Parasitology Research (2019) 118:1331–1335
https://doi.org/10.1007/s00436-019-06229-6

PROTOZOOLOGY - SHORT COMMUNICATION

# Isolation of viable *Toxoplasma gondii* from organs and Brazilian commercial meat cuts of experimentally infected pigs

Bruna Farias Alves[1] · Solange Oliveira[1] · Herbert Sousa Soares[1] · Hilda Fátima Jesus Pena[1] · Carlos Adam Conte-Junior[2] · Solange Maria Gennari[1]

Received: 24 August 2018 / Accepted: 24 January 2019 / Published online: 19 February 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

## Abstract

The present study evaluated the distribution and viability of *Toxoplasma gondii* tissue cysts in the organs and Brazilian commercial cuts of experimentally infected pigs. The pigs were infected with $3 \times 10^3$ oocysts of the *T. gondii* isolate TgCkBr57 (Type BrII). Mouse bioassays were performed on the brain, retina, tongue, diaphragm, and heart as well as the following muscle cuts: loin (*longissimus*), coppa (*longissimus*, *spinalis dorsi*, *rhomboideus*), tenderloin (*psoas major*), outside flat (*biceps femoris*), topside (*semimembranosus*), and top sirloin (*gluteus medius*). *Toxoplasma gondii* was isolated from the coppa, heart, diaphragm, and tongue of three pigs; from the tenderloin, outside flat, and brain of two pigs; and from the top sirloin and loin of one pig. Thus, the viability of *T. gondii* cysts was observed in all of the organs and cuts evaluated (except for the topside and retina), demonstrating the broad distribution of this parasite in pig organs and commercial meat cuts, and the importance of this species as a source of human infection.

**Keywords** Bioassay in mice · Pork · Swine · Toxoplasmosis · Viability · Zoonosis

*Toxoplasma gondii* is one of the most successful parasites in the world; it is able to infect warm-blooded animals (mammals and birds), including humans. Approximately, one third of the human population has been exposed to this parasite. *Toxoplasma gondii* sexual reproduction occurs in the gut of felids, which excrete the oocysts in their feces. In the environment, these oocysts sporulate and become infectious. After the ingestion of oocysts by an intermediate host, microscopic tissue cysts form in the organs and muscles. Thus, the ingestion of raw or undercooked meat is one of the main sources of human infection by *T. gondii*, and it is responsible for most human cases of toxoplasmosis (Dubey 2010).

Pigs are the most efficient production animal reservoirs of *T. gondii* cysts (Dubey 2009). Epidemiological studies have shown a high seroprevalence of this parasite in pigs around the world (Steinparzer et al. 2015; Samico-Fernandes et al. 2017). In recent decades, biosafety and biosecurity practices on pig farms have resulted in a reduction of *T. gondii* infection in confined pigs; however, the increasing demand for organic pork has caused the free-range pig breeding system to grow, thereby increasing access of these animals to grassland and organic material contaminated with feline feces and thus increasing the risk of infection with *T. gondii* by ingesting oocysts (Kijlstra and Jongert 2008).

Studies on the distribution of *T. gondii* cysts in pork have been previously performed (Dubey et al. 1986; Dubey 1988; Yai et al. 2003; Garcia et al. 2006; Tsutsui et al. 2007), but there are no recent studies, and no study has evaluated *T. gondii* distribution in Brazilian commercial cuts with an isolate circulating in the country (Type BrII). Furthermore, whether *T. gondii* distribution is influenced by the genotype of the parasite is unknown. In light of the worldwide epidemiological importance of *T. gondii* transmission through contaminated pork, the present study evaluated the distribution and viability of *T. gondii* tissue cysts in the organs and Brazilian commercial cuts of experimentally infected pigs.

