Original Article

Molecular characterization of *Toxoplasma gondii* and *Sarcocystis* spp. in raw kibbeh and other meat samples commercialized in Botucatu, Southeastern Brazil

Caracterização molecular de *Toxoplasma gondii* e *Sarcocystis* spp. em amostras de quibe cru e de outras carnes comercializadas em Botucatu, Região Sudeste do Brasil

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Abstract

Toxoplasmosis occurs worldwide causing economic losses to the animal production and problems to the public health. The study aimed to detect *Toxoplasma gondii* and *Sarcocystis* spp.in 141 meat products from commercial meat cuts of pork, beef, and kibbeh sold in commercial markets from Botucatu, SP, Brazil. Samples were bioassayed in mice to isolate the parasite, and the parasite DNA detected by PCR targeting the 529 base pairs repeat element region (PCR-529-bp). All samples resulted negative on bioassay, whereas PCR positive for 9 (6,38%), distributed as 5/48 beef, 3/49 pork, and 1/44 kibbeh. PCR-positive were investigated for the the parasite genotype using multiplex-, nested-, and RFLP-PCR for 11 markers (SAG1, 5'-3'SAG2, alt.SAG2, SAG3, B-TUB, GRA6, L358, c22-8, c29-6, PK1, Apico). Complete genotype was determined on just one PCR-positive sam
le that matched MAS, TgCkBr89 and TgCkBr147 isolates already identified. In addition, nested- and RFLP-PCR targeting 18S rRNA was run for all PCR-positive samples and, the products, sequenced and aligned to the GenBank at NCBI website. Four samples showed 100% homology with *T. gondii* (GenBank #L37415.1), three with *Sarcocystis hominis* (GenBank #AF006471.1), two *Sarcocystis cruzi* (GenBank #AF176934.1), and one *Sarcocystis hirsuta* (GenBank #AF006469.1), indicating the circulation of *T. gondii* and *Sarcocystis* spp.

Keywords: Toxoplasmosis, sarcocystosis, meat products, food safety, public health, molecular techniques.

Resumo

A toxoplasmose está mundialmente distribuída e causa perdas na produção animal e problemas de saúde pública. Objetivou-se detectar *Toxoplasma gondii* e *Sarcocystis* spp. em 141 produtos cárneos de origem suína (49), bovina (48) e de quibe cru (44), comercializados em mercados de Botucatu, SP, Brazil. Realizou-se bioensaio das amostras em camundongos para isolamento do parasita, e detecção do DNA pela reação em cadeia pela polimerase, tendo como alvo a região do elemento repetitivo de 529 pares de bases (PCR-529-bp). Todas as amostras foram negativas ao bioensaio e 9 (6,38%) positivas à PCR, sendo 5/48 bovinas, 3/49 suínas e 1/44 quibe. Determinou-se a genotipagem das amostras positivas pela multiplex-, nested- e RFLP-PCR com 11 marcadores (SAG1, 5'-3'SAG2, alt.SAG2, SAG3, B-TUB, GRA6, L358, c22-8, c29-6, PK1, Apico). Obteve-se genótipo completo em uma amostra,

semelhante a outros já identificados (MAS, TgCkBr89 e TgCkBr147). Nested- e RFLP-PCR do gene 18S rRNA das amostras positivas à PCR foram realizadas, e os produtos da nested-PCR, sequenciados e alinhados com dados do GenBank no NCBI. Quatro apresentaram 100% de homologia com *T. gondii* (L37415.1), duas *Sarcocystis hominis* (AF006471.1), duas *Sarcocystis cruzi* (AF176934.1), uma *Sarcocystis hirsuta* (AF006469.1), indicando a circulação de *T. gondii* e *Sarcocystis* spp.

Palavras-chave: Toxoplasmose, sarcocistose, produtos cárneos, segurança alimentar, saúde pública, técnicas moleculares.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite, which causes toxoplasmosis, a worldwide distributed zoonosis, infecting homoeothermic hosts, including humans (Acha & Szyfres, 2003). Felids are the definitive hosts, while livestock animals develop tissue cysts (bradyzoites) in different tissues, e.g. the musculature (intermediate hosts). Contaminated food increases the importance of this infection to the public health. This anthropozoonosis can cause severe disease on newborns and immunosuppressed patients (Millar et al., 2007). Many different risk factors can be associated to this infection, including the ingestion of food containing the cysts as well as its handling by butchers and people who manipulate it.

