Research Note

Toxoplasma gondii in Commercially Available Pork Meat and Cured Ham: A Contribution to Risk Assessment for Consumers

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ABSTRACT

Toxoplasmosis is an infection caused by Toxoplasma gondii, whose transmission has usually been attributed to ingestion of undercooked or raw meat. Dry-cured ham is a high-quality meat product of increasing economic relevance, and epidemiological studies point to cured meat products as a risk factor for acquiring toxoplasmosis. With the aim of contributing to the risk assessment process, 50 samples of fresh pork meat and commercial cured ham were collected in the city of Zaragoza (northeastern Spain), and the presence of viable forms of T. gondii was analyzed. A mouse concentration bioassay technique was used, and the presence of the parasite in mice was determined by indirect immunofluorescence assay. T. gondii was detected in two samples of rib, reflecting a frequency of 8% positive fresh pork meat (4% positivity of total samples analyzed). Brains of seropositive mice were analyzed by histology and PCR, although the parasite was not isolated in the seroconverted mice. No viable forms were detected either in other types of fresh meat or in the samples of cured ham.

Toxoplasmosis is one of the most common parasitic zoonoses worldwide. Its causative agent, Toxoplasma gondii, is a protozoan that has developed several potential routes of transmission between different host species. Ingestion of oocysts, congenital infection, and ingestion of undercooked infected tissues are the three main modes of transmission of T. gondii (12). Infection can produce a severe disease in immunocompromised people and abortions in pregnant women, as well as perinatal death, fetal abnormalities, or reduced quality of life in children who survive prenatal infection (4, 12, 15).

The consumption of raw or undercooked pork meat containing tissue cysts, which can persist for a long time, is considered one of the principal sources for human infection (5, 9, 23). Any part of infected pork can be a source of infection; the parasite has been found in the seemingly most edible tissues or cuts of meat (12). Despite the accumulating evidence for the role of meat in human exposure to T. gondii, little information about the presence and viability of the parasite in retail meats and meat products is available to evaluate the risk to consumers (5, 11, 14, 23).

From all different meat products made with pork meat, dry-cured ham stands out among them as a high-quality product of increasing economic relevance. It is a nonsmoked product manufactured by curing with salt and nitrates, and stabilized by decreasing water activity. The entire process takes several (from 6 to 36) months, and it is consumed without heat treatment. In the market, it is possible to find several presentations of dry-cured ham. Consumers can buy unpackaged entire or sliced ham (sold on request), as well as refrigerated vacuum-packaged dry-cured ham cuts, which can be either distributed to specialized butchers or directly sold in supermarket displays.

Some researchers have suggested that tissue cysts of T. gondii are killed during commercial curing procedures with salt (7, 16, 17). In contrast, other studies have indicated the potential failure of curing to inactivate T. gondii (27), and in epidemiological studies of risk factors for recent Toxoplasma infection in pregnant women, a strong association was found between infection and eating cured pork or raw meat (3, 4, 19). Recently, Bayarri et al. (2) evaluated the influence of processing of cured ham on the viability of T. gondii. These authors evidenced the importance of curing time, and indicated the need of additional studies to evaluate the safety of cured ham to complete the risk assessment, including the analysis of other products cured under different conditions of time, salt, and nitrite concentration.

As hams of different compositions and curing times are available in the market, and with the aim of contributing to the risk assessment process, the present study was conducted to gain information about the prevalence of viable T. gondii in fresh pork meat and to evaluate the risk of ingestion of commercially available cured ham.

MATERIALS AND METHODS

Samples were processed in order to study the prevalence of viable T. gondii in retail pork meat and cured ham. A mouse
concentration bioassay technique was used, and the presence of the parasite in mice was determined by indirect immunofluorescence assay test (IFA). Positive results were also analyzed by histology and PCR.

**Sampling of fresh meat and commercial cured ham.** Fifty samples of fresh meat and commercial cured ham were collected in Zaragoza. This city is located in northeastern Spain, in the Ebro River Valley, and has a Mediterranean continental climate.

To ensure that samples were not from the same animal, sampling was carried out in different weeks and in different shops (supermarkets and butchers) distributed in different quarters of the city. In brief, 25 pieces of fresh pork meat were sampled, corresponding to tongue, rib, loin, and shoulder loin. In addition, 25 samples of commercial cured ham were randomly collected for analysis, including paleta and ham, white and Iberian, and package sliced of different trademarks and cut on request; information on the length of curing process was not always provided on the label.

**Mouse bioassay of tissues for *T. gondii***. A concentration bioassay technique with the acid pepsin digestion procedure was used to demonstrate viable bradyzoites of *T. gondii* in fresh meat and dry-cured hams, as described previously (2, 8). A 0.5-ml aliquot of digestion extract was inoculated intraperitoneally into each mouse (five mice per sample). The digestion procedure and inoculation were done in triplicate for each sample (150 g total). All experiments included negative control mice, which were analyzed at the end of the process.

**IFA of mouse sera.** Blood samples were drawn from mice that survived 60 days after inoculation. Serum from each mouse was diluted 1:10, 1:20, 1:40, 1:80, and 1:160 and tested for *T. gondii* antibodies with IFA (Toxo-Spot IF, bioMérieux, Inc., Marcy l’Étoile, France) with polyclonal rabbit anti-mouse immunoglobulins (DakoCytomation, Carpinteria, CA). The slides were immediately viewed at 400× total magnification with a BH2 fluorescence microscope (Olympus, Center Valley, PA). A positive result was recorded when a clear, whole-perimeter tachyzoite fluorescence was observed at dilutions higher than or equal to 1:20.

