

Effect of Commonly Used Enhancement Solutions on the Viability of *Toxoplasma gondii* Tissue Cysts in Pork Loin

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ABSTRACT

Retail meat cuts of pork are frequently enhanced with salt solutions to improve flavor and texture and to extend shelf life through reductions in microbial contamination. A study of the effect of commonly used meat enhancement solutions on the viability of *Toxoplasma gondii* tissue cysts was performed using tissues from experimentally infected mice and pigs. Brains of *T. gondii*-infected mice were injected to 110% of the original weight of the brain with solutions containing sodium chloride (1 and 2%), sodium diacetate (0.1 and 0.2%), sodium tripolyphosphate (0.25 and 0.5%), potassium lactate (1.4 and 1.96%), or sodium lactate (1.4, 1.5, and 2.0%) alone or in combination and stored at 4°C for 7 days before feeding to *T. gondii*-seronegative cats. Loins were collected from pigs experimentally infected with *T. gondii* and injected as above and stored for 7, 28, or 45 days at 4°C before feeding to *T. gondii*-seronegative cats. Cat feces were examined for 14 days to assess oocyst shedding. The present study demonstrated that injection of mouse brains or pork loins with solutions containing 2% sodium chloride or $\geq 1.4\%$ potassium or sodium lactate, alone or in combination with other components, prevented transmission of *T. gondii* to cats.

The protozoan parasite *Toxoplasma gondii* causes one of the most common parasitic infections of humans and other warm-blooded animals (10). This parasite has been found worldwide from Alaska to Australia, and nearly one third of the human population has been exposed (10). Infection does not cause serious illness in most adults, but it can cause blindness and mental retardation in congenitally infected children and devastating disease in immunocompromised individuals. The most recent U.S. National Health and Examination Survey revealed that the national seroprevalence of 15% has remained stable for the past 10 years (20). Transmission of *T. gondii* can occur by the fecal-oral route through accidental ingestion of infectious oocysts and by transplacental transfer from mother to fetus (10, 18). Consumption of raw or undercooked meat products containing *T. gondii* tissue cysts also is considered a major risk factor for *T. gondii* infection. The U.S. Department of Agriculture estimates that 50% of *T. gondii* infections in the United States are caused by consumption of raw or undercooked meat containing viable *T. gondii* tissue cysts (1, 5). A study of Seventh Day Adventists in Maryland demonstrated decreased seroprevalence in non-meat eaters compared with meat eaters (26); in another study, an estimated 50% of deaths caused by *T. gondii* infection each year resulted from consumption of improperly prepared infected meat products (22). Although the actual risk to U.S. consumers of acquiring toxoplasmosis from contaminated meats is currently unknown, bioassays and serological and molecular studies have demonstrated high levels of infec-

tion in pigs and lambs destined for human consumption (14, 17, 21). Commercial meat cuts may therefore contain viable *T. gondii* tissue cysts; Dubey et al. (16) demonstrated that virtually every edible portion of an infected pig carcass may contain viable 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* in meat.

In a previous study, Dubey (7) demonstrated that a 6% sodium chloride (SC) solution was lethal to isolated tissue cysts; however, the effect of this and other salt solutions on the viability of *T. gondii* tissue cysts contained in typical intracellular locations in meat is unknown. Retail meat cuts of pork are frequently enhanced with salt solutions to improve flavor, extend shelf life, reduce microbial contamination, and improve tenderness (3, 6, 30). In this study, we investigated the effect of the most commonly used enhancement solutions on tissue cyst viability in mice and in pork loins from market-weight pigs experimentally infected with *T. gondii*.

MATERIALS AND METHODS

Experiment 1. *T. gondii* VEG strain tissue cysts (9) containing viable bradyzoites were generated in the brains of Swiss-Webster mice by subcutaneous inoculation of bradyzoites. Mice were sacrificed 6 to 8 weeks after infection, and the brain from each mouse was removed and weighed. Brains were injected to 110% of the original weight of the tissue with enhancement solutions (Table 1) using a 1-ml syringe and a 20-gauge needle. The solutions were prepared to produce the final concentrations in injected brains listed in Table 1. Each solution was injected into 10 brains, at 15 injection sites per brain. The brains injected with

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TABLE 1. *Toxoplasma gondii* transmission to cats by ingestion of *T. gondii*-infected mouse brains or pork loins

Solution	Final concn of enhancement solution injected ^a	Oocyst shedding	
		Mouse brains ^b	Pork loin ^c
1	0.25% STP	+, +	+, +, +
2	1.5% SL + 0.25% STP	-, -	-, -, -
3	2.0% SL + 0.20% SD	-, -	-, -, -
4	1.0% SC	+, -	+, +, -
5	0.10% SD + 0.25% STP	+, +	+, +, +
6	0.25% STP + 1.4% potassium lactate (PL) + 0.10% SD ^d	-, -	-, -, -
7	0.25% STP + 1.4% SL + 0.10% SD ^d	-, -	-, -, -
8	0.25% STP + 1.96% PL + 0.14% SD ^d	-, -	-, -, -
9	2.0% SL	-, -	-, -, -
10	1.0% SC + 0.25% STP	+, +	+, +, +
11	2.0% SC + 0.50% STP	-, -	-, -, -
12	Control-sterile water	+, +	+, +, -
13	Control-0.85% saline	NT ^e	NT, +, +
14	Uninjected	NT	NT, +, +

^a All chemicals, unless otherwise stated, obtained from Sigma Chemical (St. Louis, Mo.).

