



Genotyping of *Toxoplasma gondii* isolated from pigs for human consumption

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Abstract

The present study aimed to isolate and genotype strains of *T. gondii* from pigs slaughtered for human consumption in South Brazil. Blood and tissues (heart, diaphragm, liver, tongue, and masseter) from 400 animals were collected at two slaughterhouses. Sera were obtained, and antibodies against *T. gondii* were detected by both indirect fluorescence antibody test (IFAT) and modified agglutination test (MAT). The tissues of animals that tested positive in MAT, IFAT, or both (cut-off ≥ 64) were bioassayed. Twenty-six (6.5%) of the 400 animals were positive by serology. A total of 18 (69.2%) out of those 26 were positive in the mouse bioassay. The isolates were characterized by using 10 PCR-RFLP genetic markers. Fourteen isolates were fully genotyped, and four isolates were genotyped using nine of the 10 markers. All isolates belonged to ToxoDB PCR-RFLP genotype #206. The present study reports on genotype #206 in pigs for the first time, and it confirms the atypical nature of the Brazilian *T. gondii* isolates. Additionally, even with low levels of antibodies detected in pig herds, pork presents a *T. gondii* infection risk for humans.

Keywords Mouse bioassay · PCR - RFLP · Seroprevalence · Genetic diversity · Zoonosis · Population structure

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects most warm-blooded animals and causes toxoplasmosis, a zoonotic disease with a worldwide distribution (Boothroyd et al. 1998; Dubey and Beattie 1988). Epidemiological studies suggest that the ingestion of cysts from raw or undercooked meat and raw pork sausage is one of the most common means of *T. gondii* transmission in the human population (Dubey 2009; Fialho and Araújo 2003; Jamra et al. 1969; Navarro et al. 1992). Furthermore, *T. gondii* is the fourth leading cause of deaths

attributed to foodborne pathogens, responsible for approximately 24% of all such deaths in the USA (Scallan et al. 2011). In Europe, 40 cases of confirmed congenital toxoplasmosis cases were reported in 2012, and toxoplasmosis remains one of the most prevalent parasitic zoonotic diseases (ECDC 2014). Of all human infections, 30% to 63% are related to consuming undercooked meat or meat products, mainly from pork (Djokic et al. 2016).

The detection of *T. gondii* in meat using current methods of sanitary inspection is highly challenging (Dubey and Jones 2008), and advances in genotyping techniques, such as PCR-RFLP (10 markers), have revealed greater complexity not only in Central and South America but also in North America; therefore, prevention, control, and knowledge of its epidemiology are very important for public health. Furthermore, studies evaluating the genetic characteristics of this parasite at a high resolution should be performed to elucidate the role of pigs in the transmission of *T. gondii* to humans and to investigate the population structure of this parasite, considering that approximately 62% cases of congenital toxoplasmosis caused by *T. gondii* genotypes have been previously reported in animals (Carneiro et al. 2013). Thus, the present study aimed to isolate and genotype strains of

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T. gondii from the tissues of pigs slaughtered for human consumption in Paraná state, South Brazil.

Materials and methods

Sample collection

The samples were collected from two swine slaughterhouses with official inspection services located 40 km apart from each other in north Paraná state, south Brazil. A total of 400 pigs were used, 140 from one slaughterhouse and 260 from the second. Pigs came from nine farms ($n = 19, 48, 41, 50, 60, 42, 55, 55$) from Paraná state and two farms ($n = 15, 15$) from Santa Catarina state. Samples were collected from August/2013 to March/2014. Blood and tissue samples (heart, diaphragm, liver, tongue, and masseter) were collected individually and transported under refrigerated conditions to the laboratory. Sera were obtained and tested for *T. gondii* antibodies using the indirect fluorescence antibody test (IFAT) and the modified agglutination test (MAT) within 24–48 h. The meat samples with positive sera in at least one of the serological tests were subjected to mouse bioassays no more than 24 h after sampling. The experiments conducted in this work comply with current Brazilian regulations and were approved by the Ethics Committee on Animal Use at the State University of Londrina (CEUA/UDEL) permit no. 206/12.

Serological tests

Sera from pigs were tested for the presence of anti-*T. gondii* IgG antibodies using IFAT and MAT, according to the protocols from Camargo (1964) and Desmonts and Remington (1980), respectively. Serum sample from previously known positive and negative animals were included as controls. A cut-off titer of ≥ 64 (Garcia et al. 1999) was used for both tests. Tachyzoites of the RH strain were used to perform both tests.

