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SHORT COMMUNICATION

# A new strain of *Toxoplasma gondii* circulating in southern Brazil

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**Abstract** Recently, an outbreak of human toxoplasmosis was identified in Santa Maria city, Southern Brazil. However, the suspected vehicle of *Toxoplasma gondii* contamination in this region remains unclear. This study was conduct to analyze whether pork meat samples collected in supermarkets from Santa Maria city, RS, could be infected with *T. gondii*. Thus, we analyzed the presence of *T. gondii* DNA in 20 pork hearts, 20 pork tongues and 20 sausages. DNA was extracted from each sample and real-time PCR was performed using 529-bp and B1 markers. *T. gondii* genotyping was performed by PCR–RFLP analysis. *T. gondii* DNA was detected in 2 of 20 (10%) heart samples and in 1 of 20 (5%) tongue samples using 529-bp marker. Besides, those 2 (10%) heart samples also were positive for *T. gondii* using B1 marker. All sausage samples were negative for both markers. Genotyping revealed a new atypical genotype in the pork meat. Our findings were not able to confirm whether these food samples were involved in some outbreak. However, we can conclude that food samples containing *T. gondii* can be displayed in Santa Maria supermarkets. In addition, a new *T. gondii* genotype was identified circulating in southern Brazil.

**Keywords** Genotype · Outbreak · Pork meat · Real-time PCR · Sausage · *Toxoplasma gondii*

## Introduction

Toxoplasmosis is caused by the Apicomplexa protozoon *Toxoplasma gondii*, which has felids as definitive hosts and all warm-blooded animals as intermediate hosts, including man (Tenter et al. 2000).

Studies have identified risk factors for *T. gondii* infection, such as eating raw or undercooked meat, exposure to contaminated water, contact with the soil, and eating unwashed vegetables or fruits (de Moura et al. 2006; Lass et al. 2012; Franco-Hernandez et al. 2016).

In several countries, including Brazil, pigs are considered the most important source of *T. gondii* infection to humans (Belfort-Neto et al. 2007; Dubey 2009). In Rio Grande do Sul, a state located in southern Brazil, the consumption of pork and handmade sausages is high. It could be an important source of *T. gondii* contamination (Dias et al. 2005).

In 2006, water was the cause of a human toxoplasmosis outbreak in Santa Isabel do Ivaí, southern Brazil (de Moura et al. 2006). Recently, another outbreak of human toxoplasmosis was identified in Santa Maria city, Rio Grande do Sul, Brazil. However, the suspected vehicle of *T. gondii* contamination in this region remains unclear.

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Considering the high prevalence of infected meat in southern Brazil, this study was conducted to analyze whether pork meat samples collected in supermarkets from Santa Maria city, RS, could be infected with *T. gondii*.

## Materials and methods

### Samples collection and DNA extraction

A total of 20 pork hearts, 20 pork tongues and 20 sausages were purchased from different supermarkets in Santa Maria city, located in the west central region in Rio Grande do Sul state, southern Brazil (Fig. 1).

After collection, a piece of sample weighting 2 g was cut in small pieces and added to 10 mL of Buffer EB for digestion. Then, total DNA was extracted using a commercially DNeasy Mericon Food Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol.

### Real-Time Polymerase Chain Reaction (qPCR) analysis

qPCR was performed using SYBR Green PCR Master Mix (PE Applied Biosystems, Foster City, CA, USA), targeting the *T. gondii* B1 gene (forward: AGA GAC ACC GGA ATG CGA TCT and reverse: TTC GTC CAA GCC TCC GAC T) and *T. gondii* 529-bp gene (forward: GAA AGC CAT GAG GCA CTC CA and reverse: TTC ACC CGG ACC GTT TAG C). Briefly, we used 10 μL of SYBR Green Master Mix, 0.4 μL of forward and 0.4 μL of reverse (primers concentration: 10 μM), 7.2 μL of nuclease- and DEPC-free water (Invitrogen, Carlsbad, CA, USA), and 2 μL of DNA, with a total reaction volume of 20 μL. Genomic DNA from *T. gondii* RH strain was used as positive control and molecular grade water as negative control. Amplification and data analysis were performed on an ABI Prism 7500 DNA sequence detection system.