Three female Large White × Landrace pigs that were approximately 90 days old and had previously tested negative for anti-*T. gondii* antibodies via an indirect fluorescent antibody

Section Editor: Berit Bangoura

✉ Solange Maria Gennari
sgennari@usp.br

[1] Laboratório de Doenças Parasitárias, Departamento de Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, São Paulo, Brazil

[2] Departamento de Tecnologia de Alimentos, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil

test (IFAT; Camargo 1964) at a 1:64 threshold (Garcia et al. 1999) were used. The animals were inoculated orally with a suspension of $3 \times 10^3$ oocysts of the TgCkBr57 isolate (Dubey et al. 2003), Type BrII (Dubey et al. 2008). Throughout the experiment, the pigs were kept in screened pens, fed rations formulated according to the basic needs of the species and age group, and supplied with water ad libitum. Blood collection was performed weekly to monitor anti-*T. gondii* serum antibody titres. The animals were slaughtered after 60 days and weighed approximately 90 kg; the brain, retina, tongue, diaphragm, and heart were collected, and the carcasses were kept at 4 °C ± 0.5 °C for 24 h. The carcasses were subsequently deboned, and the following commercial cuts were separated (Fig. 1): loin (*longissimus*), coppa (*longissimus*, *spinalis dorsi*, *rhomboideus*), tenderloin (*psoas major*), outside flat (*biceps femoris*), topside (*semimembranosus*), and top sirloin (*gluteus medius*).

To perform the mouse bioassays, the whole organs and commercial cuts were individually cut into small pieces. Then, tissue samples of up to 50 g were separated according to the protocol described by Dubey (1998). Briefly, the organs and commercial cuts were grinded in a blender in five volumes (*v/v*) of aqueous 0.85% NaCl (saline) and an aliquot of each sample was collected for a PCR assay. The resulting homogenate was mixed with the same volume of prewarmed (37 °C) acid pepsin solution (1.3 g pepsin—porcine stomach 1:10,000 biological activity; 2.5 g NaCl; 3.5 mL HCl; distilled water to a total volume of 250 mL, pH 1.1–1.2). The mixture was incubated in a shaking water bath for 1 h at 37 °C, centrifuged, and neutralized with 1.2% sodium bicarbonate. Antibiotics (2000 units penicillin and 200 μg streptomycin (*v/v*) were added to a final suspension of approximately 5 mL, and 1 mL of the homogenate was inoculated subcutaneously into each of five 2-month-old Swiss albino mice. Instead of centrifuging only half (250 mL) of the digested and strained homogenate, as recommended by Dubey (1988), the whole homogenate (500 mL) was used to get an increased inoculum at the end of the process.

Mice showing signs of acute toxoplasmosis (ruffled or starry stiff coat and hunched appearance or reluctance to move) were culled using an isoflurane chamber, after sedation with an association of xylazine (10 mg/kg) and ketamine (100 mg/kg) administered intramuscularly. The mice were examined for the presence of *T. gondii* tachyzoites in the lungs and cysts in the brain, as described by Dubey (2010). Mice that survived for 6 weeks post-inoculation (p.i.) were examined serologically using the modified agglutination test (MAT) according to the protocol described by Dubey and Desmonts (1987). Briefly, dilution of the sera was performed in a microplate (96 wells) using buffered saline (0.146 M NaCl, 0.0026 M $NaH_2PO_4$, 0.008 M $Na_2HPO_4$, pH 7.2). Subsequently, 150 μL of antigen stock (formalin-fixed whole tachyzoites) was diluted in 2.5 mL buffered alkaline solution (0.12 M NaCl, 0.05 M $H_3BO_3$, 0.03 M $NaN_3$, 0.4% bovine serum albumin, pH 8.95), 35 μL of 0.2 M 2-mercaptoethanol and 50 μL of 0.2% Evans Blue. This mixture was homogenized and immediately distributed into a U-bottom microplate, with 25 μL of reagents per well. Diluted sera were transferred to this microplate and mixed with the reagents (*v/v*). The plate was sealed and incubated overnight at 37 °C in a moist incubator. To confirm MAT results, the mice were euthanized as described, and they were examined for the presence of *T. gondii* cysts in the brain.