Mouse bioassay

Tissue samples from serum-positive pigs (IFAT or MAT) were subjected to mouse bioassay according to the protocol described by Dubey (1998). Briefly, a mix of tissues (10 g each of heart, diaphragm, liver, tongue and masseter, a total of 50 g) were subjected to peptic digestion and neutralized with sodium bicarbonate, and the homogenate was inoculated subcutaneously (1 ml per mouse) in three Swiss Webster mice. Mice were observed daily, and those who developed symptoms (bristly hair, tearing, weight loss, diarrhea, and abdominal distension) were euthanized for collection of peritoneal fluid and tachyzoites. In case of death of the mouse, an impression of brain, liver, and lung between the slide and the cover slip was

used for detecting cysts or tachyzoites, and those organs were macerated and re-inoculated into another mouse. Mice that survived until 6 weeks post-inoculation (p.i.) were euthanized. IFAT was used as the serological test for anti-*T. gondii*, and brain and lung tissues were analyzed for the presence of cysts or tachyzoites, respectively. Mice were considered positive when titers were ≥ 16 . A brain squash specimen was prepared between the slide and the cover slip for microscopic examination. Mouse bioassay data were analyzed by the standard Kaplan–Meier survival curves, using the computer program GraphPad Prism.

DNA extraction and PCR

Mice that tested positive for *T. gondii* in the acute and chronic stage of infection had their lung, liver, heart, and brain tissues collected 45 days after bioassays. DNA was extracted from these tissues using a commercial kit (PureLink™ Genomic DNA Kit, Invitrogen ® USA) following the manufacturer's instructions. These samples were subjected to PCR to confirm infection by *T. gondii*. Amplification of DNA from *T. gondii* was performed according to the technique described by Homan et al. (2000) with no modifications. TOX-4 and TOX-5 primers were used to amplify 529-bp repeat element. A suspension of RH strain tachyzoites (10^4 tachyzoites/mL) was used as the positive control, and ultrapure water was used as negative control.

Genotyping

Genotyping was performed by PCR-RFLP using 10 genotypic markers, including SAG1, SAG2 (5'-3' SAG2 and alt. SAG2), SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico, as previously described by Su et al. (2006) with no modifications. DNA from strains GT1, PTG, CTG, TgCgCa1, MAS, TgCatBr5, TgCatBr64, and TgRsCr1 were used as positive controls.

The target DNA sequence was first amplified by multiplex PCR using external primers for all markers, and then subjected to nested PCR for individual markers. The nested PCR products were digested with restriction enzymes under temperature and time conditions specific for each marker. All products were visualized by electrophoresis on a 2.5% or 3% agarose gel, stained with Sybr Safe DNA Gel Stain and visualized using the Safe Imager™. The results were identified, compared, and ranked according to the genotypes present in the ToxoDB. The evolutionary history was inferred from a phylogenetic network by the neighbor-joining method using SplitsTree 4.0 (Huson and Bryant 2006). The patterns of genotypic data from PCR-RFLP were transformed into binary data and tabulated. The phylogenetic tree was constructed using the genotype isolated in the present study and others in ToxoDB.

Statistical analyses

The levels of agreement between the serological tests (IFAT and MAT) were evaluated using the kappa agreement (Landis and Koch 1977).

Results

Antibodies against *T. gondii* were detected in 6.5% (26/400) of the pigs tested either by IFAT or by MAT. The most frequent titers by IFAT were 256 (1.75%) and 64 (1.25%). In MAT, 5.0% (20/400) tested positive with a titer of 64. Of those 26 positive animals, 9 (34.6%) and 6 (23.07%) were positive in MAT and IFAT, respectively, and 11 (42.3%) were positive in both tests. The kappa agreement between the two tests was considered moderate ($k = 0.57$; standard error = 0.0989; 95% CI 0.3812 to 0.769).

T. gondii strains were isolated from the tissues of 18 out of 26 (69.2%) seropositive pigs by mouse bioassay (Table 1). All isolates (18) were confirmed to be *T. gondii* based on PCR amplifying an amplicon of 529 bp. From these isolates, 11 (61.1%) had only tachyzoites, 6 (33.3%) had both tachyzoites and bradyzoites, and 1 (5.5%) had only bradyzoites. The virulence of the strains was evaluated, and 16.6% of the *T. gondii* isolates were highly pathogenic to mice. These strains exhibited a 100% mortality rate (Fig. 1), with deaths ranging from 8

to 18 days p.i., and the average death occurring at 6.5 days p.i. (61.1%).

