Effects of Time and Temperature on the Viability of *Toxoplasma gondii* Tissue Cysts in Enhanced Pork Loin

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ABSTRACT

Enhanced or pumped pork products represent a significant proportion (40 to 50%) of the commercially available pork cuts available to consumers at the retail level. In a previous study, pork loins containing viable *Toxoplasma gondii* tissue cysts were pumped with solutions containing 2% sodium chloride or 1.4% or higher potassium or sodium lactate and stored at 4°C for 7 days. This treatment prevented transmission of *T. gondii* to cats. In the present study, enhanced pork loins were stored for 0, 8, 16, 24, 32, or 40 h at 4°C and then fed to *T. gondii*–seronegative cats to determine how quickly the loss of tissue cyst viability occurred. In a second experiment, pork loins collected from pigs experimentally infected with *T. gondii* were stored at temperatures found in retail meat cases and then fed to *T. gondii*–seronegative cats to determine the effect of typical meat case storage temperatures on tissue cyst viability. In both experiments, cat feces were examined for 14 days after the infected meat meal to assess oocyst shedding. The results indicate that solutions containing 2% sodium chloride or 1.4% potassium or sodium lactate are effective within 8 h of injection for killing *T. gondii* tissue cysts in pork loins and that storage at meat case temperatures at or below 0°C (32°F) for 7 days also killed *T. gondii* tissue cysts in pork loins.

The use of enhancement solutions in retail meat products has increased in recent years as a result of consumer concerns about meat quality and safety. Retail cuts of pork are frequently enhanced with salt solutions to improve flavor and texture and to extend shelf life by reducing microbial contamination (1–4). The protozoan parasite *Toxoplasma gondii* is one of the most common parasitic infections of humans and other warm-blooded animals (8). The parasite can cause serious illness in healthy adults and is dangerously virulent in immunocompromised individuals and congenitally infected children. The U.S. national seroprevalence of 23% in humans has remained stable for the past 10 years, as reported by the National Health and Nutrition Evaluation Survey (22). Transmission of *T. gondii* occurs through accidental ingestion of infectious oocysts from cat feces in contaminated food or water, by transplacental transfer from mother to fetus, and by consumption of raw or undercooked meat products containing *T. gondii* tissue cysts (8, 12, 13). The recently completed National Retail Meats Survey (NRMS) for *T. gondii* (11) revealed that among retail beef, chicken, and pork, pork was the only meat that contained viable *T. gondii* tissue cysts. Dubey et al. (13) found that virtually every edible portion of an infected pig carcass may contain viable *T. gondii* tissue cysts. Considering this risk from infected pork, it is important to understand how meat handling and processing might affect the viability of *T. gondii* cysts in meat.

In a previous study, Hill et al. (19) found that injection of 2.0% NaCl or 1.4% or higher lactate-based salt solutions into pork loins containing infective tissue cysts prevented transmission of *T. gondii*. In the present study, we investigated the effect of postenhancement time and meat case storage temperature on tissue cyst viability in pork loins from market weight pigs experimentally infected with *T. gondii*.

MATERIALS AND METHODS

Infection of pigs with *T. gondii* oocysts. *T. gondii* (VEG strain) oocysts were collected by sucrose flotation from feces of cats fed tissues of mice experimentally infected with *T. gondii* as previously described (9, 12, 19). Oocysts were sporulated in 2% H2SO4 while shaking for 7 days at room temperature. Sporulated oocysts were washed in water by centrifugation, treated for 5 min in 3.0% sodium hypochlorite, and washed in Hanks balanced salt solution until the pH of the wash solution was neutral. Ten *T. gondii*–seronegative pigs (~50 kg each, 5 months of age; Ernst Farms, Clear Spring, Md.) were infected orally with 1,000 sporulated *T. gondii* oocysts. Serum antibodies to *T. gondii* were evaluated in infected pigs with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Safepath Laboratory, Carlsbad, Calif.) using a 1:50 serum dilution on the day of infection and at the time of sacrifice. Positive and negative control swine sera were included on each ELISA plate. A positive cutoff was established as five times the mean + standard deviation of the mean of a set of five *T. gondii*–negative swine serum samples. Pigs were sacrificed at 60 days postinfection, and both loins were removed from each animal. A 50-g sample was removed from each loin and fed to individual cats to verify *T. gondii* infection; an additional 50 g was removed for collection of tissue fluids for the ELISA. The positive cutoff for the tissue fluid ELISA was established using *T. gondii*–negative tissue fluid collected from five *T. gondii*–negative pigs.