The presence of *T. gondii* DNA in organs and meat cuts from pigs was assessed using both nested and classical PCR protocols targeting a specific 155-bp fragment of the B1 gene (Yai et al. 2003) and the 529-bp RE repetitive fragment (Homan et al. 2000) of the *T. gondii* genome. DNA extracted from tachyzoites of the *T. gondii* reference strain RH was used as a positive control. The cytochrome

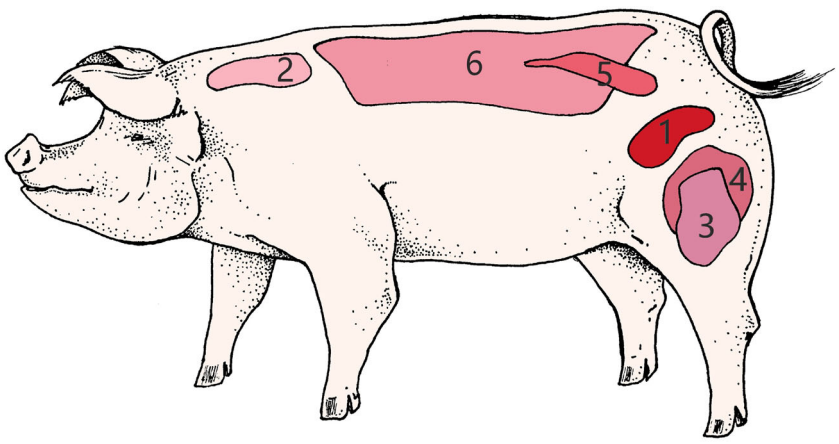


ILLUSTRATION: Daniel Torres

**Fig 1** Illustration of Brazilian commercial pig cuts evaluated in this study with regard to the distribution of *T. gondii* cysts in experimentally infected animals. 1) Top sirloin, 2) coppa, 3) outside flat, 4) topside, 5) tenderloin, 6) loin

b (cytb) genes of the vertebrate animals were used as endogenous control, as described by Parson et al. (2000).

The three inoculated pigs became infected with *T. gondii*, as demonstrated by the MAT results and mouse bioassays. *Toxoplasma gondii* antibody seroconversion was detected at the collection performed on day 14 p.i., and the titres ranged from 64 to 1024 during the experimental period (Fig. 2).

With regard to the commercial cuts, *T. gondii* was isolated from the coppa of the three pigs, from the outside flat and tenderloin of two pigs, and from the top sirloin and loin of one pig. No parasites were isolated from the topside. Parasites were isolated from the heart, diaphragm, and tongue of three pigs and from the brains of two pigs The retinal samples did not cause any infection in the mouse bioassays (Table 1).

Of the 165 inoculated mice, 61 were infected with *T. gondii* (37%) and among these, 44 animals were acutely infected (72%) and 17 (28%) were chronically infected. The pig tissues had viable parasites that infected at least one mouse inoculated with the homogenate from one of the pigs, demonstrating the broad distribution of the parasite and the risk to the human population associated with consumption of pork contaminated with *T. gondii*. A total of 73% (11 infected/15 inoculated) of the mice were infected when inoculated with the coppa homogenates of the three pigs, and 67% (10 infected/15 inoculated) were also infected when inoculated with the tongue homogenates of the three pigs. The coppa is one of the most consumed pork cuts in Brazil, and pork tongue is also widely used in Brazilian cuisine.

*Toxoplasma gondii* DNA was not detected in the tissues of pigs tested by PCR amplification of the B1 gene or the 529-bp RE fragment. The cytb gene was amplified in all the DNA samples, indicating successful extraction of the genetic material from the samples and the absence of PCR inhibitors.

Dubey et al. (1986) evaluated the distribution of *T. gondii* cysts in the cuts and organs of two pigs experimentally infected with strain GT1 (Type I). *Toxoplasma gondii* was isolated from the tongue, diaphragm, heart, and brain of 100% of the pigs, a result that is similar to that of the present study, with the exception of the brain, which was infected in only 66% of the pigs. With regard to the commercial cuts, parasites were isolated from the ham of two pigs and the loin of one pig. In addition to other cuts, the ham includes the top sirloin, topside, and outside flat, which were evaluated separately in the present study; putting these cuts together, *T. gondii* was isolated in two of the three pigs evaluated (66%).