The origin of the seropositive slaughtered pigs was traced, and it was found that all of the pigs were from 2 out of 11 farms. These two farms are 300 km from each other and do not trade pigs with each other. Investigating these two properties, it was possible to determine that farm-1 was responsible for the eight seropositive animals from which *T. gondii* was not isolated via mouse bioassay, representing a total of 1500 pigs and 470 sows, being a commercial farm attending to biosecurity rules for the purpose of providing pig products for human consumption. Farm 2 was responsible for all 18 seropositive animals from which *T. gondii* was isolated by mouse bioassay. Farm 2 was a small-scale farm with a herd of 300 pigs and 50 sows. The source of water was spring water and food (a mix of corn and soybean) was prepared on site without rodent or cat control programs; therefore, the food was not protected from these pests, and no biosecurity measures had been implemented.

According to PCR-RFLP, a new genotype ToxoDB#206 was identified in 14 isolates. The other four isolates were genotyped at 9 out of the 10 markers, and the available typing data indicate that they were likely an atypical strain of genotype no. 206 (Table 2). Clonal types I, II, and III were not detected in this study (Table 2). The phylogenetic tree of *T. gondii* isolates from pigs is shown in Fig. 2.

Table 1 Biological characteristics of *T. gondii* isolated from pigs (*Sus domesticus*) by mouse bioassay

Pig no.	IFAT		Positive micea/inoculated mice	Mortality (%)	Day of death p.i.	Tachyzoites	Tissue cysts
191	256	< 16	3/3	3 (100)	8,9,12	+	–
192	64	< 16	3/3	2 (66.6)	10,18	+	–
193	256	≥ 64	3/3	2 (66.6)	6,8	+	+
197	256	≥ 64	3/3	3 (100)	16,16,17	–	+
198	1024	≥ 64	3/3	2 (66.6)	12,13	+	–
203	256	16	2/3	2 (66.6)	8,16	+	–
205	64	< 16	3/3	2 (66.6)	13,14	+	+
212	16	≥ 64	3/3	3 (100)	14,16,18	+	–
220	256	≥ 64	3/3	1 (33.3)	8	+	–
222	256	≥ 64	2/3	2 (66.6)	8,20	+	–
225	< 16	≥ 64	2/3	1 (33.3)	16	+	–
226	< 16	≥ 64	3/3	2 (66.6)	10,11	+	+
228	1024	≥ 64	2/3	1 (33.3)	8	+	+
234	< 16	≥ 64	1/3	1 (33.3)	8	+	–
235	1024	≥ 64	3/3	2 (66.6)	8,13	+	–
236	< 16	≥ 64	3/3	2 (66.6)	12,14	+	–
240	< 16	≥ 64	3/3	1 (100)	16	+	+
246	1024	≥ 64	2/3	1 (33.3)	8	+	+

p.i. post-inoculation

^a Positive mice were either by parasite detection in their tissues or through positive IFAT (cut-off ≥ 16)

