Rev. Inst. Med. trop. S. Paulo
47(4):185-189, July-August, 2005

Toxoplasma gondii IN FRESH PORK SAUSAGE AND SEROPREVALENCE IN BUTCHERS FROM FACTORIES IN LONDRINA, PARANÁ STATE, BRAZIL(1)

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SUMMARY

The aims of this study were to verify the presence of *Toxoplasma gondii* cysts in fresh pork sausage and the presence of antibodies against *T. gondii* in serum of workers from factories with Municipal Inspection Service, in Londrina, PR, Brazil. 149 samples of sausage were collected from eight factories and blood samples from 47 workers. We also took information about the practices that were adopted in the factories and the workers' habits that could influence the prevalence of toxoplasmosis. After bioassay in mice, 13 (8.7%) sausage samples were positive, in one of them *T. gondii* was isolated and in the other 12 the mice seroconverted. Of 47 workers, 36 (76.6%) worked in sausage production and 11 (23.4%) were involved in other functions; 59.5% (28/47), 55.5% (20/36) and 72.7% (8/11), respectively, had *T. gondii* antibodies. There were no significant differences in the variables of industries' practices and workers' habits related to *T. gondii* infection. We concluded that fresh pork sausage could be important in the transmission of toxoplasmosis.

KEYWORDS: *Toxoplasma gondii*; Swine; Sausage; Butcher; Bioassay.

INTRODUCTION

Toxoplasmosis is an infection caused by *Toxoplasma gondii*, an obligatory intracellular parasite distributed worldwide that attacks warm-blooded animals, including human beings[13]. Felines are the definitive hosts where the parasite carries out its sex cycle resulting in oocysts that are taken to the environment by the feces[4]. Both man and animals acquire the infection by ingesting oocysts that contaminate water, foodstuffs and pastures in the environment, by hunting or ingesting raw or uncooked meat containing *T. gondii* cysts or by placenta[9,23]. In man the symptoms predominate in individuals with damaged immune systems, congenitally infected children and in those with eye toxoplasmosis[5]. The infection acquired orally is considered the main form of dissemination of the pathogen in the human and animal population[7]. *T. gondii* outbreaks are not frequent but the infection is very common. However, the diseases could be severe or fatal and occurs mainly in immunosuppressed people, children who became infected congenitally and people with the ocular form[9].

Prevalence of *T. gondii* in market pigs is worldwide[8,20]. In Brazil, in Paraná State, the seroepidemiological investigation of *T. gondii* on farms in the Londrina region revealed a prevalence of 37.84% in the early 1990s, ten years later this rate had decreased to 15.35%[21,23]. Similarly, in 424 samples of swine blood collected in 13 slaughterhouses throughout Paraná the prevalence was 4%[2]. The presence of *T. gondii* cysts in pork, viscera and by-products has also been studied. Bioassay verified 20.26% positiveness in 153 meat and brain samples from butcheries in the municipality of Londrina, PR[15]. The presence of the parasite was detected in 28 out of 40 pieces of commercial pork from animals experimentally infected with *T. gondii*; of these, 26 were positive by the bioassay, nine by the Polymerase Chain Reaction (PCR) and seven by both methods[22]. In another study, 33 out of 70 fresh pork sausage samples obtained from commercial establishments in the municipality of Botucatu, SP were positive by PCR but not by the bioassay[14].

Regarding workers in swine slaughter and sausage production, butchers were assessed in Finland and Egypt and 25% and 52.4% positiveness to *T. gondii* was detected, respectively[12,19]. In Brazil, Minas Gerais State, the prevalence of anti-*T. gondii* antibodies in workers from a swine slaughterhouse was 72%[17]. The *T. gondii* dissemination and the fact that the tissue cysts are not visible in post-mortem inspection, make pork and its by-products potential to toxoplasmosis transmission in man.

The objectives of this study were to determine the presence of *T. gondii* cysts in fresh pork sausages, produced in factories with Municipal Health Inspection (SIM) in Londrina-PR, to verify the

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prevalence of anti-*T. gondii* antibodies in employees in these factories and to associate the epidemiological data to the serological results.

MATERIAL AND METHODS

1. Study location: The analyses were performed in the Animal Protozoon and Zoonoses and Public Health laboratories in the Department of Preventative Veterinary Medicine at Londrina State University.

2. Collection location: Fresh pork sausage samples, manufactured from pork meat, and blood samples from the workers were collected in eight factories in the municipality of Londrina, PR. Factories inclusion criteria were the consent of the owners and that they use the Municipal Health Inspection.

3. Sampling: The universe to be sampled was two months production, time stipulated for sausage sample collection, totaling 184 batches. The Epi Info 6.04 statistical package was used for the calculation[3], a 15% prevalence was estimated at 2.5% precision and 95% confidence level, thus 149 samples were obtained.