### PCR–RFLP for *T. gondii* genotyping

*T. gondii* genotypes in heart and tongue samples were performed using multilocus PCR–RFLP (restriction fragment length polymorphism), as previously described (Su et al. 2006; Ferreira et al. 2011). The genetic markers were SAG1, SAG2 (5′-and 3′-SAG2), nSAG2, SAG3, BTUB, GRA6, C22-8, c29-2, L358, PK1 and Apico. The set of reactions included: (1) multiplex PCR; (2) nested PCR; and (3) amplified product treatment with restriction enzymes. Each reaction set included two negative controls (ultrapure water and a negative DNA for toxoplasmosis); and 6 positive controls using the RH, GTI, MAS, VEG, ME-49 and PTG strains (as reference). After thermal cycles, PCR products were electrophoresed in 2.0–3.0% agarose gel,

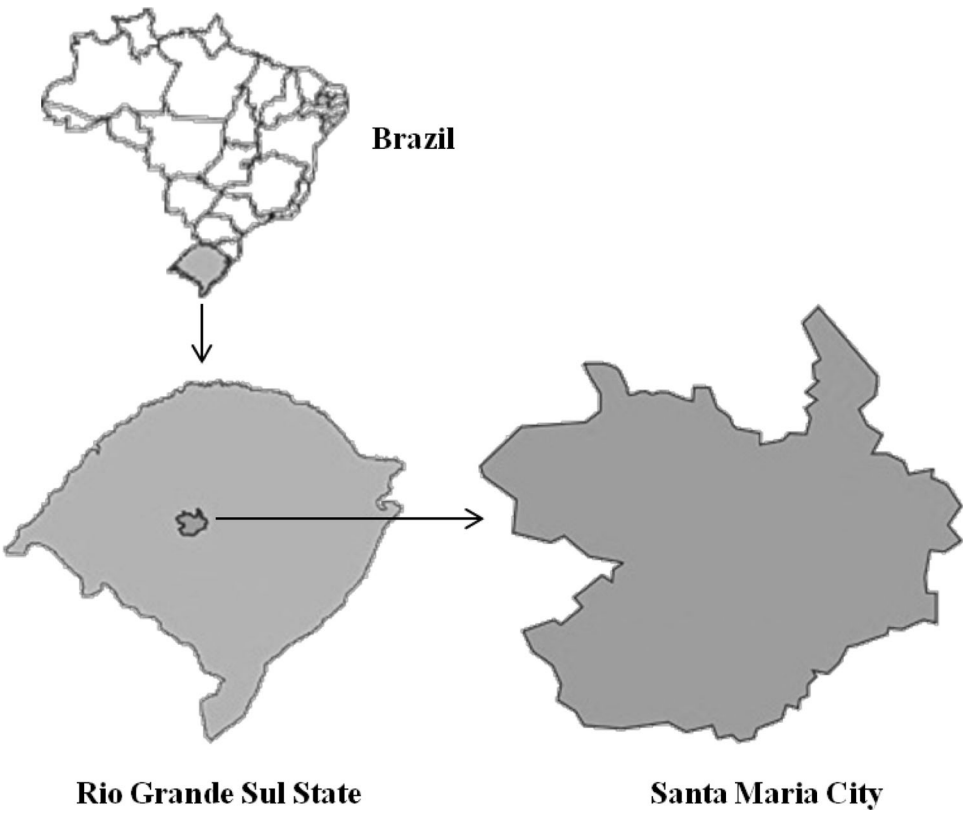


**Fig. 1** Santa Maria city, located in the west central region of Rio Grande do Sul state, southern Brazil, where heart, tongue and sausages samples were collected for *T. gondii* detection

stained with ethidium bromide and visualized under UV illumination.

### Data analysis

DNA profiles after restriction enzyme digestion were compared with those from reference strains. Next, they were compared, identified and matched to those listed in ToxoDB at http://toxodb.org/toxo/ and described in previous genotyping studies of Brazilian *T. gondii* isolates between 2010 and 2018.

## Results and discussion

Since 1967, several outbreaks have been described in different regions of Brazil. These outbreaks were associated with soil, raw meat, water and green vegetables (Magaldi et al. 1967; Coutinho et al. 1982; Bonametti et al. 1997; de Moura et al. 2006; Ekman et al. 2012). Recently, a considerable outbreak of human toxoplasmosis was identified in Santa Maria city, Rio Grande do Sul, Brazil. However, until now, the suspected vehicle of *T. gondii* contamination in this region still remains unclear.