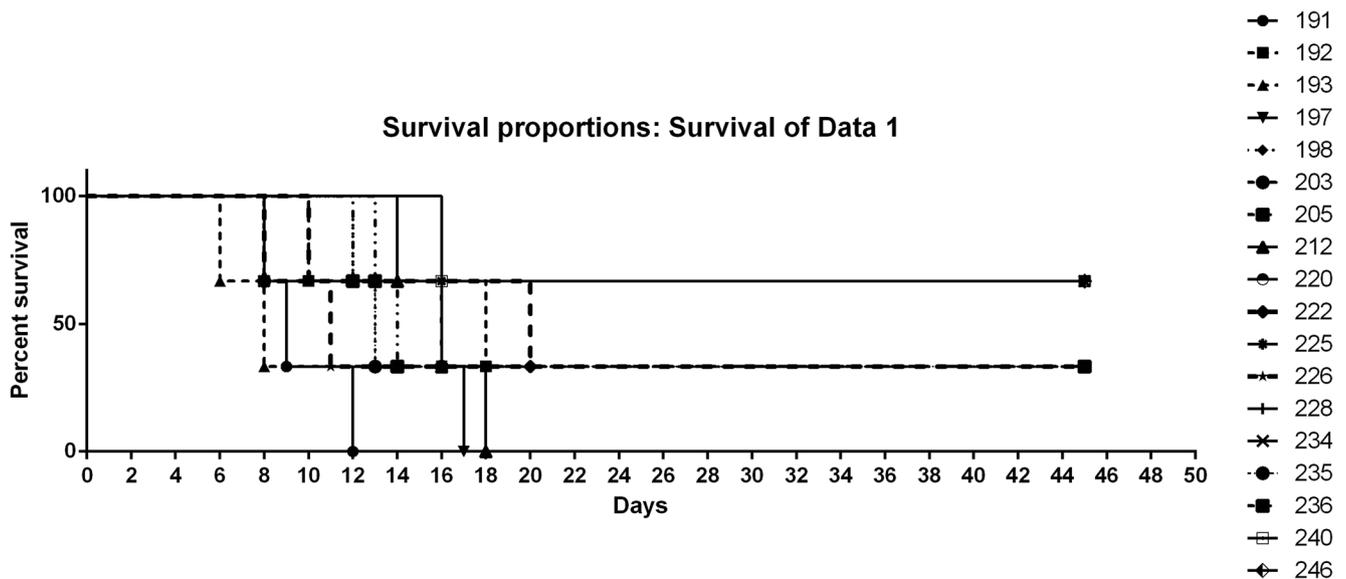


Fig. 1 Survival proportions from mice infected with *Toxoplasma gondii* isolates from pig tissues. There were 18 isolates from pigs, which animal numbers are shown at right

Discussion

The total serum occurrence from pigs in our study was 6.5% (26/400), which was lower than in previous studies (Tsutsui et al. 2003; Garcia et al. 1999). This result is substantial because nearly 32 million pigs are slaughtered in Brazil per year (ABIPECS 2012), and based on the prevalence presented here, it is possible that up to almost 2.1 million *T. gondii* infected pigs can enter the Brazilian food chain or could be exported each year. Hill et al. (2010) described that a single market weight pig (113 kg) yields 620 individual 113 g servings. Even when one-half of the harvested meat is processed (salting, cooking, freezing, etc.) by methods that are known to kill *T. gondii* (Hill et al. 2010), we can speculate that nearly 644,000,000 individual 113 g servings of *T. gondii*-infected pork are available for consumption in Brazil.

The 6.5% prevalence of *T. gondii* obtained using serological tests in this study was similar to the prevalence in South Brazil, as described by Carletti et al. (2005) and De Moura et al. (2007). These authors used IFAT and observed a prevalence of 4.0% and 8.5% respectively. Likewise, Da Silva et al. (2008) and Djokic et al. (2016) reported a prevalence of 7.2% and 6.9%, respectively, using only MAT. In Brazil, studies in pigs from slaughterhouses detected a prevalence of anti-*T. gondii* antibodies ranging from 4.0% to 54.3% (Carletti et al. 2005; Dos Santos et al. 2005; Cademartori et al. 2014; Bezerra et al. 2009; Fernandes et al. 2015). Around the world, the seroprevalence of *T. gondii* varies dramatically from 0% to 94% (Alvarado-Esquivel et al. 2011; De Sousa et al. 2006; Dubey and Jones 2008; Dubey 2009; García-Bocanegra et al. 2010; Klun et al. 2011; Veronesi et al. 2011). These results could be explained due to differences in the region, the category of animals, diagnostic methods used, cut-offs used,

Table 2 Genotyping of *Toxoplasma gondii* isolates obtained from pigs (*Sus domesticus*) slaughtered for human consumption in South Brazil

Isolate	Markers											Genotype
	SAG1	SAG2	alt.SAG2	SAG3	BTUB	GRA6	c22–8	c29–2	L358	PK1	Apico	
191, 192, 193, 197, 203, 212, 220, 222, 225, 228, 234, 235, 236, 246.	u-1	I	II	III	III	III	II	III	I	III	I	ToxoDB no. 206
198	u-1	I	II	III	nd	III	II	III	I	III	I	Likely no. 206
205	u-1	I	II	III	III	III	II	III	I	nd	nd	Likely no. 206
226	u-1	I	II	III	III	III	II	III	I	nd	I	Likely no. 206
240	u-1	I	II	III	III	III	II	III	I	III	nd	Likely no. 206