Injection of loins with enhancement solutions. Each loin was divided into three samples, weighed, and injected to 110%...
of the original weight with enhancement solutions listed in Table 1. Injections were performed using a hand-held four-needle meat pumping station (Dayton Electric Manufacturing Company, Niles, Ill.). Each solution was formulated to obtain the final concentrations (wt/wt) listed in Table 1 in each pork loin. Each solution was injected into five loin samples using 24 to 28 injection sites per loin. The enhanced loins were then individually heat-sealed in plastic bags.

Effect of time postenhancement on tissue cyst viability. To test how rapidly the enhancement solutions killed *T. gondii* tissue cysts in the loins, enhanced loins were stored at 4°C for 0, 8, 16, 24, 32, and 40 h. At the times indicated, the loins were removed from storage, and 50 g from each of five similarly treated loins was removed, pooled, and blended; the blended pool of 250 g of tissue was then fed to one cat. The remaining loins were resealed in plastic bags and returned to storage at 4°C until the next sampling time. Cats (Liberty Research, Waverly, N.Y.) used in this and subsequent experiments were seronegative for *T. gondii* antibodies at a 1:25 serum dilution as determined with a modified agglutination test and the ELISA. Feces from each cat were examined for oocysts daily for 14 days beginning on day 3 after ingestion of the infected meal.

Effect of storage temperature on tissue cyst viability. To determine the effect of a range of observed meat case temperatures on the viability of *T. gondii* tissue cysts in retail meat, a survey of retail meat case temperatures was performed during the NRMS for *T. gondii* (11). Temperatures were measured with a digital infrared noncontact thermometer (Radioshack, Ft. Worth, Tex.) at three places (front, center, and back) as close as possible to the center in three pork cases in each of 14 different retail outlets. The temperature was taken between the first and second layers of meat packages or beneath a single package when there was only one layer. The mean meat case temperature was calculated for each of the cases in each retail outlet. Pork case temperatures ranged from −3.2 to +4.6°C in the 14 stores. A total of eight pork loins from experimentally infected pigs were enhanced with solution 8 (0.85% NaCl plus 0.25% sodium tripolyphosphate [STP]), and these eight loins and another eight loins that were unenhanced were packaged as described above. Two loins that were enhanced with solution 8 and two loins that were unenhanced were stored for 7 days at each temperature tested: −5°C (23°F), 0°C (32°F), 3.8°C (39°F), and 6.1°C (43°F). The storage temperatures were selected to bracket the observed temperatures in meat cases. The loins were then removed from storage, and 100 g from each of the two similarly treated loins was removed and pooled. This pool of 200 g of tissue was fed to one cat, and the feces from each cat were examined for oocysts as described above. The sampled loins were resealed in plastic bags and returned to storage at the experimental temperatures described. At day 14 postenhancement, loins were again removed from storage and retested as described for day 7.

RESULTS

Infection of pigs with *T. gondii* oocysts. Serology from pigs inoculated with *T. gondii* oocysts indicated that each pig became infected as a result of the oral oocyst inoculation and developed high titers of anti-*T. gondii* antibody that could be detected by day 60 postinoculation with the ELISA using sera diluted 1:50 (Table 2). Antibody titers in tissue fluids diluted 1:10 were lower than those in corresponding serum samples; however, all ELISA optical density in tissue fluids from experimentally infected pigs exceeded the positive cutoff value for the tissue fluid ELISA (Table 2). Bioassay results from 10 cats fed infected tissues from each *T. gondii*-inoculated pigs confirmed the serology results; all cats fed loin tissue collected prior to enhancement shed oocysts by day 5 postinoculation.

