

ARTÍCULO ORIGINAL

## *Detection of Toxoplasma gondii in swine sausages*

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### ABSTRACT

*In order to evaluate the importance of swine sausages in toxoplasmosis epidemiology, Toxoplasma gondii presence was investigated in 70 samples of the product commercialized in the city of Botucatu-SP. Samples were analyzed by bioassay in mice and DNA amplification by Polymerase Chain Reaction (PCR). Although the parasite was not isolated from any sample in the bioassay, 33 (47.14%) samples were positive in the PCR. These results indicate that swine sausages probably have low importance as a source of infection for human toxoplasmosis in the studied region. Nevertheless, the great number of PCR positive samples shows that the protozoan may be present, but may be inactivated by salt added in sausage manufacture.*

**Key words:** *Toxoplasma*, swine sausage, PCR, isolation.

### INTRODUCTION

Toxoplasmosis is an infection, sometimes a disease, caused by the protozoan *Toxoplasma gondii*<sup>1</sup>. Cats are definitive hosts, while human, other mammals and birds are intermediate hosts<sup>2</sup>. Human toxoplasmosis is widespread with infection ranges varying from zero to 100%<sup>3</sup>. In Brazil, studies indicate rates of up to 81%<sup>4</sup>.

Among food animals, pigs are considered to be the major meat source of *T. gondii* for humans in the United States<sup>5-7</sup>. In Brazil, toxoplasmosis in pigs was diagnosed for the first time in 1959<sup>8</sup>. Nowadays, prevalence of infection in swineherds varies widely from place to place<sup>9</sup>.

The detection of *T. gondii* in meat is

fundamental in order to assess its importance as a source of infection. Serological studies solely are insufficient, since seropositive animals are not necessarily potential sources of the agent to humans<sup>10</sup>.

Martins et al. isolated *T. gondii* from only one of 40 swine sausage samples commercialized in the city of Erechim-RS<sup>11</sup>. In an attempt to elucidate the difficulty to isolate the agent, Jamra et al. studied salt effect on the viability of *T. gondii* cysts and tachyzoites, and concluded that salt (in a concentration equal to 3%) inactivated the parasite when exposure time was at least three days<sup>12</sup>.

On the other hand, Navarro et al. studying sausages manufactured from pigs experimentally inoculated with *T. gondii*, concluded that salt in

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concentration equal to 2.0% and 2.5% inactivated the parasite in 48 hours of the beginning of the cure process<sup>13</sup>.

The aims of the present study were to detect the presence and viability of the protozoan *T. gondii* in swine sausage samples and to evaluate the importance of this kind of food in the epidemiology of toxoplasmosis.

## MATERIALS AND METHODS

Seventy swine sausage samples (weighting at least 50 g each) were collected from 55 commercial establishments in the city of Botucatu-SP. Samples were identified and transported under refrigeration to NUPEZO's ("Núcleo de Pesquisas em Zoonoses") laboratory, where they were analyzed.

For the bioassay in mice, sausages were digested by pepsin<sup>10,14</sup>. For each sample, five mice (Swiss, albino, 30 days old) were inoculated with one milliliter of the digested sample by subcutaneous route. Mice were observed for up to 45 days. Samples of lung, liver, spleen and brain were collected from mice that died during the observation period for cytological analysis, and were stained by Giemsa. After the observation period, blood samples from surviving mice were collected to serologic analysis (indirect fluorescent antibody assay - IFA<sup>15</sup>).

PCR was performed according to a recommended protocol<sup>16</sup>. For each sample, DNA extraction was performed in sausages submitted or not to pepsin digestion.

In order to compare results between digested and non-digested samples, the Qui-square and McNemar tests were used to evaluate success proportions in dependent samples<sup>17</sup>.

## RESULTS

All samples were negative in the bioassay.

From 350 mice inoculated (five mice per sausage tested), nine died during the observation period. They belonged to different groups and none of them showed positive reaction to *T. gondii* in the cytological tests. In the other 61 groups, all mice survived until the end of the observation period. Serum samples were collected from them to detect antibodies against *T. gondii*. However, none was positive up to a dilution equal to 1:16 (Square 1).

In relation to PCR, 33 samples (47.14%) were positive. Twenty-one (30.0%) and 25 (35.71%) were positive using, respectively, digested and non-digested samples. Among positive samples, 13 (39.39%) were positive in both treatments, 12 (36.36%) only in non-digested samples, and eight (24.24%) only in digested samples (Table 1 and Figure 1). Statistical analysis did not show significant difference between treatments ( $\chi^2 = 0.45$ ;  $p = 0.50$ ;  $k = 0.3548$ ).

