**Taenia solium** Metacestode Viability in Infected Pork after Preparation with Salt Pickling or Cooking Methods Common in Yucatán, México

R. RODRIGUEZ-CANUL,1* F. ARGAEZ-RODRIGUEZ,2 D. PACHECO DE LA GALA,2 S. VILLEGAS-PEREZ,2 A. FRASER,3 P. S. CRAIG,3 L. COB-GALERA,2 AND J. L. DOMÍNGUEZ-ALPIZAR2

1Laboratorio de Inmunología y Biología Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN) Unidad Mérida, Carretera Antigua a Progreso Km 6, AP 73, CP 97310, Mérida, Yucatán, México; 2Laboratorios de Parasitología e Inmunología y Salud Pública Veterinaria, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán (FMVZ-UADY), AP 4-116, Colonia Itzimna, 97100 Mérida, Yucatán, México; and 3Cestode Zoonoses Research Group, Division of Biological Sciences, School of Environment and Life Sciences, University of Salford, Salford M5 4WT, UK

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**ABSTRACT**

The cestode parasite *Taenia solium* is an important cause of foodborne infection throughout tropical and subtropical regions. Ingestion of pork meat infected with *T. solium* larvae can lead to taeniasis infection in humans. With tourism and the consumption of native food increasing, it is important to investigate potential risks of transmission associated with food preparation methods. In this study, traditional Mexican salt pickling and two methods of pork preparation (as roast pork [*cochinita pibil*] and in pork and beans [*frijol con puerco*]) were evaluated in order to determine their effects on *T. solium* cyst viability in infected tissue. In the control groups, all metacestodes isolated were 100% viable, and only small changes in pH (from 6.0 to 5.9) and temperature (29 to 30 °C) were recorded. No viable cysts were detected after 12 and 24 h of salt pickling. The pH of the meat during salting dropped from 6.0 to 5.3. Osmotic changes and dehydration from the salting, rather than a change in pH, could be considered the main cause of cyst death. Temperatures of \(65^\circ\)C damaged *T. solium* metacestodes in roast pork and in pork and beans. The results of this study indicate that if traditional pork dishes are prepared properly, *T. solium* cysts are destroyed. The criteria used in this study to evaluate the viability of tissue cysts are discussed.

The cestode *Taenia solium* is an important zoonotic parasite throughout Central America, South America, West Africa, and non-Muslim areas of Indonesia and Southeast Asia. It is transmitted between humans, who carry the adult worm in the intestine, and pigs, which carry the parasite in its larval (cyst or metacestode) stage in muscle tissue (6). Infection of humans (the definitive hosts for adult *T. solium*) occurs after the ingestion of undercooked infected pork meat. Although the pathogenicity of adult *T. solium* organisms in humans is asymptomatic, infection with the parasite’s eggs can lead to massive metacestode infection in the natural intermediate host (the pig) and in the accidental host (the human). In humans, cysts can lodge in the central nervous system, producing neurocysticercosis, which is considered a public health issue because of the severe and irreversible neurological conditions it can cause (18). Neurocysticercosis is thought to be responsible for up to 25% of all cases of epilepsy in tropical areas (10).

In Mexico, *T. solium* is endemic, and cultural practices, poor sanitary conditions, and traditional methods of pig husbandry promote the transmission of this parasite (12, 14). In rural communities, pork meat is heavily consumed and the raising and slaughtering of swine are improperly managed. Low family income is also associated with *T. solium* transmission for several reasons, including a reluctance to discard infected pork because of the loss of food represented by such an action (12). The hygiene of slaughterhouses in these areas is often inadequate, making the sale of infected pork meat possible. In addition, reports of porcine cysticercosis from rural slaughterhouses are underestimated (1). However, some epidemiological studies on cysticercosis and taeniasis in rural communities throughout Yucatan State in southern Mexico showed a mean porcine cysticercosis seroprevalence of 23%. Seroprevalence was higher in free-roaming pigs (33%) and much lower in confined pigs (2%), indicating that husbandry had a crucial impact on infection levels (12). In the same region, a human cysticercosis seroprevalence of 3.4% and a taeniasis prevalence of 1.7% were also detected (13). These findings could indicate a significant intake of poorly cooked or raw pork among the inhabitants of this region. However, it is not clear which dishes are responsible for the infection.

In southern Mexico, pork meat is common in local dishes and is consumed on a daily basis and during family and religious festivals. The Yucatan area is becoming increasingly popular as a tourist destination, and travelers are now often more willing to try local delicacies, increasing the risk of their becoming infected with *T. solium* and possibly reintroducing it into countries where it is no longer endemic (16, 17).

The purpose of this study was to determine whether
traditional native dishes from southern Mexico involving either salting, stewing, or slow roasting allowed *T. solium* cyst survival in infected meat. These dishes are in high demand with locals and tourists, so the identification of possible risks of consumption is important for epidemiological studies and control programs and for preventing the importation or reemergence of *T. solium* infection in other countries.