These samples were collected in December 2002 and January 2003 on the week days when fresh pork sausages were produced. The amount of samples that were collected from each factory was realized according to its production capacity. About 300 g of sausage meat was obtained, placed in a plastic container and transported to the laboratory at 10 ºC.

Blood was collected by an auxiliary nurse using a vacuum blood collector without anticoagulant from 47 workers in all the factories involved, 36 of them linked to sausage production and 11 from other sectors. The blood collected was kept at 56 ºC for 30 minutes and later the serum was separated, placed in Ependorf® and stored at –15 ºC.

4. Research instruments: Three questionnaires were used to obtain the epidemiological data. One of them for data of sausage samples (temperature, day and time of production), other about the production, products and health environment of the factory and the other for each worker that included habits during production and lifestyle.

5. Bioassay: Five hundred Swiss albino mice, average weight 25 g, were used for the bioassay. The sausage samples were processed on the day they were collected with a maximum interval of 30 minutes between collection and the start of processing. Each sample (50 g) was submitted individually to peptic digestion. The material resulting from the digestion was inoculated in three mice that were observed daily. Those that presented clinical signs were put down to collect the peritoneal liquid and observed tachyzoite presence. In its absence, brain, liver and spleen were squashed and inoculated in a further three mice. At the end of six weeks, the surviving animals were put down and the blood collected for later serology[15]. Brain fragments were removed from these animals and placed between slides and slide covers for tissue cyst research in optical microscopy. The mice were considered negative to *T. gondii* in the absence of the cysts in brain and of specific serum antibodies.

6. Indirect Fluorescence Antibody Test: The sera stored at -15 ºC were researched for anti-*T. gondii* IgG class antibodies by the Indirect Fluorescence Antibody Test (IFAT). Conjugates marked with fluoresceine specific for human and mice (Sigma Chemical Co.) were used; dilutions greater or equal to 1:16 were considered positive for both species[15].

7. Statistical analysis: The results obtained from the questionnaire and the serology was submitted to statistical analysis by the Chi-square test, using the Epi Info 6.04 statistical package. A confidence level of 95% was adopted[3].

RESULTS

13 of the 149 sausage meat samples assessed by the bioassay technique (8.72%; CI 95%: 4.72 - 14.45) were positive and the parasite (tachyzoites) was isolated in one of them. All the other inoculated mice remained alive until end of the six week observation period and positive IFAT was obtained in 12 (Table 1).

In the sample where there was isolation by the bioassay in mice, one of the three animals inoculated died and the other two were slaughtered, and in these the presence of *T. gondii* tachyzoites was ascertained in peritoneal exudates, by optical microscopy.

Table 1
Toxoplasma gondii detection by bioassay in mice in fresh pork sausage samples collected in factories with Municipal Health Inspection, Londrina, PR, 2003

Factory	Workers		Factory	Week sausage	Sausage samples		Prevalence %		Positive sausage samples	
	Production	Office	area	production (Kg)	Collected	Positive	Factory	Total	*T.gondii* *	IFI**
A	02	01	Until 100 m²	51 to 200	07	0	0	0	—	—
B	06	01	Until 100 m²	201 to 500	18	03	16.7	2.01	01	02
C	08	01	Until 100 m²	Upper 500	25	02	8.0	1.34	—	02
D	03	01	Until 100 m²	51 to 200	10	02	20.0	1.34	—	02
E	04	0	Until 100 m²	51 to 200	10	02	0	0	—	—
F	02	01	Until 100 m²	51 to 200	09	01	11.1	0.68	—	01
G	11	06	101 to 500 m²	Upper 500	25	02	8.0	1.34	—	02
H	#	#	Until 100 m²	51 to 200	45	03	6.7	2.01	—	03
Total	36	11			14	13		8.72	01	12

Not realized; * Tachyzoite isolation; ** Indirect Fluorescence Antibody Test (title ≥ 16)

All the swine destined for fresh sausage production were slaughtered in slaughterhouses with official inspection; 75% of the factories acquired the animals specifically for this purpose and 25% used scraps left over from pork commercialization, also 12.5% of the factories sporadically used beef scraps in fresh sausage manufacture.

Of the 47 workers, 59.5% (28/47; CI 95%: 44.96 - 73.63) were positive to *T. gondii*. Among the 36 that worked in sausage handling and production, 55.5% (20/36; CI 95%: 38.09 - 72.06) were positive and 72.7% (8/11; CI 95%: 39.02 - 93.97) of the 11 that performed administrative tasks and product transport were positive. All the workers were over than 25 years old.

The serological titers for the workers linked to sausage handling and production were: one with 16, eight with 64, ten with 256 and one with 1024. The titers of those involved in administrative and transport functions were: three with 16, two with 64 and three with 256.