In another study, Dubey (1988) infected 16 pigs with the *T. gondii* strains GT-1 (Type I), ME-49 (Type II), TS-2, and TC-2, and 14 became infected. The largest number of isolates was collected from the brain (12 animals), followed by the heart (11 animals), tongue (10 animals), and diaphragm (6 animals). In the evaluation of muscle cuts, parasites were isolated from the ham of five pigs.

*Toxoplasma gondii* was isolated from all of the animals in the present study. This is different from the results obtained by Yai et al. (2003), who isolated *T. gondii* from only four of eight pigs experimentally infected with the AS-28 strain. Importantly, the dose used by Yai et al. (2003) was higher than that used in the present study, and it was from a different isolate. These authors isolated parasites from the retina of two pigs, but no parasites were isolated from the brains, which was an unexpected result.

Garcia et al. (2006) infected 10 pigs with the *T. gondii* strain VEG (Type III) and performed mouse bioassay with brain tissue and a mixture of masseter muscle and organs (tongue, masseter, diaphragm, and heart). *Toxoplasma gondii* was isolated from the brain in eight pigs and from the masseter/organ mixture in 10 animals. This result is similar to that of the present study, as parasites were not isolated from the brain of all the pigs, but when evaluating the organs together (tongue, masseter, diaphragm, and heart) parasites were isolated from all the pigs. Tsutsui et al. (2007) also infected 10 pigs with the *T. gondii* strain VEG (Type III) and, using mouse

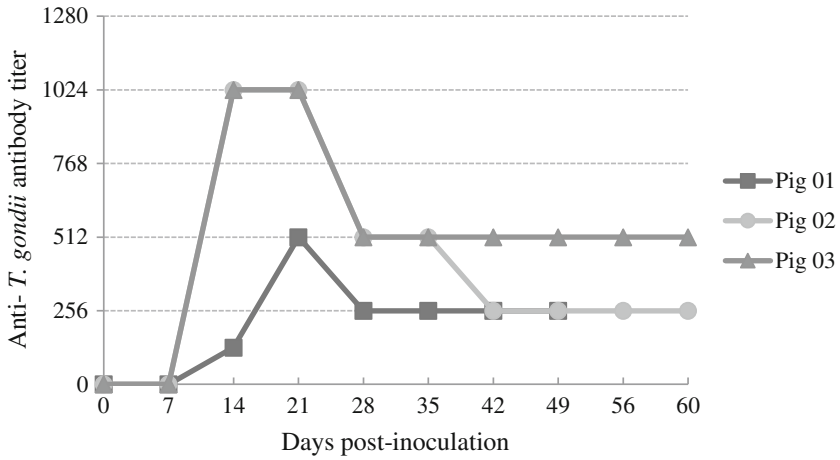


**Fig 2** Titers of anti-*Toxoplasma gondii* IgG antibodies in three pigs challenged with $3 \times 10^3$ oocysts of the Brazilian strain TgCkBr57 (Type BrII)

**Table 1** Number of infected/inoculated mice (%) according to the bioassay of organs and Brazilian commercial meat cuts of pigs experimentally infected with a Brazilian strain of *Toxoplasma gondii* (TgCkBr57, Type BrII)