In addition, our group found a higher prevalence of *T. gondii* DNA in the pork tongue samples (Belfort-Neto et al. 2007), and in pork products (Costa et al. 2018) from Rio Grande do Sul, South of Brazil. Besides the high prevalence of *T. gondii* DNA in meat samples in Brazil, *T. gondii* DNA was also described in pig farms in an area of intensive swine production in northern Italy (Gazzonis et al. 2018), and in sausages, smoked meat products, ham, and minced meat from Poland (Sroka et al. 2019). These studies may indicate the potential risk for consumers.

Considering the high prevalence of infected meat, this study was conduct to analyze whether pork meat samples collected in supermarkets from Santa Maria city, RS, could be infected with *T. gondii*.

Our results demonstrated that only 5% (1/20) of the pork tongues were qPCR positive for 529-bp marker. No positive tongues were observed for B1 marker (Table 1). *T. gondii* DNA was also detected in 10% (2/20) of pork heart samples by both 529-bp and B1 markers (Table 1). On the other hand, all sausage samples were negative for *T. gondii* infection using both B1 and 529-bp markers (Table 1). In contrast, in a recent publication, we showed a high prevalence of *T. gondii* DNA in sausages (47.5%) from Rio Grande do Sul (Costa et al. 2018).

Several studies have used 529-bp and B1 primer sets to detect and characterize *T. gondii* DNA in meat by qPCR (Marino et al. 2017; Papini et al. 2017; Vergara et al. 2018). Our study confirms that these primer set are good markers for the detection of *T. gondii* DNA in meat samples.

New non-archetypal genotypes of *T. gondii* have been described in pigs (Feitosa et al. 2017a; Bezerra et al. 2012) and in free-range chickens (Feitosa et al. 2017b) from northeastern Brazil; in pigs (Belfort-Neto et al. 2007) and in free-range chickens (Vieira et al. 2018) from southern Brazil; in free-range chickens from southeast Brazil (Dubey et al. 2003); and in free-range chickens from northern Brazil (Dubey et al. 2007, 2008). These strains are genetically distinct from the clonal type I, II, and III strains of *T. gondii* common to North America and Europe (Howe and Sibley 1995).

In our study, PCR–RFLP was performed in the 3 qPCR positive samples, after checking the quality and purity of extractions using the primer pair β1/β2. All of them were genotyped as: a type I allele at SAG1, BTUB, GRA6, c29-2, PK1 and Apico; type II allele at c22-8; and type III allele at 5′ + 3′ SAG2, nSAG2, SAG3 and L358 (Table 2). These results revealed a new non-archetypal *T. gondii* genotype (TgPkSMBra) in Santa Maria/RS, accordingly to the ToxoDB and the literature between 2010 and 2018 (Table 2).

**Table 1** Number of food samples and *T. gondii* positivity in qPCR

| Food samples | | Positive qPCR—n (%) | |
|---|---|---|---|
| | | Primer set | |
| Types | n | B1 | 529-bp |
| Tongue | 20 | 0 | 1 (5) |
| Heart | 20 | 2 (10) | 2 (10) |
| Sausage | 20 | 0 | 0 |

**Table 2** New *T. gondii* genotypes determined in food samples collected in Santa Maria city (RS, Brazil)

| Food samples | SAG1 | 5′ + 3′ SAG2 | nSAG2 | SAG3 | BTUB | GRA6 | c22-8 | c29-2 | L358 | PK1 | Apico | Genotype |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pork tongue (L1) | I | III | III | III | I | I | II | I | III | I | I | TgPkSMBra |
| Pork heart (C4) | I | III | III | III | I | I | II | I | III | I | I | TgPkSMBra |
| Pork heart (C12) | I | III | III | III | I | I | II | I | III | I | I | TgPkSMBra |

Our findings were not able to confirm whether these food samples were involved in some outbreak, since we detected only the parasite DNA by molecular methods (qPCR and PCR–RFLP). However, we can conclude that food samples containing *T. gondii* can be displayed in Santa Maria supermarkets. In addition, these samples had a good DNA quantity. Thus, a new *T. gondii* genotype circulating in southern Brazil was identified. Likewise, other studies are necessary to investigate the parasite viability and relationship between disease and the strain genotype.

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**Authors' contributions** RBJ, AGC, CS, MLRP and DFC designed the study. DFC, MLRP, RG, and VLPC carried out the laboratory experiment. While RBJ, AGC, DFC and VLPC drafted the manuscript. The final version of the manuscript was read, revised critically and approved by all the authors.

**Compliance with ethical standards**

**Conflict of interest** No authors have conflicts of interest to declare.

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