat. Int. J. Parasitol. 47, 875–884.

Guo, M., Abhinav, M., Buchanan, R.L., Dubey, J.P., Hill, D.E., Gamble, H.R., Jones, J.L., Du, X., Pradhan, A.K., 2016. Development of dose-response models to predict the relationship for human *Toxoplasma gondii* infection associated with meat consumption. Risk Analysis 36, 926–938.

Halonen, S.K., Weiss, L.M., 2013. Toxoplasmosis. Handb. Clin. Neurol. 114, 125–145.

Hosein, S., Limon, G., Dadios, N., Guitian, J., Blake, D.P., 2016. *Toxoplasma gondii* detection in cattle: A slaughterhouse survey. Vet. Parasitol. 228, 126–129.

Hsu, J., 1996. Multiple Comparisons: Theory and Methods. CRC Press. Chapman & Hall/CRC, US.

Jennes, M., De Craeye, S., Devriendt, B., Dierick, K., Dorny, P., Cox, E., 2017. Strain- and dose-dependent reduction of *Toxoplasma gondii* burden in pigs is associated with interferon-gamma production by CD8+ lymphocytes in a heterologous challenge model. Front. Cell. Infect. Microbiol. 7, 232.

Juránková, J., Basso, W., Neumayerová, H., Baláž, V., Jánová, E., Sidler, X., Deplazes, P., Koudela, B., 2013. Brain is the predilection site of *Toxoplasma gondii* in experimentally inoculated pigs as revealed by magnetic capture and real-time PCR. Food Microbiol. 32, 167–170.

Opsteegh, M., Langelaar, M., Sprong, H., den Hartog, L., De Craeye, S., Bokken, G., Ajzenberg, D., Kijlstra, A., van der Giessen, J., 2010. Direct detection and genotyping of *Toxoplasma gondii* in meat samples using magnetic capture and PCR. Int. J. Food Microbiol. 139, 193–201.

Opsteegh M., Schares G., Blaga R and van der Giessen J.,, 2016. Experimental studies of Toxoplasma gondii in the main livestock species (GP/EFSA/BIOHAZ/2013/01). Final report. 2016:EN-995.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States–major pathogens. Emerg. Infect. Dis. 17, 7–15.

Staggs, S.E., See, M.J., Dubey, J.P., Villegas, E.N., 2009. Obtaining highly purified *Toxoplasma gondii* oocysts by a discontinuous cesium chloride gradient. JoVE 33, 1420.

Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30, 1217–1258.

Verhelst, D., De, C.S., Jennes, M., Dorny, P., Goddeeris, B., Cox, E., 2015. Interferon-gamma expression and infectivity of *Toxoplasma* infected tissues in experimentally infected sheep in comparison with pigs. Vet. Parasitol. 207, 7–16.