Sarcocystis spp. are obligate protozoan parasites causing sarcocystosis, another worldwide distributed zoonosis. More than hundred species of *Sarcocystis* infect mammals, but only two are known to parasitize the human intestine, ie. *S. suihominis* and *S. hominis*. Other species, ie. *S. hirsuta, S. miescheriana, S. gigantea*, *S. cruzi*, and others, affect animals (Acha & Szyfres, 2003). All *Sarcocystis* spp. have obligatory heteroxenous life cycle. Carnivores and omnivores are usually definitive (sporogony – sexual stage) hosts while omnivores and herbivores are intermediate hosts (merogony – asexual stage). The definitive host acquires them upon ingesting meat from intermediate hosts infected with mature cysts (sarcocysts), containing the parasite (infective bradyzoites). The intermediate host acquires the infection upon consuming oocysts or mature sporocysts shed by the definitive hosts in their feces (Lindsay et al., 1995; Dehkordi et al., 2017).

It is extremely important to search for *T. gondii* (Acha & Szyfres, 2003) and *Sarcocystis* spp. (Dehkordi et al., 2017) in meat products used for human consumption since they constitute a transmission route responsible for food-borne outbreaks (Acha & Szyfres, 2003). The infection in livestock animals can be detected by serological tests and bioassay in mice (Kijlstra & Jongert, 2009).

Serology may be used to have a screening of the *T. gondii* infection (Acha & Szyfres, 2003). de Macedo et al. (2012) observed 48.3% dairy cows and 3.7% fetuses seropositive for *T. gondii* using the indirect fluorescent antibody technique (IFAT), while bioassay in mice resulted positive in only 10% dairy cows and 23.3% fetuses. Spagnol et al. (2009) reported 11.83% seropositive cattle among 600 slaughtered animals compared to 41.4% observed by Daguer et al. (2004) among 348 slaughtered animals. Furthermore, Da Rosa et al. (2011) observed 30.4% seropositive pigs among 353 sampled, while Da Silva et al., (2011) detected 10.96% seropositive slaughtered sheep among 602 sampled.

Since meat products participate to the transmission chain of toxoplasmosis to animals and humans, the present study aimed to detect *T. gondii* and *Sarcocystis* spp. in meat products sold at commercial markets in Botucatu, SP, Brazil.

Materials and Methods

Sampling

Meat samples were sampled from different markets from Botucatu, a city placed at the Mid-West region of Sao Paulo State, at the Southeastern Region from Brazil (22º52'20"S; 48º26'37"W). Samples of pork, beef cuts, and raw kibbeh were obtained from four butcher shops (A, B, C and D) and one supermarket, monthly, along 10 months to increase the diversity of the origin of the commercialized meat products sold by the suppliers. One hundred forty-one meat cuts were sampled, as follows: swine gammon (49), bovine shank (48), and raw kibbeh (44). Samples were individually packed in sterile plastic bags, identified, and stored in an isothermal box, at 4°C, and processed within 1 hour. The present study was approved by the Ethics Committee on Animals Use (CEUA) of the School of Veterinary Medicine and Animal Science, UNESP, Botucatu, SP (#155/2010).

Bioassay in mice

All meat samples were bioassayed in 30 day-old, female albino Swiss mice (*Mus musculus*). The samples were weighed (50 g) and eight fragments from different sites of each meat sample were pooled and mixed. Each pool (10 g) was individually ground, diluted 1:5 (w/v) in 0.18% saline solution and digested by the acid action of pepsin (Dubey, 1998). Aliquot of each pool (1 mL) was stored at –80°C for molecular assay. Five mice per pool were bioassayed with 1 mL suspension (per mice), using subcutaneous inoculation. Bioassayed mice were observed along 60 days. After the observational period, blood samples were drawn from all alive mice by the retro-orbital sinus puncture into a microtube without anticoagulant. The blood samples were centrifuged at 1600 x *g* for 10 minutes, and the sera sample searched for *T. gondii* IgG antibodies using a serological test with a cut-off 1:16. All died mice, or mice inoculated with meat cut positive for *T. gondii* DNA detection, had the brain removed and also searched of *T. gondii* DNA using molecular technique. Samples were considered positive after detected *T. gondii* DNA or *T. gondii* IgG antibodies.