**MATERIALS AND METHODS**

This study was carried out at the School of Veterinary Medicine, University of Yucatan (FMVZ-UADY), and in the community of Dzununcan (20°59′N, 89°39′W), in the municipality of Merida (capitol of Yucatan), approximately 5 km southeast of the FMVZ-UADY, in April 2000. Two infected pigs that were confirmed by tongue palpation and serology to have cysticercosis (12) were butchered in the FMVZ-UADY slaughterhouse. Meat was separated from bones and placed in plastic containers. Bones and viscera were subsequently incinerated to avoid potential environmental contamination (2). Meat was immediately transported to the community of Dzununcan, where it was prepared by community members according to traditional methods (described below), deliberately excluding the involvement of the researchers so as to avoid their possible influence on the traditional methods of preservation and cooking.

**Pork meat preparation.** Five batches of pork were prepared, one by a traditional preservation method, one as a control, two as roast pork (at two different temperatures), and one in pork and beans. Prior to preparation, 30 metacestodes were isolated from each batch as preliminary controls for the investigation of metacestode viability.

**Pork meat preparation: salt pickling.** In rural areas, where there is rarely access to a freezer or a refrigerator, the preservation method of choice for pork fillets is known as *salado* and involves pickling the meat with salt. For this process, approximately 1 kg of meat was cut into 7 fillets of approximately 19 by 9 by 0.5 cm. Each slice was covered with 10 to 15 g of sea salt and placed in a sealed plastic container overnight at room temperature (29 to 30°C).

**Pork meat preparation: control.** For the control pickling preparation, 1 kg of meat was placed in a plastic container and left at room temperature for 24 h.

**Pork meat preparation: roasted pork.** Roasted pork was cooked in two ways: the traditional way (17 h) and the commercial way (3 h). In both cases, 10 pieces of approximately 5 kg of meat were marinated in a paste made of annato seeds (*Bixa or-ellana*), sour orange juice, pepper, and sea salt. The marinated meat was wrapped in a banana leaf and placed in a sealed tin container. The container was placed underground in a rustic oven that was approximately 1 m long, 0.50 m wide, and 1.50 m deep (9).

**Pork meat preparation: pork and beans.** To prepare pork for use in pork and beans, 1 kg of meat was cut into chunks of approximately 3 cm. Beans were briefly boiled and then simmered in water, and then the chopped pork meat was added and the dish was cooked gently for approximately 2 h (9).

For all preparation methods, the temperature was measured with a standard thermometer (0 to 120°C). For the roasted pork, the thermometer was placed in a steel tube in contact with the food during cooking. A portable pH meter (Sigma Chemical Co., St. Louis, Mo.) was used to measure pH for all preparation methods. Once the preparation periods were complete, all of the batches were transported immediately to the FMVZ-UADY, where metacestode cysts were isolated from each lot to evaluate viability as described below.

**Criteria of viability.** Three criteria were used to define viability: assessment of metacestode morphology and movement according to Fan et al. (4, 5), staining of metacestodes and observation of the flame cells, and in vitro evagination of the scolex (head). The staining of metacestodes and the observation of the flame cells were carried out by placing the metacestodes in 10 ml of a 0.1% solution of the vital colorant Blue Nile (Sigma) for 10 min, then placing them in phosphate-buffered saline (pH 7.4) for 10 min, and then observing them with a stereoscopic microscope (×4). The excretory system of cestodes consists of a nephridial system with flame cells (bulbs) as excretory units. These flame cells are located on each side of the scolex in dorsal and ventral positions, forming a loop. Blue Nile dye is one of several stains recommended for use in dye exclusion procedures for viable parasites. This method is based on the principle that live (viable) parasites take up certain colorants, while dead (non-viable) parasites do not. Staining facilitates the visualization of the flame cells (15). The in vitro evagination of the scolex was carried out by placing the metacestodes in 10 ml of fresh bovine bile (pH 8.4) at 37°C for 120 min, with observations being made every 5 min (3).

Metacestodes were considered viable if they exhibited undulatory movements of the cyst, an intact (white-yellowish) wall, a semitransparent membrane, active movement of the neck and scolex (suckers and hooks) within the vesicle, transparent cyst fluid, and complete or incomplete evagination of the scolex. Metacestodes were considered nonviable if they exhibited a damaged wall; an absence of cyst fluid; an absence of movement in the cyst, neck, and scolex; a lack of metacestode staining; a lack of flame cell movement; or the absence of scolex evagination in bovine bile.

**RESULTS AND DISCUSSION**

The purpose of this study was to determine if pork preservation and preparation methods currently in use in Yucatan were responsible for the transmission of *T. solium*. To this end, *T. solium*-infected pork was prepared by local methods and subsequently tested for metacestode viability. In the pickling process, a total of 280 metacestodes were isolated and checked for viability (160 metacestodes after 12 h and 120 metacestodes after 24 h). Pork meat was salt pickled overnight to represent the shortest time for which people from rural communities preserve meat before consumption (11). In the control group, 240 metacestodes were isolated (120 metacestodes after 12 h and 120 metacestodes after 24 h).