nd no data

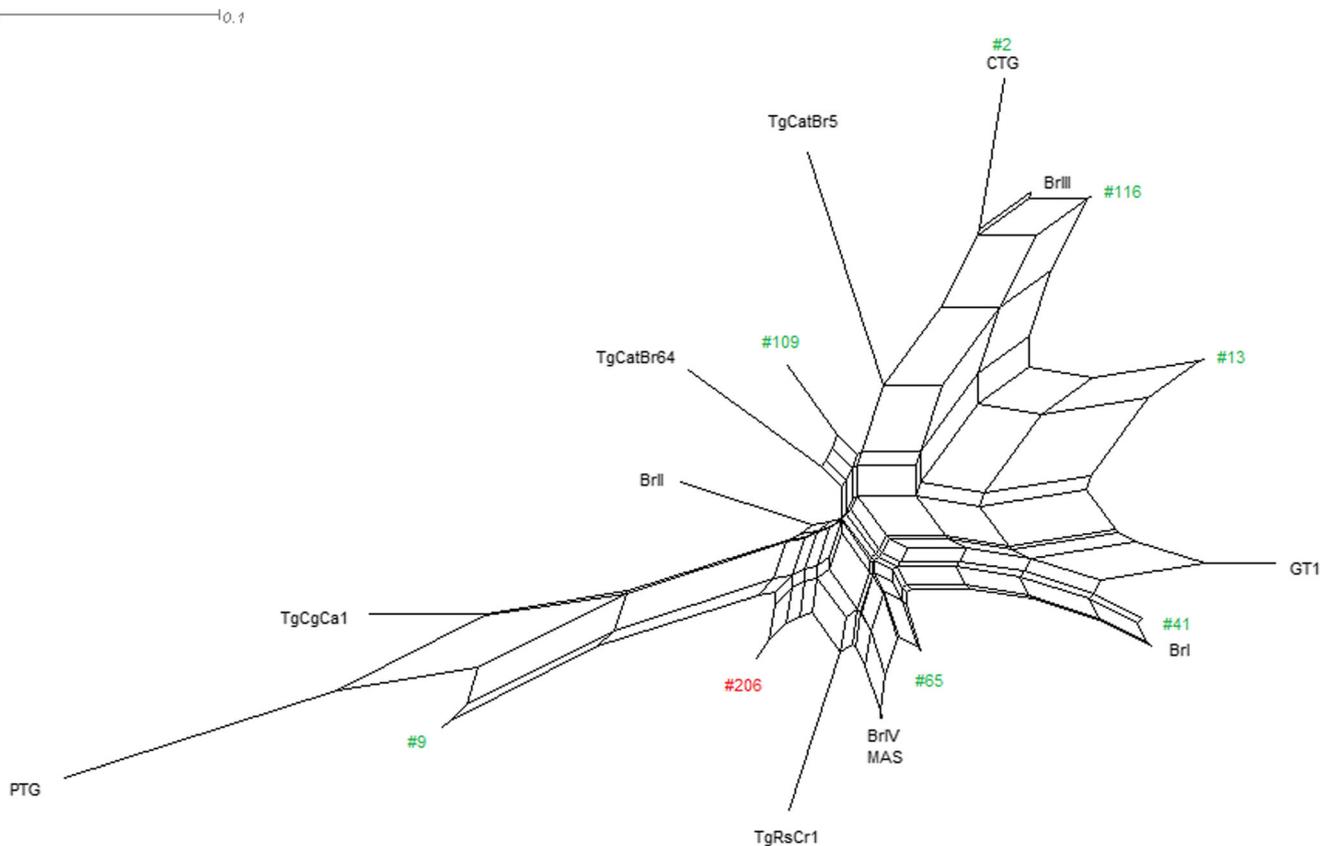


Fig. 2 Phylogenetic network of *Toxoplasma gondii* isolates in pigs from slaughterhouses. ToxoDB genotype observed in this study is marked in red, reference genotypes in black and other genotypes described in pigs from Brazil in green

climate, socioeconomic factors, production systems (indoor pigs with a biosecurity system versus free-range), and cultural factors, all resulting in highly variable seroprevalence values for *T. gondii* (Dos Santos et al. 2005; Fialho et al. 2009).

In the present study, the meat of 69.2% of the seropositive pigs (18/26) was infected by *T. gondii*, different from previous studies in Brazil; 62.5% in Rio Grande do Norte state (Clementino-Andrade et al. 2013), 47.2% in Rio Grande do Sul state (Cademartori et al. 2014), 25% in São Paulo state (Dos Santos et al. 2005), 35.1% in Paraíba state (Feitosa et al. 2014), and 2% in Pernambuco state (Fernandes et al. 2015).

The results of our genotype analysis revealed the presence of a single genotype no. 206 in the 18 isolates. Genotype no. 206 is an atypical genotype, and it is phenotypically closer to BrIV and no. 65 Brazilian isolates. This is the first description of genotype no. 206 in pork; however, it has been described previously in newborns with ocular lesions in the Minas Gerais state (Carneiro et al. 2013), 982 km from the region where our study was performed. Additionally, the same genotype was described in chickens in the Espírito Santo state (Pena et al. 2013), which is approximately 1200 km away. One of the most important reasons to isolate and genotype isolates from *T. gondii* is to characterize the transmission relationship between animals and

human beings; thus, for genotype no. 206, chickens and pigs could be the sources of infection for ocular toxoplasmosis.

This genotype can be highly or intermediately virulent to mice, and as presented in Table 2, the mortality rate was 61.1% (54/33). Carneiro et al. (2013) and Pena et al. (2008) determined genotype no. 206 to be virulent in mice because of the high mortality rates; however, mouse virulence depends on several factors, including the stage of the parasite, route of transmission, dose, types of mice used, and strain of the parasite. In the present study, the mice were inoculated intraperitoneally, a route that can increase parasite virulence (Dubey et al. 2002).