Effect of postenhancement time on tissue cyst viability. Cats that were fed a 250-g pooled sample of pork loin enhanced with solution 8 (0.85% NaCl plus 0.25% STP) or a pooled sample of unenhanced loin that had been stored at 4°C shed oocysts by day 6 postinoculation at each of the time periods assayed (0, 8, 16, 24, 32, and 40 h postenhancement; Table 3).

Cats that were fed pooled loins that had been enhanced with solutions 1 through 7 and stored at 4°C for 0 h shed oocysts, but cats fed pooled loins stored at 4°C for 8, 16,
24, 32, and 40 h did not shed oocysts (Table 3), indicating that these enhancement solutions rendered the tissue cysts in the meat nonviable within 8 h of exposure.

**Effect of storage temperature on tissue cyst viability.** When temperatures were evaluated in pork display cases of 14 retail outlets, a range of temperatures was found (−3.2 to +4.6°C; Table 4). In the laboratory, loins were stored for 7 and 14 days at −5, 0, 3.8, and 6.1°C, removed from storage, and fed to cats as described above. Cats fed loins that were stored for 7 or 14 days at −5 or 0°C did not shed oocysts, but cats fed loins stored for 7 and 14 days at 3.8 and 6.1°C did shed oocysts, indicating that storage of *T. gondii*-infected pork loins at 0°C or below rendered *T. gondii* tissue cysts nonviable by day 7.

### DISCUSSION

Postharvest technologies such as chilling and pumping with various solutions are commonly used as pathogen reduction methods in the pork industry. Widespread application of these technologies and the advent of confinement rearing of pigs in biosecure housing facilities has improved the safety of pork with regard to formerly common zoonotic parasites, such as *Trichinella spiralis* and *T. gondii*. The results of the current study indicate that injection of specific formulations of lactate- or NaCl-based enhancement solutions is effective for killing *T. gondii* tissue cysts in pork loin within 8 h and that meat case storage temperatures at or below 0°C for 7 days also can be effective for killing these cysts.

Previous studies have indicated that chilling or pumping of pork products with NaCl- or lactate-based solutions reduces microbial contamination, improves flavor and texture, and prolongs shelf life (7, 18, 20, 25, 27, 29, 30). Pumping resulted in a loss of viability of *T. gondii* tissue cysts in fresh pork (19).

Results of the 1990, 1995, and 2000 National Animal Health Monitoring System (NAHMS) serological surveys indicate a decreasing level of *T. gondii* infection in market-weight pigs (5, 15, 17), although management systems in which pigs are allowed access to the outdoors result in high levels of infection (10, 14). Currently, a low but measurable prevalence (0.8%) of *T. gondii* persists in market-weight hogs (5), and *T. gondii*-infected pigs harbor viable tissue cysts. Meat from these infected animals is shipped to retail stores and is available for purchase by consumers, posing a risk to consumers from improperly prepared meat.

Results of the recently completed NRMS for *T. gondii* (11) support the NAHMS seroprevalence data. The survey of 698 retail outlets nationwide determined the prevalence of viable *T. gondii* tissue cysts in commercially available fresh pork products to be 0.38%, approximately half of the reported seroprevalence in live market-weight hogs. A slightly higher percentage of the samples (0.57%) were serologically positive for *T. gondii* based on results of a commercially available ELISA of collected tissue fluids (16).

Because 40 to 50% of fresh pork is pumped with enhancement solutions of various formulations, some of the differences between the observed seroprevalence in live hogs and the measured prevalence of viable *T. gondii* tissue cysts in fresh pork products probably is due to killing of tissue cysts by the enhancement solutions.

Previous studies have demonstrated the efficacy of lactate- and NaCl-based solutions in inhibiting spoilage organisms and pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella Typhimurium*, *Campylobacter*, and *Clostridium* sp. that are leading causes of foodborne illnesses (6, 24, 28). Enhancement of meats with lactate-based products increases the shelf life of pork products by 30 to 60% (1, 2, 32). Enhancement solutions in this study were prepared with formulations similar to those used in commercial pork products; in a previous study, solutions 1 through 7 killed *T. gondii* tissue cysts in pork loin within 7 days (19). The rapid time frame (8 h) in which the lactate- and NaCl-based enhancement solutions killed the tissue cysts in the meat is important because the time between harvest of the pork product and display of fresh products on store shelves can be as little as 12 h, although frequently it is much longer (20, 31).