Results from the pickling analysis revealed that *T. solium* metacestodes did not survive after 12 and 24 h of preservation, in stark contrast to the control group, which was 100% viable at 12 and 24 h. Metacestodes isolated from pickled meat exhibited damaged walls, the absence of cyst fluid, the absence of movement, cysts failing to stain, no flame cells, and scolecites failing to evaginate (Fig. 1). In contrast, in the control group, all metacestodes exhibited active intact walls and active undulatory movement of the cysts and the neck and head (suckers and hooks) (Fig. 2A...
and 2B). The actual means by which the pickling abolished viability were not investigated. The pH level may have had some effect, although pH decreased in both the pickled and the control preparations. The pH of the pickled meat decreased from 6.3 before conservation to 6.1 at 12 h and, finally, to 5.3 at 24 h, whereas in the control preparation it changed from 6.2 to 6.0 at 12 h and, finally, to 5.9 at 24 h with no effect on viability in the latter case. In both cases, differences in pH were not statistically significant in relation to metacestode death ($P = 0.8$).

In a study performed by Keittivuti et al. (8), metacestodes isolated from meat dipped in a 20 to 25% saline solution died with pH values of 5.5 to 5.7. However, this treatment’s effectiveness may be more directly linked to the salt itself. It evidently affected metacestode viability, probably causing changes in osmotic potential and membrane rupture, damaging cyst walls, and causing an absence of cyst fluid. This finding is supported by Keittivuti et al. (8), who demonstrated that salt interfered with absorption by the metacestode membranes, resulting in death by dehydration. Meat temperatures in both the pickling and the control groups oscillated between 29.3 and 30°C (mean, 29.3°C) during the 24-h experiment and were not related to cyst death.

For the roast pork, 30 metacestodes isolated before cooking were 100% viable, and after 17 h of cooking (the traditional method), none of the 237 isolated metacestodes were viable. The cooking temperature was 85°C during the first hour, 70°C after 10 h, and 65°C at the end of the cooking time. The meat’s pH was initially 6.3 but increased slightly to 6.5 after marination and cooking. For the pork cooked in the commercial way (3 h), none of 233 isolated cysts were viable, in contrast to the 30 metacestodes isolated before cooking, which were 100% viable. The cooking temperature was 90°C during the first hour, 100°C after 2 h, and 90°C at the end of the cooking time. The meat’s initial pH was 6.3, increasing to 6.5 after marination and cooking. For both cooking conditions, all metacestodes isolated from the pork preparations exhibited damaged membranes, lack of movement, and loss of viability according to the criteria used in this study (Fig. 3). Both roast pork

preparation methods effectively annulled metacestode viability; the temperatures, ranging from 60 to 85°C, of the traditional preparations and the temperatures, ranging from 90 to 100°C, of the commercial method destroyed all $T. solium$ metacestodes. This corresponds well with studies showing that metacestodes cooked at more than 45 to 50°C can be destroyed within 15 to 20 min (3, 7).

For the pork-and-bean dish, 30 metacestodes isolated

FIGURE 1. Nonviable metacestodes from the pickling process. Bladder wall dehydration and a lack of evagination were the main features observed. Bar = 5 mm.

FIGURE 2. (A) Intact metacestodes isolated from the control group. The bladder wall is intact and cyst fluid is seen inside it. Bar = 5 mm. (B) Evaginated metacestodes from the control group. The remainder of the cyst (C) follows the scolex (S) and neck (N). Bar = 5 mm.

FIGURE 3. Nonviable metacestodes from roasted pork. Cysts exhibited damaged membranes, lack of movement, and failure to evaginate. Bar = 5 mm.
before cooking were 100% viable, while 271 metacestodes isolated after cooking exhibited 0% viability. Metacestodes were intact but deformed, still containing cyst fluid; flame cells were not observed and scolices failed to evaginate (Fig. 4). The pork-and-bean preparation effectively annulled metacestode viability. The cooking temperature was initially 36°C, increasing to 100°C within 30 min and remaining at this level until cooking was completed around 2 h later. The meat’s initial meat pH was 6.3, increasing to 6.8 after cooking. It has been shown that temperatures above 76.6°C for more than 10 min will destroy metacestodes (17). The pH actually increased during cooking and thus was likely unrelated to metacestode death.

The use of a variety of methods in this study is novel. The application of these different methods represents a comprehensive approach to the identification of potential methods of *T. solium* transmission. By assessing morphological features, vital colorants, the presence or absence of flame cells, and in vitro evagination of scolices with bovine bile, the viability of *T. solium* metacestodes can be determined without employing pepsin, trypsin, or artificial gastric juices. In developing tropical countries, where *T. solium* is endemic, these substances can be prohibitively expensive and of limited availability. Promotion of this approach can make this important procedure more accessible in areas where it is most needed.

Overall, the preparation methods tested in this study resulted in nonviable *T. solium* metacestodes, meaning that these methods effectively prevent *T. solium* transmission from pork. However, the larger question of the specific route(s) by which cysts of *T. solium* are transmitted to humans in Yucatan still remains to be answered. An extension of this research, in which other pork preparation methods are being evaluated for their effect on *T. solium* metacestode viability, is currently in progress.

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