| | | No. pig | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Total of mice |
| Commercial cut | Top sirloin | 0/5 (0) | 5/5 (100) | 0/5 (0) | 5/15 (33) |
| | Coppa | 5/5 (100) | 4/5 (80) | 2/5 (40) | 11/15 (73) |
| | Outside flat | 3/5 (60) | 4/5 (80) | 0/5 (0) | 7/15 (47) |
| | Topside | 0/5 (0) | 0/5 (0) | 0/5 (0) | 0/15 (0) |
| | Tenderloin | 1/5 (20) | 1/5 (20) | 0/5 (0) | 2/15 (13) |
| | Loin | 0/5 (0) | 3/5 (60) | 0/5 (0) | 3/15 (20) |
| Organs | Brain | 3/5 (60) | 2/5 (40) | 0/5 (0) | 5/15 (33) |
| | Heart | 4/5 (80) | 1/5 (20) | 2/5 (40) | 7/15 (47) |
| | Diaphragm | 2/5 (40) | 5/5 (100) | 4/5 (80) | 11/15 (73) |
| | Tongue | 1/5 (20) | 4/5 (80) | 5/5 (100) | 10/15 (67) |
| | Retina | 0/5 (0) | 0/5 (0) | 0/5 (0) | 0/15 (0) |

bioassays with meat cuts, they isolated the parasite from five pigs; isolates were retrieved from the ham of four pigs and from the loin of three pigs. All the animals developed anti-*T. gondii* antibodies, and the range of titres was similar to that found in the present study (64–1024).

The markers used in the present study for *T. gondii* DNA detection are widely used in the scientific literature, but they have limitations in chronically infected animals, since the cysts are not homogeneously distributed in the tissues. In contrast to the present study, Yai et al. (2003), Garcia et al. (2006), and Tsutsui et al. (2007) detected *T. gondii* from 63% (5/8), 100% (10/10), and 70% (7/10) of animals, respectively, using the same molecular methods but some differences should be considered, as the animals used by Garcia et al. (2006) and Tsutsui et al. (2007) were treated to promote encystation of the parasite; possible biological changes in the VEG strain after successive passages since its isolation in 1996 (Dubey 1996) and natural biological differences between strains may also have caused differences in the results.

Viable *T. gondii* cysts were isolated in all of the organs and cuts from pigs evaluated in the present study, except for the retina and topside. These findings demonstrate the broad distribution of this parasite in pigs and the importance of this species as a source of infection in humans. *Toxoplasma gondii* cysts are sensitive to traditional cooking (i.e., temperatures above 60 °C; Dubey et al. 1990) and are inactivated when the meat reaches − 12 °C during freezing (Kotula et al. 1991). Our results confirm the need for special attention to the preparation of these meats for human consumption.

**Acknowledgements** The authors thank the São Paulo Research Support Foundation (FAPESP, 2015/20649-7) for the Master's scholarship awarded to B. F. Alves and the National Council for Scientific and Technological Development of Brazil (CNPq) for the productivity grant awarded to S. M. Gennari and C. A. Conte-Junior. The authors also thank artist Daniel Torres for the illustration.

**Funding information** This study was financially supported by the Rio de Janeiro Research Support Foundation (FAPERJ, 26/010.001556/2014). A departmental grant from the Coordination for the Improvement of Higher Education Personnel—Brazil (CAPES, finance CODE 001) also supported in part this study.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Research involving animals** The protocols used in this study were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine (CEUA/FMVZ/6626071114) of the University of São Paulo, Brazil.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References

Camargo ME (1964) Improved technique of indirect immunofluorescence for serological diagnosis of toxoplasmosis. Rev Inst Med Trop Sao Paulo 6:117–118

Dubey JP (1988) Long-term persistence of *Toxoplasma gondii* in tissues of pigs inoculated with *Toxoplasma gondii* oocysts and effect of freezing on viability of tissue cysts in pork. Am J Vet Res 49:910–913

Dubey JP (1996) Infectivity and pathogenicity of *Toxoplasma gondii* oocysts for cats. J Parasitol 82:957–960

Dubey JP (1998) Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. Vet Parasitol 74:75–77

Dubey JP (2009) Toxoplasmosis in pigs — The last 20 years. Vet Parasitol 164:89–103. https://doi.org/10.1016/j.vetpar.2009.05.018

Dubey JP (2010) Toxoplasmosis of animals and humans, 2nd edn. CRC Press, Florida

Dubey JP, Desmonts G (1987) Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet J 19:1987