Modified agglutination test (MAT)

T. gondii IgG antibodies were searched in the blood serum samples of bioassayed mice using modified agglutination test (MAT) (Desmonts & Remington, 1980), on 96-well U-shaped-bottom microplates (Cralplast, Brazil). The cut-off titer was 16. Positive result was considered after the formation of a pellicle covering at least half of the well, and negative when a sediment ("button" or a "ring") was formed at the bottom of the well.

Detection of T. gondii *using PCR of the 529 bp fragment (PCR-529-bp)*

DNA extraction and purification of the meat samples were done using the extraction kit Illustra Tissue and cells genomic Prep Mini spin kit (GE Healthcare, USA), according to the manufacturer instructions. The concentration and the quality of the extracted DNA were determined using a Nanovue spectrophotometer (GE Healthcare, USA).

PCR-529-bp was assayed using TOX4 and TOX5 primers, targeting 529-base pairs (bp) repeat element region, which is a 200- to 300-fold repetitive in the parasite genome, and result on an amplification product of 529-bp (Homan et al., 2000). All amplified products were visualized on electrophoresis using 1.5 agarose gel. The products were visualized using the digital documentation system, Gel-Doc-it System (UVP, USA), and VisionWorks Software (UVP, USA).

Genotyping

Genotyping was assayed using 11 genetic markers (SAG1, 5'-3'SAG2, alt-SAG2, SAG3, B-TUB, GRA6, c22-8, c29-2, L358, PK1 and Apico) as Su et al. (2006), Ferreira et al. (2006) and Pena et al. (2008). The marker CS3 was included in the present study to evaluate the virulence (Khan et al., 2005). Reference strains (GT1, PTG, ME49, CTG, M7741, TgCgCa1, MAS, and TgCatBr5) were used as controls. During genotyping, sequences of the target DNA were initially amplified by multiplex-PCR employing external primers for all biomarkers, followed by nested-PCR for individual biomarker (Su et al., 2006; Ferreira et al., 2006; Pena et al., 2008). In addition, possible co-infection with other Apicomplexa parasitic protozoa was determined using multiplex-, nested-, and RFLP-PCR of the 18S ribosomal RNA (18S rRNA) gene, according to Da Silva et al. (2009). The expected amplification product for *Sarcocystis neurona* (*S. neurona*), *Neospora caninum* (*N. caninum*), *Hammondia hammondi* (*H. hammondi*), and *T. gondii* was 290-bp, whereas for other *Sarcocystis* spp. was 310-bp. Positive controls for *Sarcocystis tenella* (*S. tenella*), *S. neurona*, *T. gondii*, *H. hammondi*, and *N. caninum* were employed.

All amplified and digested products were stained with ethidium bromide and electrophoresis using 2.5% or 3% agarose gel (depending on the marker used) was performed. The products were visualized using the digital documentation system, System Gel-doc-it.

Sequencing

The products of PCR for primers TOX4 and TOX5, and that of nested-PCR for gene 18S rRNA were purified using the purification kit Centrifugal Filter Units, MRCF0R030 (Millipore, USA) or ExoStar1-Step, US77705 (GE Healthcare, USA). DNA was quantified using the spectrophotometer Nanovue (GE Healthcare, USA), and electrophoresed on agarose gel.

Sequencing was run on an automated sequencer, Applied Biosystems 3100 (Applied Biosystems, USA), employing ABI BigDye kit and GE DYEnamic ET terminator kit (GE Healthcare, USA), and analyzed by the software Sequence

Analyzer, using Base Caller Cimarron 3.12. The obtained sense and antisense sequences were aligned and visualized using the software BioEdit Sequence Alignment Editor 7.0.9.0. Nucleotide sequences were analyzed using Nucleotide Basic Local Alignment Search Tool (BLASTn) and compared to those deposited on GenBank nucleotide database.

Statistical analyses

All data were tabulated on Excel spreadsheet. Fisher's exact test was used to analyze the association of the PCR results, the type of meat sample (beef, pork and kibbeh), and the origin of samples (butcher shops or supermarket). A significance level of 5% was adopted (Triola, 2005).

Results

PCR-529-bp resulted positive for 9/141 (6.38%; 95% CI 3.43-11.69%) meat samples, comprehending 5/9 (55.56%; 95% CI 26.24-81.29%) beef samples, 3/9 (33.33%; 95% CI 12.16-65.25%) pork samples, and 1/9 (11.11%; 95% CI 2.52-44.50%) kibbeh sample (p-value > 0.05). Concerning to the markets, 7/9 (77.78%; 95% CI 44.39-93.33%) PCR-positive samples were sold by butcher shops and 2/9 (22.22%; 95% CI 6.67-55.61%) from a supermarket (Table 1). All mice survived to the bioassay and resulted negative to the serology, including those bioassayed with PCR-positive meat samples.