There was no statistically significant difference in the characteristics studied for *T. gondii* presence in fresh sausage and for the presence of anti-*T. gondii* antibodies in the workers (Tables 2 and 3).

Table 2
Results of *Toxoplasma gondii* presence in fresh pork sausage samples associated with the characteristics of production and distribution of the eight factories with Municipal Health Inspection, Londrina, PR, 2003

Variables	Samples Positives/Total (%)	p value	Chi-square (χ^2)
Week production of sausage:			
51 to 200 kg	6/81 (7.4)	0.441	1.64
201 to 500 kg	3/18 (16.6)		
> 500 kg	4/50 (8)		
Storage's temperature of processed meat:			
0 to 3 °C	2/35 (5.7)	0.398	1.84
4 to 10 °C	8/96 (8.3)		
11 to 25 °C	3/18 (16.6)		
Storage's temperature of the product that will be inlay:			
0 to 3 °C	2/35 (5.7)	0.449	1.60
4 to 10 °C	8/95 (8.4)		
11 to 25 °C	3/19 (15.7)		
Use of swine's viscera in sausage's production:			
Yes	6/62 (9.6)	0.957	0.00
No	7/87 (8)		
Kinds of viscera used:			
Brain			
Yes	0/10 (0)	0.601*	-
No	13/139 (9.3)		
Tonghe + intestines			
Yes	6/52 (11.5)	0.377*	-
No	7/97 (7.2)		
Sausage storage's temperature:			
0 to 3 °C	4/60 (6.6)	0.174	3.50
4 to 10 °C	3/52 (5.7)		
11 to 25 °C	6/37 (16.2)		
Kind of fresh sausage:			
Pure pork	3/19 (15.7)	0.270	3.92
Type "Toscana"	1/3 (33.3)		
Type herbs	6/82 (36.5)		
Pure pork + herbs	3/45 (66.6)		
Products' validity:			
8 to 15 days	3/52 (5.7)	0.157	3.69
16 to 20 days	5/28 (17.8)		
> 20 days	5/69 (7.2)		
Presence of felines:			
Yes	5/38 (13.1)	0.317*	-
No	8/111 (7.2)		

* Fisher

Table 3
Results of *Toxoplasma gondii* serology (IFAT-IgG) in workers from eight factories that produce fresh pork sausage, associated with their habits and lifestyle, Londrina, PR, 2003

Variables	Workers Positives/Total (%)	p value	Chi-square (χ^2)
Work at sausage production:			
Yes	20/36 (55.5)	0.484*	
No	8/11 (72.7)		
Working time at production:			
Until 2 hours / day	3/3 (100)	0.382	3.06
3 to 5 hours / day	1/3 (33.3)		
6 to 8 hours / day	7/13 (59.8)		
More than 8 hours / day	9/17 (52.9)		
Eat the sausage producted:			
Yes	27/44 (61.3)	0.557*	
No	1/3 (33.3)		
Time of working in the factory:			
until 1 year	7/12 (58.3)	0.611	1.82
2 to 4 years	11/19 (57.8)		
5 to 7 years	5/6 (83.3)		
> 7 years	5/10 (50)		
Use to eat undercooked meat:			
Yes	20/30 (66.6)	0.314	1.01
No	8/17 (47)		
Use to taste the meat during the sausage production:			
Yes	8/15 (53.3)	0.909	0.01
No	12/21 (57.1)		
Use to eat undercooked sausage:			
Yes	10/15 (66.6)	0.719	0.13
No	18/32 (56.2)		
Work only in the sausage production:			
Yes	14/24 (58.3)	0.715	0.13
No	6/13 (46.1)		
Have cats in home:			
Yes	2/3 (66.6)	1.000*	
No	26/34 (47)		
Have contact with cats:			
	2/2 (100)	0.507*	
	26/45 (57.7)		
Worker's gender:			
Male	25/43 (58.1)	0.637*	
Female	3/4 (75)		

* Fisher

DISCUSSION

Pork and its byproducts are important in the toxoplasmosis transmission chain to human beings[7], this statement can be proved by the positiveness of 13 (8.72%) fresh pork sausage samples among the 149 samples collected in this study, including one isolate. In contrast, studies carried out in Botucatu, SP that compared the Bioassay and PCR techniques did not detect viable cysts in any of the 70 samples of fresh sausages collected in various commercial establishments in that municipality. Only *Toxoplasma gondii* DNA segments were detected by PCR in 33 samples that indicated the presence of the parasite but not its viability[14].

This difference may be due to several factors, including the fact that the samples in this study were collected directly in the factories that produce fresh pork sausage, where the production and commercialization process is fast so that the sausage meat has a short contact time with the added sodium chloride. It is known that a quantity of 2.0 to 2.5% salt acting for 48 h is sufficient to make *T. gondii* cysts non viable. Moreover in all the sausage's production, including delivery, the temperature remains upper than 0 ºC, which is essential for the parasite survival. Another relevant characteristic is that some of these industries did not measure the quantity of seasonings added, that could lead to the use of smaller quantities of sodium chloride than recommended. Because the population is not informed, fresh pork sausages consumed without proper heat treatment (> 67 ºC) may become potential transmission route for *T. gondii* to man[16].