^b Values are for two cats per solution.

^c Values are for three cats per solution that ingested samples at 7, 28, and 45 days after enhancement, respectively.

^d Food grade mixtures of PL and SD or SL and SD were kind gifts of Purac America (Lincolnshire, Ill.).

^e NT, not tested.

enhancement solutions were heat sealed in plastic bags and stored at 4°C. After 7 days, the brains were removed from storage, and five brains from each package were fed to one cat, for a total of two cats per solution. Cats used in this and subsequent experiments (Liberty Research, Waverly, N.Y.) were seronegative for *Toxoplasma* antibodies at a 1:25 serum dilution as determined by the modified agglutination test and enzyme-linked immunosorbent assay (ELISA) (11). Feces from each cat were examined for oocyst shedding daily for 14 days beginning on day 3 after feeding. Bioassay in cats is the most sensitive method for detecting *T. gondii* in meat because cats can shed millions of oocysts after ingesting a few bradyzoites (8).

Experiment 2. *T. gondii* (VEG strain) oocysts were collected by sucrose flotation from feces of cats fed tissues of mice experimentally infected with *T. gondii*; these procedures have been previously described (12, 15). 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 *Toxoplasma*-seronegative pigs (~50 kg each, 5 months of age; Ernst Farms, Clear Spring, Md.) were infected per os with 1,000 sporulated *T. gondii* oocysts. Serum antibodies to *T. gondii* were determined in infected pigs by the modified agglutination test and ELISA using a 1:25 serum dilution. Serum samples were collected from each pig on the day of infection and at three additional times during the 8 weeks of infection. Pigs were sacrificed at 60 days postinfection, and both loins were removed from each animal. Each loin was cut into two sections to approximate the average size of a retail cut of meat (~0.8 kg each). A 100-g sample was removed from each loin before injection with enhancement solutions and fed to cats to verify *T. gondii* infection; an additional 50 g was removed for collection of tissue fluids for ELISA. Each loin was weighed and then injected to 110% of the original weight of the loin with enhancement solutions (Table 1). Injections were performed using a handheld, four-needle meat-pumping station (Dayton Electric Manufacturing Company, Niles, Ill.). Each solution

was formulated to result in a final concentration in each loin as listed in Table 1. Each solution was injected into three loins, using 24 to 28 injection sites per loin. The loins injected with enhancement solutions were individually heat sealed in plastic bags and stored at 4°C. The loins were removed from storage after 7 days, and a pool of 600 g of tissue (200 g from each of three loins) was fed to one cat. Feces from each cat were examined for oocysts daily for 14 days beginning on day 3 after feeding. Each loin was stored for an additional 21 days (28 days postenhancement), and 275 g of tissue was fed (in pools of three loins per cat) to a second set of *Toxoplasma*-free cats; feces from these cats were examined for oocysts as described above. The remaining loin was stored for an additional 17 days (45 days postenhancement), and 250 g of tissue was fed to a third set of *Toxoplasma*-free cats (in pools of three loins per cat); feces from these cats were examined for oocysts as described above.

RESULTS

Results from experiment 1 demonstrated that the viability of *T. gondii* tissue cysts was affected by exposure to enhancement solutions containing SC (2%), sodium lactate (SL, ≥1.4%), or potassium lactate (PL, ≥1.4%) for a period of 7 days. Solutions 2, 3, 6, 7, 8, 9, and 11 all prevented transmission of *T. gondii* to cats fed infected brains enhanced with these solutions. SC (1%, solution 4), sodium tripolyphosphate (STP, 0.25%, solution 1), and sodium diacetate (SD), either alone or in combination with other solutions (solutions 5 and 10), had no effect on tissue cyst viability in mouse brain; cats that ingested brains treated with these solutions shed oocysts in numbers comparable to those shed by cats fed control brains (solution 12; Table 1). Solutions containing STP or SD in addition to SC (2%), SL, or PL did prevent oocyst shedding; however, SL solutions alone had the same effect as did the solutions in combination with STP or SD, and solutions containing a

combination of STP and SD had no effect on oocyst shedding unless either SC (2%) or PL was also present.

