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Prevalence of *Toxoplasma gondii* DNA in Processed Pork Meat

Deise F. Costa,[1] Flávio Fowler,[1] Claudio Silveira,[2] Mario Junqueira Nóbrega,[3] Heloisa Amaral Junqueira Nobrega,[3] Heloisa Nascimento,[1] Luiz Vicente Rizzo,[4] Alessandra G. Commodaro,[1] and Rubens Belfort, Jr.[1]

Abstract

Toxoplasma gondii infection may be attributed to the ingestion of pork meat and contaminated water. In southern Brazil, the prevalence of blindness caused by *T. gondii* is the highest in the world. Our purpose is to determine the frequency of *T. gondii* DNA in commercial fresh sausage and cured salami samples from Rio Grande do Sul state, south of Brazil. A total of 118 samples (sausage and salami) from 8 different producers were collected and DNA was extracted. Real-time polymerase chain reaction (qPCR) technique was performed to detect *T. gondii* DNA using B1 marker. The frequency of *T. gondii* DNA among the total number of samples (sausage and salami) was 39% (46/118). Among these, a higher frequency of positivity was observed in the sausage samples (47.5%) when compared with the salami samples (17%). However, the mean parasite concentration was significantly higher in the salami samples. The prevalence of *T. gondii* DNA in fresh sausage and cured salami may indicate that infected pigs may be an important source of infections and a public health hazard to be considered.

Keywords: *Toxoplasma gondii*, real-time PCR, fresh sausage, cured salami

Introduction

The protozoan parasite *Toxoplasma gondii* is a relevant medical and veterinary pathogen, and an estimated one-third of the human population worldwide is chronically infected with *T. gondii* (Dubey, 2010).

In southern Brazil, about 80% of the population is seropositive for *T. gondii* infection and the frequency of ocular involvement is high (Silveira *et al.*, 2001). The cause for this incidence and severity of toxoplasmic infection in the southern region of Brazil remains unclear; one possibility might be the high contamination of pork meat (Belfort-Neto *et al.*, 2007).

In this study, our goal was to analyze the frequency of *T. gondii* DNA in fresh sausage and cured salami samples from Rio Grande do Sul (RS) state, Brazil.

Materials and Methods

Sample collection

A total of 118 samples (fresh sausages, $n = 59$ and cured salamis, $n = 59$) were purchased from 8 different producers, under municipal (local survey) and federal surveys. Samples were obtained from January to October 2015 in RS state, Brazil. All samples were stored individually at −20°C.

The typical ingredients of the fresh sausages and cured salamis included pork meat, salt, acids, nitrates, nitrites, and condiments.

Sample preparation and DNA extraction

After collection, a piece of sample weighting 2 g was cut in small pieces and added to 10 mL of buffer EB for digestion. It was repeated two more times with pieces from different parts of the sample to avoid false negative results. Then, total DNA was extracted from the sausage and salami samples using a commercial DNeasy Mericon Food Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol.

DNA amplification by real-time polymerase chain reaction (qPCR)

Real-time polymerase chain reaction (qPCR) was performed using SYBR Green Master Mix (Applied Biosystems, CA) and targeting the *T. gondii* B1 gene (Forward: AGA

[1]Department of Ophthalmology, Federal University of Sao Paulo, Sao Paulo, Brazil.
[2]Clinica Silveira, Rio Grande do Sul, Brazil.
[3]Department of Ophathalmology, University of Joinville, Santa Catarina, Brazil.
[4]Hospital Israelita Albert Einstein, Sao Paulo, Brazil.

GAC ACC GGA ATG CGA TCT and Reverse: TTC GTC CAA GCC TCC GAC T). Amplification was performed according to the manufacturer on an ABI Prism 7500 DNA sequence detection system (Ibrahim *et al.*, 2017). *T. gondii* DNA was supplied by Dr Michel Grigg, NIH, Bethesda/USA, and was used as positive control and molecular-grade water as negative control in the qPCRs.

Parasite quantification

Suspensions with sausage and salami samples (with PCR negative for *T. gondii*) and serial dilutions of tachyzoites were made ranging from 10^7 to 10^0 parasites to establish a standard curve of parasites. A standard curve was constructed according to Opsteegh *et al.* (2010).