Pork plays an important role in *T. gondii* transmission because it is one of the most commonly consumed meats around the world. The current emerging market trends of organic and environmentally conscious animal-friendly farming systems can increase exposure to infection by pig's outdoor access as indicated by studies (Bacci et al. 2015; Herrero et al. 2016; Kijlstra and Jongert 2008; Pipia et al. 2018; van der Giessen et al. 2007; Wallander et al. 2016). Furthermore, a high prevalence may indicate greater environmental contamination, and a flawed technical production system combined with inadequate sanitary management can facilitate contact with felines and rodents (Fernandes et al. 2015).

The isolates of *T. gondii* obtained from pig tissues at the present study were all from a small familiar farm with poor sanitary control and presence of cats, chicken, and dogs. This is likely to be the reason of high level of *T. gondii* in those animals as described by Djokic et al. (2016) where small farms presented elevated toxoplasmosis risks.

No test is able to detect antibodies against *T. gondii* with 100% accuracy (Basso et al. 2013), but the use of IFAT and MAT in parallel may improve detection of antibodies anti-*T. gondii* (Vieira et al. 2018) and consequently to increase the isolation rate. We credited the high isolation rate (69.2%) obtained here because of the sampling of different tissues (heart, diaphragm, liver, tongue and masseter), as suggested by Dubey and Jones (2008). A cut-off titer of ≥ 64 was established to prevent false-positive results caused by cross-reactions (Garcia et al. 1999).

More studies are needed to investigate the circulation of *T. gondii* in other animal species and its relationship to pigs, especially related to human transmission, due to the high zoonotic potential of this genotype. Given that *T. gondii* in Brazil is highly diverse, the mechanisms that allow a single genotype to be transmitted in a large area are important for future investigations.

Conclusions

In summary, pig herds, even those with a low seroprevalence of antibodies against *T. gondii*, are still an important source of *T. gondii* infection in humans. Additionally, pig management systems may contribute to risk factors for *T. gondii* infection and should be examined further. This is the first report of the genotype no. 206 of *T. gondii* in pork.

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Compliance with ethical standards

The experiments conducted in this work comply with current Brazilian regulations and were approved by the Ethics Committee on Animal Use at the State University of Londrina (CEUA/UDEL) permit no. 206/12.

Conflict of interest The authors declare that they have no competing interests.