A total of 3/7 (42.86%; 95% CI 15.70-75.51%) PCR-positive samples was commercialized by butcher shop A (two beef and one pork samples), while 3/7 (42.86%; 95% CI 15.70-75.51%) samples from butcher shop C (one beef, one pork, and one kibbeh samples), and 1/7 (14.29%; 95% CI 3.19-52.65%) from butcher shop B (one beef sample). No positive result for PCR was observed on meat samples from butcher shop D. Positive samples from the supermarket were from beef (one) and pork (one) (Table 2). No statistical difference was observed between the meat origin and the PCR-529-bp results (p-value > 0.05).

The genotyping of the nine PCR-positive samples resulted in just one (11.11%; 95% CI 2.52-44.50%) complete genotypic pattern, which matched to *T. gondii* (Table 3), specifically to MAS, TgCkBr89, and TgCkBr147 strains. In addition, 4/9 (44.44%; 95% CI 18.71-73.76%) samples (#51, #107, #116 and #119) resulted positive for nested-PCR (18S rRNA), amplifying 290-bp amplicons, which is suggestive of *T. gondii*. On the other hand, 5/9 other and different samples (55.56%; 95% CI 26.24%-81.29%) resulted a single ~310-bp amplicon to the same 18S rRNA target, a non-expected pattern. Just two of that (#115 and #118) presented RFLP-PCR pattern that suggested *S. hominis*. The remaining 3/5 samples (#100, #106, and #112) had no pattern to RFLP-PCR.

Sequencing of the nested-PCR amplicons for 18S rRNA gene matched *T. gondii* (GenBank accession number L37415.1) genome on four samples (44.44%; 95% CI 18.71-73.76%), confirming the expected above for those samples presenting 290-bp amplicons. Concerning the samples with ~310-bp amplicons, 2/5 (22.22%; 95% CI 6.67-55.61) matched *Sarcocystis cruzi* (*S. cruzi*) (GenBank accession number AF176934.1), one (11.11%; 95% CI 2.52-44.50%) matched *Sarcocystis hirsuta* (*S. hirsuta*) (GenBank accession number AF006469.1), and two (22.22%; 95% CI 6.67-55.61) matched *S. hominis* (GenBank accession number AF006471.1) (Table 2).

Table 1. Percentage *T. gondii* DNA detection using PCR-529-bp in beef, pork and kibbeh commercialized in markets from Botucatu, SP, Brazil.

Markets	N (%)	PCR-529-bp results		
		n	% of positive/market (95% CI)	% of positive/total (95% CI)
Butcher shop A	29 (20.57)	3	10.35 (3.76-26.53)	33.33 (12.16-65.25)
Butcher shop B	30 (21.28)	1	3.33 (0.79-16.70)	11.11 (2.52-44.50)
Butcher shop C	30 (21.28)	3	10.00 (3.63-25.75)	33.33 (12.16-65.25)
Butcher shop D	24 (17.02)	0	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Supermarket	28 (19.85)	2	7.14 (2.19-22.77)	22.22 (6.67-55.61)
Total	141 (100.00)	9	6.38 (3.43-11.69)	100 (-)

Legend: N: number of samples per each market; n: number PCR-positive samples; %: percentage; 95% CI: 95% confidence interval.

Table 2. DNA research for different parasites from meat products using different molecular techniques targeting 529-bp repeat element region and 18S ribosomal RNA (18S rRNA).

#	Sample	Market	529-bp repeat element region	18S rRNA gene				
			PCR	nested-PCR[†]	RFLP-PCR	Sequencing		
						GenBank	Identity	Agent
51	kibbeh	Butcher shop C	positive	~290bp	*Toxoplasma gondii*	L37415.1	100%	*Toxoplasma gondii*
100	beef	Butcher shop A	positive	~310bp	ND	AF176934.1	100%	*Sarcoystis cruzi*
106	beef	Supermarket	positive	~310bp	ND	AF006469.1	100%	*Sarcocystis hirsuta*
107	pork	Supermarket	positive	~290bp	*Toxoplasma gondii*	L37415.1	100%	*Toxoplasma gondii*
112	beef	Butcher shop B	positive	~310bp	ND	AF176934.1	100%	*Sarcoystis cruzi*
115	beef	Butcher shop C	positive	~310bp	*Sarcocystis hominis*	AF006471.1	100%	*Sarcocystis hominis*
116	pork	Butcher shop C	positive	~290bp	*Toxoplasma gondii*	L37415.1	100%	*Toxoplasma gondii*
118	beef	Butcher shop A	positive	~310bp	*Sarcocystis hominis*	AF006471.1	99%	*Sarcocystis hominis*
119	pork	Butcher shop A	positive	~290bp	*Toxoplasma gondii*	L37415.1	100%	*Toxoplasma gondii*