## DISCUSSION

In the present study, viable *T. gondii* was not detected in any sample. However, results obtained in the PCR assay showed its presence in almost half of the samples (47.14%).

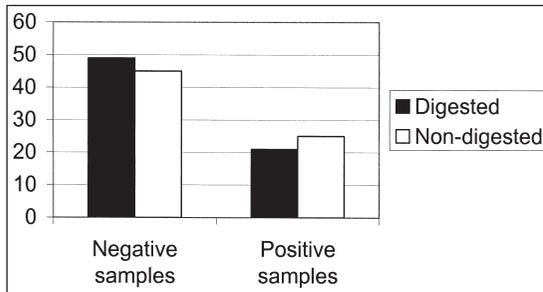
In relation to PCR methodology, primers used in the present study identify a 200 - to 300 - fold repetitive 529 bp DNA fragment in the *T. gondii*

**Table 1. Comparison between the number of positive and negative samples observed in *Toxoplasma gondii* detection in swine sausages using PCR, according to the treatment of the sample. Botucatu-SP, 2003**

	Samples digested by pepsin	Non-digested samples
Positive samples	21	25
Negative samples	49	45
Total	70	70

**Square 1. Results of bioassay in mice for *Toxoplasma gondii* detection in swine sausages. Botucatu-SP, 2003**

Number of samples	70
Number of mice inoculated per sample	05
Number of mice that died during the observation period	09
Number of samples positive to the presence of <i>T. gondii</i> collected from mice that died during the observation period, according to the cytological tests	00
Number of mice that survived after the observation period	341
Number of samples positive to the presence of antibodies against <i>T. gondii</i> in surviving mice, according to serological tests	00



**Figure 1.** Detection of *Toxoplasma gondii* in swine sausages, using PCR, in digested and non-digested samples. Botucatu-SP, 2003.

genome. In such case this methodology is much more sensitive than the amplification of gene B1, one of the most used at the moment, according to Homan et al<sup>16</sup>. These authors have tested the primers using several parasites genetically close to *T. gondii* and did not detect any cross-reaction. The influence of spices used in the curing process on the viability of *T. gondii* cysts has been studied since the 70's. Venkatachalam & Zimmerman studied *T. gondii* viability in relation to different meat processing methods. They inoculated 18 pigs and then prepared some foods with their meat, including sausage. *T. gondii* was not isolated from the sausages and the authors concluded that nitrite, nitrate and salt used in the cure process inactivated the parasite<sup>18</sup>.

Navarro et al. evaluated *T. gondii* viability in relation to different swine sausage curing processes<sup>13</sup>. The protozoan was isolated from all salt-free samples. These sausages were seasoned using garlic and black pepper, showing that these condiments do not inactivate the cysts. In relation to samples treated with different salt concentrations (1.25%, 2.00% and 2.50%), the greatest the salt concentration and its time of exposure, the most difficult the *T. gondii* isolation. It was concluded that it is possible to isolate *T. gondii* from swine sausage cured with salt in concentrations commonly used in industrial and domestic manufacturing, in up to 24 hours of refrigeration<sup>13</sup>.

Jamra et al, studied the viability of *T. gondii* cysts and tachyzoites submitted to different salt concentrations. Parasites were obtained from mice inoculated previously. The authors concluded that salt concentration equal to 3.0% was enough to inactivate cysts and tachyzoites after at least three days of exposure<sup>12</sup>.

In the present study, parasites present in the sausages, whose DNA was amplified by PCR, probably were inactivated by the salt, what is in accordance with the studies mentioned above.

Further studies on the importance of swine meat to toxoplasmosis epidemiology in Brazil are necessary as this meat represents an important *T. gondii* source to human infection in other countries.

## RESUMEN

Con el objeto de estudiar la importancia de la longaniza de cerdo en la epidemiología de la toxoplasmosis, se investigó la presencia de *Toxoplasma gondii* en 70 muestras comercializadas en Botucatu-SP. Las muestras fueron procesadas por las pruebas de aislamiento in ratones y por la amplificación del ADN por la Reacción en Cadena de la Polimerasa (PCR). Aún que el *T. gondii* no ha sido aislado de ninguna muestra por la prueba de aislamiento in ratones, 33 (47,14%) muestras han sido positivas por la PCR. Estos resultados indican que la longaniza de cerdo probablemente tiene poca importancia como fuente de infección para la toxoplasmosis humana en la región estudiada. Mientras tanto, el gran número de muestras positivas por la PCR indica que el protozoario puede estar presente, pero es inactivado por la sal usada en el aderezo de las longanizas.

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