**Histological and PCR analysis.** Positive mouse brain samples were analyzed by histology to search for cysts and/or lesions compatible with *T. gondii* infection; five samples from each mouse brain were also analyzed by PCR, as described previously (2, 18).

**RESULTS**

*T. gondii* was detected in 2 of the 50 samples analyzed. A serological response was observed in 8 of 25 mice inoculated with homogenates of rib, with IFA titers of 1:20 to 1:40, while no response was observed in mice inoculated with any other sample of fresh meat. No response was observed in mice inoculated with samples of cured ham. All inoculated mice except four survived. The parasite was not isolated from positive mouse samples by histological and PCR analysis of brains. *T. gondii* was not detected in control mice.

**DISCUSSION**

In our study, we found *T. gondii* in 2 of the 50 samples studied. *T. gondii* was detected only in rib, while the parasite was not identified in other types of fresh meat or any of the samples of cured ham studied.

Despite the accumulating evidence for the role of meat in human exposure to *T. gondii*, little information is available on risk to the consumer from retail meats and meat products (11). As well, there is little information on the positivity in the different commercial meat pieces. In our work, *T. gondii* was detected in 8% of the fresh meat samples analyzed (4% of the total samples analyzed). Similar results were obtained by Dias et al. (5) in studies carried out with fresh pork sausages, who detected a positivity of 8.7%. Lower detection was obtained by Galván-Ramírez et al. (14), who analyzed meat samples of pork meat from butcher shops in Ocotlán (Jalisco, Mexico), detecting 2.1% positivity. As well, in a comprehensive study carried out by Dubey et al. (11) in the United States, the prevalence of viable *Toxoplasma* in retail pork was very low (0.5%). As with our study, all these studies evaluated the viability of the parasite and were carried out by bioassay technique.

However, Aspinall et al. (1) analyzed 58 pork meat product samples obtained from United Kingdom retail outlets, and found that 20 (34.5%) were *T. gondii* positive by PCR detection. The higher positivity obtained might be because unlike the previously mentioned studies, detection of *Toxoplasma* in this work was purely by PCR, and they only demonstrated the presence of *T. gondii* and not the presence of viable parasites capable of initiating a human infection. The demonstration of *T. gondii* viable is the only way to confirm that a product constitutes a risk factor for contracting toxoplasmosis.

Tissue cysts have a high affinity for neural and muscular tissues. They are located predominantly in the central nervous system, the eye, as well as skeletal and cardiac muscles, and to a lesser extent, they might also be found in visceral organs, such as lungs, liver, and kidneys (6, 13). However, any part of infected pork can be a source of infection, because *T. gondii* has been found in seemingly most edible tissues or cuts of meat, both in experimentally and naturally infected pigs (12). In our study, we found the parasite only in rib samples, and to date we have no evidence that there is a greater presence of *Toxoplasma* cysts in this area. Nevertheless, no cysts were found through PCR and histological analysis in mouse brain despite evidence of serological infection. It might be the parasite was present in unexamined brain tissue.

In relation to the other product analyzed, dry-cured ham, there is little knowledge of the effects of several processing conditions on the inactivation of the parasite, such as organic acids, nitrites and nitrates, or the combination of salt concentration, time, and temperature during its maturation (24). Sommer et al. (25) showed that encysted *T. gondii* survived for 4 days in 8% NaCl, but neither this group nor Work (28) could demonstrate viable parasites in *T. gondii*-infected pork subjected to various curing processes. Results of Hill et al. (16) demonstrated that solutions containing 2% sodium chloride or 1.4% potassium or sodium lactate were effective in eliminating viability of *Toxoplasma* tissue cysts in pork loins. Lundén
and Ugla (22) reported the absence of viable *Toxoplasma* in mutton meat after curing and smoking. Warnekalusuriya et al. (27), who used tissue culture in order to isolate viable parasites, detected *T. gondii* by PCR in 1.5% of ready-to-eat cured meat samples, and these authors point out the potential failure of the curing process to inactivate *T. gondii*. A recent study evidenced that curing time is a major factor in ensuring that the consumption of this product does not pose a risk of contracting toxoplasmosis (22). Other authors state that the inactivation of *T. gondii* cysts depends on the synergistic interaction between salt concentration, maturation time, and temperature of storage (20). Both salt composition of the hams and curing time can vary according to the manufacturer or producer countries, and we did find in the markets (and use) hams with different composition and curing times. However, there is a lack of information regarding curing times in the labeling of meat products, and this information should be included because it is of great value to consumers.

In our assay, we did not detect the presence of viable parasites capable of initiating human infection in cured ham. The low detection found in this meat product could either be because of the effect of curing or because of the decline of this parasitism in pigs because of the intensive management systems (21). In addition, the number of cysts per gram of tissue from food animals such as pigs might be low, often one tissue cyst in 25 ± 250 g of organ (10); thus, the sampling is very important (2). The parasite could be present in unexamined tissue (15), and a negative result from any sample does not necessarily mean that the entire tissue is free of the parasite. In any case, it should be noted that the recent trend of rearing pigs outdoors (e.g., organic farming) in European countries is likely to increase *T. gondii* seroprevalence (26); the public demand for animal-friendly production systems could however lead to a re-emergence of *T. gondii* in pork (21).

In conclusion, results reported in this article are optimistic concerning food safety, as the presence of *Toxoplasma* in cured ham was not detected, and only two samples of fresh meat were positive. However, in order to achieve a complete risk assessment on the viability of *T. gondii*, it is necessary to analyze a much larger number of samples, particularly those from organic farms, as well as cured ham considering different curing times and salt composition.

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**REFERENCES**