bp: base pairs; ND: result not determined; #: ID sample; [†]: base pair size. BLAST results: L37415.1: *T. gondii* 18S ribosomal RNA gene, complete sequence; AF176934.1: *S. cruzi* strain 12hcr60 18S ribosomal RNA gene, partial sequence; AF006469.1: *S. hirsuta* 18S ribosomal RNA gene, complete sequence; AF006471.1: *S. hominis* 18S ribosomal RNA gene, complete sequence.

Table 3. Genotypic characterization of *Toxoplasma gondii* from a PCR positive sample of kibbeh commercialized in Botucatu, SP, Brazil.

#	Sample	Survival (days)*	Genetic Markers												Genotype	Typing reference
			SAG1	5'-3'SAG2	alt-SAG2	SAG3	B-TUB	GRA6	c22-8	c29-2	L358	PK1	Apico	CS3		
51	Kibbeh	60	nd	I	II	III	III	III	u-1	I	I	III	I	nd	MAS	Su et al. (2006)
															TgCkBr89	Dubey et al. (2008)
															TgCkBr147	Dubey et al. (2008)

#: ID sample; nd: no data; u-1: unique-1. *Period of observation of mice = 60 days p.i.

Discussion

The search for zoonotic agents in meat for human consumption has high importance, considering its risk to public health (Acha & Szyfres, 2003). *In natura* meat products, and those derived from beef and pork, are important protein sources for humans. Livestock animals play an important role to the spread of *T. gondii* infection, since they harbor tissue cysts containing bradyzoites and constitute one of the possible transmission routes to humans. The ingestion of viable tissue cysts by a non-immune host may result in an infection with the parasite (Tenter, 2009).

Tissue cyst formation varies among animals and is related to the parasite organotropism (Dubey & Jones, 2008). The cysts present in carcasses and meat products remain viable and infective for a variable time. However, bioassay in mice requires a minimum infective dose, the virulence, and the absence of agents that inactivate the parasite to isolate the parasite (Mendonça et al., 2004).

The PCR-positive and bioassay-negative results in the present study indicate presence of the parasite DNA on the inoculated material, but its viability was compromised. The same was observed by Mendonça et al. (2004). These authors detected *T. gondii* DNA in 70 sausage samples sold in commercial markets, but all samples resulted negative to the bioassay in mice. Thus, the study on commercialized meat products must take into consideration the conservation period and status of the investigated meat. Although it is known that freezing inactivates bradyzoites. According to Bayarri et al. (2012), freezing is enough to inactivate *T. gondii* tachyzoites and bradyzoites.

The search for *T. gondii* in meat is important to evaluate the transmission chain of this protozoan, especially on raw kibbeh. Raw kibbeh is usually consumed *in natura*. This increases the risk to the consumers, once this type of meat is prepared from different species, i.e., sheep (Silva et al., 2003). Benitez et al. (2017) reported higher seroprevalence for *T. gondii* antibodies in dog owners (41.54%) from Londrina, PR, Brazil, than their dogs (16.32%) (p<0.01). Concerning some analyzed variables, 97.7% owners reported meat consumption and occasional undercooked meat in common dishes, ie barbecue (32.8%), fried meat (24.5%), and raw kibbeh (17,8%), while just 30.2% offered sporadically meat to the dogs. This important data reflects the importance of the food care after buying meat cuts or products even on butcher shops or supermarkets. The identification of *T. gondii* and *Sarcocystis* spp. in beef and sheep, as kibbeh, products does alert the population to adopt control measures and meat treatments before consuming or offering to the pets.