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References

- ABIPECS (2012) Associação Brasileira da Indústria Produtora e Exportadora de carne suína. <http://www.abipecs.org.br>. Accessed 15 Nov 2013
- Alvarado-Esquivel C, García Machado C, Alvarado-Esquivel D, González-Salazar A, Briones-Fraire C, Vitela-Corralles J, Villena I, Dubey JP (2011) Seroprevalence of *Toxoplasma gondii* infection in domestic pigs in Durango state, Mexico. *J Parasitol* 97:616–619
- Bacci C, Vismarra A, Mangia C, Bonardi S, Bruini I, Genchi M, Kramer L, Brindani F (2015) Detection of *Toxoplasma gondii* in free-range, organic pigs in Italy using serological and molecular methods. *Int J Food Microbiol* 202:54–56
- Basso W, Hartnack S, Pardini L, Maksimov P, Koudela B, Venturini MC, Schares G, Sidler X, Lewis FI, Deplazes P (2013) Assessment of diagnostic accuracy of a commercial ELISA for the detection of *Toxoplasma gondii* infection in pigs compared with IFAT, TgSAG1-ELISA and Western blot, using a Bayesian latent class approach. *Int J Parasitol* 43:565–570
- Bezerra RA, Paranhos EB, Del'Arco AE, Albuquerque GR (2009) Detecção de anticorpos anti-*Toxoplasma gondii* em suínos criados e abatidos no Estado da Bahia, Brasil. *Braz J Vet Parasitol* 18(3):78–80
- Boothroyd JC, Hehl A, Knoll LJ, Manger ID (1998) The surface of toxoplasma: more and less. *Int J Parasitol* 28:3–9
- Cademartori BG, Santos LMJF, Oliveira FC, Quevedo P, Oliveira PA, Ramos TS, Rocha ASR, Ruas JL, Farias NAR (2014) Isolation and pathogenicity of *Toxoplasma gondii* in naturally infected (rustic farm) pigs in southern Brazil. *Vet Parasitol* 203:207–211
- Camargo ME (1964) Improved technique of indirect immunofluorescence for serological diagnosis of toxoplasmosis. *Rev Inst Med Tropical de São Paulo* 6:116–118
- Carletti RT, Freire RL, Shimada MT, Ruffolo BB, Begale LP, Lopes FMR, Navarro IT (2005) Prevalence of *Toxoplasma gondii* infection among slaughtered swines in Paraná state, Brazil. *Semina: Ciênc Agrár* 26(4):563–568
- Carneiro ACAV, Andrade GM, Costa JGL, Pinheiro BV, Su C, Januário JN, Vitor RWA (2013) Genetic characterization of *Toxoplasma gondii* revealed highly diverse genotypes for isolates from newborns with congenital toxoplasmosis in southeastern Brazil. *J Clin Microbiol* 51(3):901–907
- Clementino-Andrade MM, Pinheiro BV, Cunha MM, Carneiro ACAV, Andrade Neto VF, Vitor RWA (2013) New genotypes of *Toxoplasma gondii* obtained from farm animals in Northeast Brazil. *Res Vet Sci* 94:587–589
- Da Silva AV, Boareto H, Isbrecht FB, Da Silva RC, Langoni H (2008) Ocorrência de anticorpos anti-*Toxoplasma gondii* em suínos da região oeste do Paraná, Brasil. *Rev Brasil Med Vet e Zoot* 15:263–266
- De Moura AB, Osaki SC, Zulpo DL, Marana ERM (2007) Ocorrência de anticorpos contra *Toxoplasma gondii* em suínos e ovinos abatidos no município de Guarapuava, PR, Brasil. *Rev Bras Parasitol Vet* 16: 54–56
- De Sousa S, Ajzenberg D, Canada N, Freire L, Correia Da Costa JM, Dardé ML, Thulliez P, Dubey JP (2006) Biologic and molecular characterization of *Toxoplasma gondii* isolates from pigs from Portugal. *Vet Parasitol* 135:133–136
- Desmonts G, Remington JS (1980) Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. *J Clin Microbiol* 11:562–568
- Djokic V, Fablet C, Blaga R, Rose N, Perret C, Djurkovic-Djakovic O, Boireau P, Durand B (2016) Factors associated with *Toxoplasma gondii* infection in confined farrow-to-finish pig herds in western France: an exploratory study in 60 herds. *Parasit Vectors* 9:466