Parasite quantification for each sample was performed in duplicate, and the predictor model was verified for its accuracy based on standard errors, determination coefficient (R^2), and correlation coefficient (r). This model was used to predict the number of parasites for each sample based on the observed number of cycles in qPCR.

Statistical analysis

The chi-square test or Fisher's test was used depending on the data analyzed. Generalized estimation equations were used to estimate and compare the mean parasite concentrations based on duplicate samples.

Analyses were performed using the R program (R Core Team, 2015) and the geepack package (Højsgaard *et al.*, 2006). $p < 0.05$ was considered significant.

Results and Discussion

In 2015, it was shown that the prevalence of *T. gondii* in cured ''Serrano'' ham in Spain was 8.84% (Gomez-Samblas *et al.*, 2015). Similar frequency was observed in Parana, southern Brazil, where 8.7% of the fresh pork sausages were positive for *T. gondii* (Dias *et al.*, 2005).

We analyzed the frequency of *T. gondii* DNA in fresh sausages and cured salami samples from southern Brazil. We demonstrated that among 118 samples, 39% (46/118) were qPCR positive for *T. gondii* (Table 1).

Therefore, the samples from producer 4 presented the highest frequency of *T. gondii* (60%), followed by samples from producer 8 (50%) and samples from producer 2 (40%). However, we did not observe a significant difference in the positivity among them ($p = 0.461$). We also observed that producers 3 and 1 presented the highest mean parasite concentration, 727.9 and 429.4, respectively (Table 1).

This high prevalence of *T. gondii* in southern Brazil was also described in pork meat samples from RS (Belfort-Neto *et al.*, 2007). They found that 34% of the diaphragm and 66% of the tongue samples were PCR positive for *T. gondii*.

A higher frequency of qPCR positive for *T. gondii* in sausage samples (61%) was observed in comparison with salami samples (16.9%) ($p < 0.001$). However, the mean parasite concentration in the salami samples (433.3) was significantly higher than the mean parasite concentration observed in the sausage samples (68.7) ($p = 0.006$) (Table 1).

The higher parasite concentration observed on the salami samples could be due to the different batches of pork meat used to make the sausages and salamis.

We have not checked the viability of the parasites in the sausage and salami samples. The effect of several processing conditions such as organic acids, nitrites, and nitrates, or the combination of salt, time, and temperature, during maturation, inactivated parasites (Mie *et al.*, 2008).

The ingredients of the sausages and salamis used for this work include salt, acids, nitrates, nitrites, and condiments, which could have affected the viability and vitality of *T. gondii*.

Recently, a study evaluated the *T. gondii* infection by the ingestion of several types of pork meat products consumed in Italy. It demonstrated that salt-cured meat products seem less risky when compared with consumption of fresh pork meat cuts that were associated with *T. gondii* infection in the Italian population (Condoleo *et al.*, 2017).

Toxoplasmosis is a cause of foodborne infections in Brazil. It may indicate that infected pigs may be an important source of infection and the need to decrease it to control the infection

Table 1. Distribution of Positive Samples and Mean Parasite Concentrations Among 118 Samples

Factors analyzed	*No. of samples*	*Positive samples* (N/%)	p	*Mean parasite concentration*	p
Type of samples					
Sausage	59	36 (61)	<0.001[a]	68.7	0.006
Salami	59	10 (16.9)		433.3	
Survey					
Federal	62	22 (35.5)	0.528[a]	81.1	0.466
Municipal	56	24 (42.9)		127.3	
Producers					
1	10	1 (10)	0.461[b]	429.4	
2	20	8 (40)		67.6	0.003
3	9	3 (33.3)		727.9	0.005
4	10	6 (60)		26.8	0.004
5	11	4 (36.4)		78.1	0.020
6	20	7 (35)		145.2	0.097
7	18	7 (38.9)		81.2	0.007
8	20	10 (50)		158.9	0.231
Total	118	46 (39)	—	102.6	—

Mean concentrations of parasites only for positive samples were obtained by generalized estimation equations. *p* Values for the chi-square ([a]) and Fisher's exact ([b]) tests.

rates. Even salt-cured pork meat products seem to pose a minor risk; further investigations are necessary to clarify the still unclear aspects.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Rubens Belfort, Jr. MD, PhD
Department of Ophthalmology
Federal University of São Paulo
Botucatu Street, 821, 1st Floor
São Paulo 04023-062
Brazil

E-mail: prof.belfort@clinicabelfort.com.br