Sodium diacetate and STP have some effect on bacterial growth in enhanced meats and are common compo-

### TABLE 3. Toxoplasma gondii transmission to cats fed enhanced pork loins that had been stored at 4°C

<table>
<thead>
<tr>
<th>Solution no.</th>
<th>Postenhancement time (h):</th>
<th>0</th>
<th>8</th>
<th>16</th>
<th>24</th>
<th>32</th>
<th>40</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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</table>

### TABLE 4. Pork display case temperatures collected during the National Retail Meats Survey (NRMS)

<table>
<thead>
<tr>
<th>Store no.</th>
<th>NRMS mean temp (°C)</th>
<th>Meat case thermometer reading (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−3.07</td>
<td>−5.0</td>
</tr>
<tr>
<td>2</td>
<td>0.73</td>
<td>−4.0</td>
</tr>
<tr>
<td>3</td>
<td>3.67</td>
<td>−3.0</td>
</tr>
<tr>
<td>4</td>
<td>4.2</td>
<td>6.66</td>
</tr>
<tr>
<td>5</td>
<td>3.87</td>
<td>7.0</td>
</tr>
<tr>
<td>6</td>
<td>3.67</td>
<td>−4.1</td>
</tr>
<tr>
<td>7</td>
<td>0.53</td>
<td>−3.3</td>
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<tr>
<td>8</td>
<td>4.07</td>
<td>6.0</td>
</tr>
<tr>
<td>9</td>
<td>−0.2</td>
<td>−3.2</td>
</tr>
<tr>
<td>10</td>
<td>−0.13</td>
<td>−3.16</td>
</tr>
<tr>
<td>11</td>
<td>1.87</td>
<td>1.6</td>
</tr>
<tr>
<td>12</td>
<td>1.47</td>
<td>3.2</td>
</tr>
<tr>
<td>13</td>
<td>−1.87</td>
<td>−2.8</td>
</tr>
<tr>
<td>14</td>
<td>2.47</td>
<td>−2.2</td>
</tr>
</tbody>
</table>

*a* Each value is the mean of nine readings (front, center, and back of three cases) from raw pork display cases in each store.
ments of enhancement formulations; thus, they were included here although in a previous study (19) STP and sodium diacetate alone had no effect on *T. gondii* tissue cyst viability.

Pork loin storage temperatures below 0°C also adversely impact tissue cyst viability and may contribute to the observed difference in prevalence of viable tissue cysts in pork and seroprevalence in live pigs. Optimal storage temperatures for fresh pork products are −1 to −1.5°C, just above the point of freezing (21). Although U.S. federal regulations recommend maintenance of a 4°C maximum raw meat display case temperature (3), retail meat case temperatures frequently fluctuate widely between the chilling and defrosting cycles of the case (18, 20, 23). The range of temperatures observed in meat cases during this study, some of which were higher than the recommended safe storage temperatures for many spoilage organisms and bacterial pathogens such as *Salmonella* and *Listeria*, suggests that stringent control of meat case temperatures is an underutilized method of pathogen reduction that could be implemented by retailers and would reduce *T. gondii* transmission from infected meat. Temperature readings displayed on the stores’ temperature monitors frequently differed markedly from the actual meat case temperature; meat case temperatures were actually higher than the store display temperature in 10 of 14 stores (Table 4); only 4 stores had pork case temperatures at or below 0°C, which would render *T. gondii* tissue cysts nonviable.

Toxoplasmosis is a major public health issue and, based on recent studies (11, 26), appears to pose a risk to public health because of its presence in pork. Demands from consumers for pathogen-free meat products have focused the attention of government regulators and the meat industry on food safety and the need to produce meat that is wholesome, safe, and of high quality. Refinement of current methods for processing and storing meat products to inactivate *Toxoplasma* could help assure a safe food supply by reducing the risk associated with ingestion of fresh meats. The results of this study indicate that some current meat processing and storage technologies, when applied effectivelly, can be useful for reducing or eliminating consumer risk of acquiring *T. gondii* infection from retail pork products.

**REFERENCES**