In experiment 2, serology and bioassay results from *T. gondii*-inoculated pigs indicated that each animal became infected as a result of the oral oocyst inoculation. ELISA optical density values for pigs at necropsy ranged from 0.402 to 0.993 (data not shown). Cats fed 100-g samples from loins from each infected pig prior to injection of loins with enhancement solutions all shed oocysts beginning on day 4 or 5 after feeding. Results obtained from feeding pork loins at 7, 28, and 45 days after injection with enhancement solutions revealed oocyst shedding patterns similar to those obtained in experiment 1 using mouse brains. Pork loins injected with enhancement solutions of 2.0% SC, $\geq 1.4\%$ SL, and $\geq 1.4\%$ PL and held 7, 28, or 45 days prior to feeding to cats resulted in loss of tissue cyst viability such that cats fed these tissues did not shed oocysts (Table 1). The presence or absence of STP or SD in the enhancement solutions did not affect tissue cyst viability. Oocysts were shed by cats fed loins enhanced with solutions 1, 4, 5, 10, 12, 13, and 14. Similar oocyst shedding results were observed in cats fed loins enhanced for 7 or 28 days. Some loss in tissue cyst viability occurred in loins held for 45 days at 4°C, as indicated by the fact that oocysts were not shed by cats fed loins injected with water. However, this loss of viability was not uniform; cats fed uninjected control loins, or loins injected with saline or with solutions 1, 5, and 10, did shed oocysts.

DISCUSSION

Food safety issues are of increasing concern to producers, regulatory agencies, and consumers. Undercooked and otherwise improperly prepared meat products have been implicated in *T. gondii* infections in humans (3, 19, 25). Although domestic pigs serve as a host for *T. gondii*, the levels of infection in retail meats are currently unknown. Significant morbidity occurs in cases of human toxoplasmosis; however, the development of risk reduction methods is hampered by the inability to determine what risk factors are of importance in human infection. Pigs and pork products are thought to be frequent carriers of *T. gondii* tissue cysts. Recent serological surveys have indicated a decreasing level of infection in market-weight pigs, although management systems in which pigs are allowed access to the outdoors often result in pigs with high levels of infection (13), and meat from these animals is shipped to retail stores and is available for purchase by consumers.

Consumer risk associated with pork might be further reduced by commercial processing practices for fresh pork products, including enhancement by injection. Pork products are frequently (40 to 50% of retail cuts) enhanced with salt solutions or flavorings to reduce microbial contamination, improve flavor and texture, and prolong shelf life (23, 27, 31). In the present study, enhancement solutions containing SC, SL, and PL, alone or in combination with other components, reduced or eliminated the viability of *T. gondii* tissue cysts in infected pork loins. Enhancement of pork products with salt- and lactate-based solutions has increased in recent years as a result of studies showing that SC, SL,

and PL solutions inhibit the growth of several species of pathogenic bacteria of food safety importance, including *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Campylobacter*, and *Clostridium* sp. (3, 4, 23, 27, 29). SD has a significant impact on bacterial growth in enhanced meats at concentrations of $\geq 0.2\%$; however, SD is typically used at lower levels ($\leq 0.15\%$) because of off flavors that result from higher concentrations (23). STP has not been shown to have a significant impact on bacterial growth in enhanced meats. Neither SD or STP affected *T. gondii* tissue cyst viability in this study.

It is not known how the enhancement solutions used here result in loss of viability of *T. gondii* tissue cysts. The bacteriostatic action of the solutions results from an increase in the lag phase of the bacteria due to a lactate effect on glycolytic energy metabolism (2), an increase in the pH of the meat after injection, and reduced availability of water as a result of increased binding of water molecules by meat proteins (23, 24). Effective bacterial growth inhibition has been achieved at concentrations of 2 to 3% for lactate-based enhancement solutions and at $\geq 0.2\%$ for SD (28, 31). Concentrations used in this study were similar for SL and PL (1.4 to 2%), SC (1 and 2%), and SD (0.1 to 0.2%). Enhancement of meats with lactate-based products increases the shelf life of pork products by 30 to 60% (2, 31). Tissue cysts remained viable in uninjected samples and samples injected with 0.85% saline, STP, and SD (without SL or PL) for at least 45 days after the loins were collected, indicating that tissue cysts in infected meat can remain viable for the predicted shelf life of the meat product. *Toxoplasma gondii* was not transmitted to cats fed water-injected loins that had been stored for 45 days at 4°C; tissue cyst viability in this sample may have been adversely affected by the temperature. Additional work is needed on average retail meat case temperature and the influence of this temperature on tissue cyst viability in enhanced and unenhanced meats.

Reduction of the human risk of contracting toxoplasmosis requires a multifaceted approach that begins with reduction of transmission to animals on the farm and promotion of processing and preparation procedures that reduce or eliminate the pathogen in infected meat products. This study demonstrates that some current meat-processing technologies may be effective in reducing or eliminating risk to consumers of acquiring *T. gondii* infection from retail pork products.

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