Dubey JP, Murrell KD, Fayer R, Schad GA (1986) Distribution of *Toxoplasma gondii* tissue cysts in commercial cuts of pork. J Am Vet Med Assoc 188:1035–1037

Dubey JP, Kotula AW, Sharar A, Andrews CD, Lindsay DS (1990) Effect of high temperature on infectivity of *Toxoplasma gondii* tissue cysts in pork. J Parasitol 76:201–204. https://doi.org/10.2307/3283016

Dubey JP, Graham DH, da Silva DS, Lehmann T, Bahia-Oliveira LM (2003) *Toxoplasma gondii* isolates of free-ranging chickens from Rio de Janeiro, Brazil: mouse mortality, genotype, and oocyst shedding by cats. J Parasitol 89:851–853

Dubey JP, Velmurugan GV, Chockalingam A, Pena HF, de Oliveira LN, Leifer CA, Gennari SM, Bahia-Oliveira LM, Su C (2008) Genetic diversity of *Toxoplasma gondii* isolates from chickens from Brazil. Vet Parasitol 157:299–305. https://doi.org/10.1016/j.vetpar.2008.07.036

Garcia JL, Navarro IT, Ogawa L, de Oliveira RC (1999) Soroprevalência do *Toxoplasma gondii*, em suínos, bovinos, ovinos e eqüinos, e sua correlação com humanos, felinos e caninos oriundos de propriedades rurais do norte do Paraná-Brasil. Ciênc Rural 29:91–97

Garcia JL, Gennari SM, Machado RZ, Navarro IT (2006) *Toxoplasma gondii*: detection by mouse bioassay, histopathology, and polymerase chain reaction in tissues from experimentally infected pigs. Exp Parasitol 113:267–271. https://doi.org/10.1016/j.exppara.2006.02.001

Homan WL, Vercammen M, de Braekeleer J, Verschueren H (2000) Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. Int J Parasitol 30:69–75. https://doi.org/10.1016/S0020-7519(99)00170-8

Kijlstra A, Jongert E (2008) Control of the risk of human toxoplasmosis transmitted by meat. Int J Parasitol 38:1359–1370. https://doi.org/10.1016/j.ijpara.2008.06.002

Kotula AW, Dubey JP, Sharar AK, Andrews CD, Shen SK, Lindsay DS (1991) Effect of freezing on infectivity of *Toxoplasma gondii* tissue cysts in pork. J Food Prot 54:687–690

Parson W, Pegoraro K, Niederstätter H, Föger M (2000) Species identification by means of the cytochrome b gene. Int J Legal Med 114:23–28. https://doi.org/10.1007/s004140000134

Samico-Fernandes EFT, Samico-Fernandes MFT, de Albuquerque PPF, de Almeida JC, Santos AS, Mota AR, Souza-Neto OL, Mota RA (2017) *Toxoplasma gondii* in backyard pigs: seroepidemiology and mouse bioassay. Acta Parasitol 62:466–470. https://doi.org/10.1515/ap-2017-0054

Steinparzer R, Reisp K, Grünberger B, Köfer J, Schmoll F, Sattler T (2015) Comparison of different commercial serological tests for the detection of *Toxoplasma gondii* antibodies in serum of naturally exposed pigs. Zoonoses Public Health 62:119–124. https://doi.org/10.1111/zph.12122

Tsutsui VS, Freire RL, Garcia JL, Gennari SM, Vieira DP, Marana ERM, Prudêncio LB, Navarro IT (2007) Detection of *Toxoplasma gondii* by PCR and mouse bioassay in commercial cuts of pork from experimentally infected pigs. Arq Bras Med Veterinária e Zootec 59:30–34

Yai LEO, Vianna MCB, Soares RM, Cortez A, Freire RL, Richtzenhain LJ, Gennari SM (2003) Evaluation of experimental *Toxoplasma gondii* (Nicolle and Manceaux, 1909) infection in pigs by bioassay in mice and polymerase chain reaction. Brazilian J Vet Res Anim Sci 40:227–234