No significant differences were observed among the variables related to sausage production and the positiveness of the samples. Thus factors such as using of swine viscera (brain, tongue and intestine) in sausage manufacture would not influence the presence of the parasite. The type of fresh pork sausage produced using different varieties of seasonings did not lead to positive association with parasite presence in the samples assessed, and neither did the presence of cats in the producing factories. The origin of swine from independent farmers or slaughterhouses used in sausage production was not a determining factor for sample positiveness. Similarly, the use of pork scraps in sausage manufacture did not give positiveness.

A prevalence of 59.5% was obtained among all the 47 workers assessed in the factories. A different situation was reported in Finland, where a seroprevalence of 25% was obtained in 159 butchers[19]. Prevalence close to that reported in this study was detected in Tanta in Egypt with 52.4% positiveness among 21 workers in a slaughterhouse.

There was no significant difference among the workers involved in administrative activities and those connected to sausage handling and production, although the positiveness rate was 72.7% (08/11) and 55.5% (29/36), respectively. In a study in a slaughterhouse in Belo Horizonte, MG, 80% seroreagents were obtained among the workers directly involved in sausage production[17]. A study with 345 residents in the rural area of the municipality of Jaguapitã, PR obtained 66% positiveness prevalence to *Toxoplasma gondii* by the indirect immune fluorescence technique among the study population[11]. In a seroepidemiological survey to ascertain the occurrence of reagents to *Toxoplasma gondii* among residents in the rural region of the municipality of Guaraci, PR, 115 samples were analyzed by IFAT showing 71.3% positiveness[10].

There was no significant difference related to washing hands after production. Similarly there was no significant difference related to eating the factory's sausage.

Regarding the serology titers of the reagent workers to *T. gondii* it was ascertained that most had low titulation suggesting a chronic infection condition. It is relevant that the workers were in the over 25 years old age group, since toxoplasmosis prevalence increases with age[18]. There was no association among the variables related to the workers´ habits and positiveness to *Toxoplasma gondii,* thus it is probable that these individuals had acquired the infection in a situation prior to working in the fresh pork sausage factory.

No association was ascertained between the workers´ habit of tasting the raw meat for sausage during production and positiveness to *T. gondii*. Because 30 of the 47 workers said they had the habit of consuming raw or insufficiently cooked meat, it was inferred that these food habits may have caused *T. gondii* infection outside the production environment of the factories. A population of Seventh Day Adventist individuals were studied whose nutritional characteristic is the non-ingestion of meats or their by- products and it was detected that this population presented a lower risk of infection by *T. gondii* than the control individuals of the same geographic area that did not follow the same diet[18].

RESUMO

Toxoplasma gondii em lingüiças frescas de porco e soroprevalência em açougueiros de fábricas de Londrina, Estado do Paraná, Brasil

Os objetivos deste estudo foram verificar a presença de cistos de *Toxoplasma gondii* em lingüiça de origem suína tipo frescal e de anticorpos anti-*T. gondii* no soro de trabalhadores de indústrias produtoras, com Serviço de Inspeção Municipal (SIM) de Londrina, PR. Buscou-se ainda, obter informações relativas às práticas adotadas nestas indústrias e aos hábitos dos trabalhadores, que pudessem influenciar na prevalência desta parasitose. Foram coletadas 149 amostras de lingüiça em oito indústrias produtoras e 47 amostras de sangue de todos os trabalhadores destes locais. Após a realização do bioensaio em camundongos, obteve-se 13 (8,72%) amostras de lingüiça positivas, sendo que em uma o parasita foi isolado e nas outras 12 os camundongos soroconverteram. Dos 47 trabalhadores, 36 (76,6%) atuavam na produção de lingüiça e 11 (23,4%) exerciam outras funções; os percentuais de soropositivos ao *T. gondii* foram, respectivamente, 59,5% (28/47), 55,5% (20/36) e 72,7% (8/11). Não houve diferença significativa entre nenhuma das variáveis relacionadas às indústrias e aos trabalhadores. Os resultados permitem inferir que lingüiças tipo frescal possuem importância na cadeia epidemiológica da toxoplasmose no Município de Londrina-PR.

ACKNOWLEDGEMENTS

The authors thank Ademir José da Silva, Elisabete Marangoni Marana and Mario Sergio Espadar Pereira.

Study funding:

1. Post Graduation and Research Coordination Department of Londrina State University (CPG-UEL).
2. Council for Development of University Staff (CAPES).

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Received: 30 September 2004
Accepted: 2 June 2005