- Dos Santos CBA, De Carvalho ACFB, Ragozo AMA, Soares RM, Amaku M, Yai LEO, Dubey JP, Gennari SM (2005) First isolation and molecular characterization of *Toxoplasma gondii* from finishing pigs from São Paulo state, Brazil. *Vet Parasitol* 131:207–211
- 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
- Dubey JP, Beattie CP (1988) Toxoplasmosis of animals and man. CRC Press, Boca Raton, pp 1–40
- Dubey JP, Jones JL (2008) *Toxoplasma gondii* infection in animals and humans in the United States. *Int J Parasitol* 38:1257–1278
- Dubey JP, Graham DH, Blackston CR, Lehmann T, Gennari SM, Ragozo AMA, Nishi SM, Shen SK, Kwok OCH, Hill DE, Thulliez P (2002) Biological and genetic characterization of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from São Paulo, Brazil: unexpected findings. *Int J Parasitol* 32:99–105
- European Centre for Disease Prevention and Control (ECDC) (2014) Annual epidemiological report 2014 food and waterborne diseases and zoonoses. Available at: <http://ecdc.europa.eu/en/publications/Publications/food-waterborne-diseases-annualepidemiological-report-2014.p>. Accessed 29 Sep 2018
- Feitosa TF, Vilela VLR, De Melo LRB, Almeida Neto JL, Souto DVO, De Moraes DF, Athayde ACR, Azevedo SS, Pena HJF (2014) *Toxoplasma gondii* and *Neospora caninum* in slaughtered pigs from northeast, Brazil. *Vet Parasitol* 202:305–309
- Fernandes EFTS, Melo RPB, Kim PP, Almeida JC, Barros LD, Garcia JL, Silva JCR, Mota RA (2015) First report of genotype #65 of *Toxoplasma gondii* in pigs. *Parasitol Res* 144:3927–3930
- Fialho CG, Araújo FAP (2003) Detecção de anticorpos para *Toxoplasma gondii* em soro de suínos criados e abatidos em frigoríficos da região da grande Porto Alegre-RS, Brasil. *Cienc Rural*, Santa Maria 33(5): 893–897
- Fialho CG, Teixeira MC, Araújo FAP (2009) Toxoplasmose animal no Brasil. *Acta Sci Vet* 37(1):1–23
- Garcia JL, Navarro IT, Ogawa L, De Oliveira RC (1999) Soroprevalência do *Toxoplasma gondii*, em suínos, bovinos, ovinos e equinos, e sua correlação com humanos, felinos e caninos, oriundos de propriedades rurais do norte do Paraná Brasil. *Cienc Rural* 29:91–97
- García-Bocanegra I, Simon-Grifé M, Dubey JP, Casal J, Martín GE, Cabezón O, Perea A, Almería S (2010) Seroprevalence and risk factors associated with *Toxoplasma gondii* in domestic pigs from Spain. *Parasitol Int* 59:421–426
- Herrero L, Gracia MJ, Pérez-Arquillué C, Lázaro R, Herrera M, Herrera A, Bayarri S (2016) *Toxoplasma gondii*: pig seroprevalence, associated risk factors and viability in fresh pork meat. *Vet Parasitol* 224: 52–59
- Hill DE, Haley C, Wagner B, Gamble HR, Dubey JP (2010) Seroprevalence of and risk factors for *Toxoplasma gondii* in the US swine herd using sera collected during the National Animal Health Monitoring Survey (swine 2006). *Zoonoses Public Health* 57:53–59
- Homan WL, Vercammen M, De Braekeleer J (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
- Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol* 23:254–267
- Jamra LMF, Deane MP, Guimarães EC (1969) On the isolation of *Toxoplasma gondii* from human food of animal origin: partial results in the city of São Paulo (Brazil). *Rev Inst Med Trop São Paulo* 11(3): 169–176
- Kijlstra A, Jongert E (2008) Toxoplasma-safe meat: close to reality? *Trends Parasitol* 25:18–22
- Klun I, Vujančić M, Year H, Nikolić A, Bobić B, Ivović V, Bradonjić S, Dupouy-Camet J, Djurković-Djaković O (2011) *Toxoplasma gondii* infection in slaughter pigs in Serbia: seroprevalence and demonstration of parasites in blood. *Vet Res* 42:17
- Landis J, Koch G (1977) An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics* 33(2):363–374
- Navarro IT, Vidotto O, Giraldo N, Freire RL (1992) *Toxoplasma gondii*: Isolamento a partir de carne e cérebro de suínos comercializados na região de Londrina. *Semina: Ciênc Agrár* 13:15–18
- Pena HJF, Gennari SM, Dubey JP, Su C (2008) Population structure and mouse-virulence of *Toxoplasma gondii* in Brazil. *Int J Parasitol* 38(5):561–569
- Pena HJF, Vitaliano SN, Beltrame MAV, Pereira FEL, Gennari SM, Soares RM (2013) PCR-RFLP genotyping of *Toxoplasma gondii* from chickens from Espírito Santo state, southeast region, Brazil: new genotypes and a new SAG3 marker allele. *Vet Parasitol* 192: 111–117
- Pipia AP, Varcasia A, Dessì G, Panzalis R, Gai C, Nonnis F, Veronesi F, Tamponi C, Scala A (2018) Seroepidemiological and biomolecular survey on *Toxoplasma gondii* infection on organic pig farms. *Parasitol Res* 117(5):1637–1641
- Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011) Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 17:7–15
- Su C, Zhang X, Dubey JP (2006) Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. *Int J Parasitol* 36:841–848
- Tsutsui VS, Navarro IT, Freire RL, Freitas JC, Prudencio LB, Delbem ACB, Marana ERM (2003) Soroepidemiologia e fatores associados à transmissão do *Toxoplasma gondii* em suínos do norte do Paraná, Brasil. *Arch Vet Sci* 8:27–34
- Van der Giessen J, Fonville M, Bouwknegt M, Merel L, Ant V (2007) Seroprevalence of *Trichinella spiralis* and *Toxoplasma gondii* in pigs from different housing systems in the Netherlands. *Vet Parasitol* 148:371–374
- Veronesi F, Ranucci D, Branciarri R, Miraglia D, Manfredi MT, Fioretti DP (2011) Seroprevalence and risk factors for *Toxoplasma gondii* infection on finishing swine reared in the Umbria region, Central Italy. *Zoonoses Public Health* 58:178–184
- Vieira FEG, Sasse JP, Minutti AF, Miura AC, Barros LD, Cardim ST, Martins TA, Seixas M, Yamamura MI, Su C, Garcia JL (2018) *Toxoplasma gondii*: prevalence and characterization of new genotypes in free-range chickens from South Brazil. *Parasitol Res* 117: 681–688
- Wallander C, Frössling J, Dórea FC, Ugglá A, Vågsholma A, Lundén A (2016) Pasture is a risk factor for *Toxoplasma gondii* infection in fattening pigs. *Vet Parasitol* 224:27–32