The consumption of kibbeh may be important to the occurrence of several food-borne outbreaks involving meat products, including toxoplasmosis (Bonametti et al., 1997; Cook et al., 2000; Kijlstra & Jongert, 2008). On a public health concern, Hashimoto Pugliesi et al. (2020) observed 16.9% pregnant women from Maringa, PR, eat raw or undercooked meat, 11.8% eat raw kibbeh, and 27.2% eat rare barbecue. Even with low rates, the *T, gondii* susceptibility is there. Bonametti et al. (1997) reported an outbreak with 17 human *Toxoplasma* cases after ingestion of raw kibbeh on a party in Bandeirantes, PR, Brazil. All patients presented *T. gondii* IgM and IgG antibodies.

T. gondii infection, with no co-infection, was detected in pork and kibbeh samples. Pigs and sheep are some of the most important animals related to the toxoplasmosis transmission chain, involving meat products (Tenter et al., 2000). In contrast to Brazil, livestock animal from Colombia are high exposed to the parasite. Franco-Hernandez et al. (2016) detected *T. gondii* DNA on 79/120 (43%) meat samples for human consumption in Colombia, being 33 from chicken, 22 from beef, and 24 from pork.

Concerning the meat products from cattle, *T. gondii* (PCR-529-bp) and *Sarcocystis* spp. (sequencing, 18S rRNA) were also detected in this study. Three distinct *Sarcocystis* species were identified, including *S. cruzi, S. hirsuta* and *S. hominis*. These results show the relevance of bovine meat to the public health, since *T. gondii* and *S. hominis* have a zoonotic impact. Cattle are intermediate hosts of *Sarcocystis* sp. (Carlton & McGavin, 2005), being affected all three identified species, e.g., *S. cruzi, S. hominis* and *S. hirsuta* (Lindsay et al., 1995). The macroscopic observation of the parasite on bovine musculature is crucial to diagnose the parasitosis during meat inspection at the slaughterhouse (Fortes, 2004).

Genotyping by employing 11 genotypic markers has been adopted worldwide for *T. gondii* DNA detection and analysis. Reports from South America have indicated significant genotypic variability identified by this technique, which allowed the characterization and phylogeny of different *T. gondii* isolates (Da Silva et al., 2009). Several studies have confirmed the high genetic diversity of the parasite in South America since it has been isolated from several animal species, such as goats from Ceará, Brazil (Cavalcante et al., 2007), dogs and cats from Colombia (Dubey et al., 2006a, Dubey et al., 2007a), and chicken from Nicarágua (Dubey et al., 2006b) and from Brazil (Dubey et al., 2007b). In Mid-West region of Sao Paulo State, Brazil, *T. gondii* isolates from sausages have already been characterized using molecular techniques (Vieira Da Silva et al., 2005).

According to the genotyping, *T. gondii* isolate was characterized as atypical and matched to MAS, TgCkBr89 and TgCkBr147, already identified isolates. MAS was isolated from a human patient in France, and it was classified as virulent (Grigg et al., 2001). In addition, TgCkBr89 was isolated from chickens from Rio de Janeiro State, Brazil (Dubey et al., 2003), whereas TgCkBr147 was isolated from chickens from Rio Grande do Sul, Brazil (Dubey et al., 2007b), both isolates virulent to mice and genotyped by Dubey et al. (2008). The other PCR-positive samples did not present RFLP-PCR patterns to determine the *T. gondii* genotype. This information suggests a low quantity of *T. gondii* DNA. Studies researching new genotypes in production animals must be encouraged in order to understand the dynamics of the distribution of classic and atypical *T. gondii* genotypes, contributing to the active vigilance and epidemiology of toxoplasmosis, especially using molecular epidemiology.

For the prophylaxis of toxoplasmosis, other measures are evidently related to the epidemiological transmission chain of this zoonosis, i.e., the care of definitive hosts like primoinfected felids (domestic cat). Domestic cat is

an important link to toxoplasmosis transmission chain since it shed oocysts on their feces, contributing to the environmental contamination, the spread and the maintenance of the infection among humans and animals. In addition, the education of cat owners, specially, limiting the hunting opportunities and avoiding feeding them with raw meat are easy to implement measures and may reduce acquiring new infections (Opsteegh et al., 2015).

Conclusions

The present study revealed the presence of *T. gondii* and *Sarcocystis* spp. in meat products commercialized in Botucatu, as well as the possible co-infection *T. gondii* and *Sarcocystis* sp. (*S. hominis, S. cruzi* or *S. hirsuta*) in beef. Special attention to *S. hominis* should be applied due to its zoonotic impact, as well as to the occurrence of *T. gondii* in beef and pork meat products.

Acknowledgements

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