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Author(s): David S. Lindsay , Marina V. Collins , Daniel Holliman , George J. Flick , and J. P. Dubey

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Effects of High-Pressure Processing on *Toxoplasma gondii* Tissue Cysts in Ground Pork

David S. Lindsay, Marina V. Collins*, Daniel Holliman*, George J. Flick*, and J. P. Dubey†, Department of Biomedical Science and Pathology, Virginia Tech, 1410 Prices Fork Road, Blacksburg, Virginia 24061; *Department of Food Science and Technology, Virginia Tech, Blacksburg, Virginia 24061; †USDA, ARS, ANRI, Animal Parasitic Diseases Laboratory, BARC-East, Beltsville, Maryland 20705; e-mail: lindsaya@vt.edu

ABSTRACT: Ingestion of *Toxoplasma gondii* tissue cysts can result in severe disease in immunocompromised individuals and pregnant women. Treatment of meat and meat products to eliminate viable *T. gondii* tissue cysts would provide a means to protect consumers. In this study, we examined the effects of high-pressure processing (HPP) on ground pork containing viable tissue cysts of the VEG strain of *T. gondii*. Ground pork containing tissue cysts was exposed to 400, 300, 200, 100, or 0 MPa treatment for 30, 60, or 90 sec in a commercial HPP unit. The HPP-treated ground pork was subjected to acid-pepsin digestion and bioassayed in mice. The results of the mouse bioassay revealed that none of the mice inoculated with tissue cysts exposed to 400 or 300 MPa became infected, whereas all mice inoculated with tissue cysts exposed to 200, 100, or 0 MPa became infected with *T. gondii* regardless of exposure time. Results indicate that HPP treatment of ground pork with 300 MPa of pressure will render tissue cysts of *T. gondii* nonviable and make pork safe for human consumption.

Toxoplasma gondii is a protozoan parasite that infects humans and most other warm-blooded animals. Humans become infected by ingesting meat containing tissue cysts or by ingesting oocysts in the environment. It is estimated that there are 1,500,000 cases of toxoplasmosis in the United States each year and about 15% of those infected having clinical disease (Mead et al., 1999; Jones, Kruszon-Moran et al., 2001). Congenital toxoplasmosis has long been recognized because of the devastating results it can have on the infected fetus (Jones, Lopez et al., 2001). The relative importance of meat or oocysts as a source of human infection in the United States is unknown.

High-pressure processing (HPP) has been shown to be an effective nonthermal means of eliminating non-spore-forming bacteria from a variety of food products (Tewari et al., 1999; Flick, 2003). The shelf life of the products is extended, and the sensory features of the food are not or only minimally affected by HPP. Other advantages of HPP over traditional thermal processing include reduced processing times, minimal heat damage problems, retention of freshness, flavor, texture, and color; no vitamin C loss; no undesirable changes in food during pressure-shift freezing because of reduced crystal size and multiple ice-phase forms; and minimal undesirable functionality alterations (Tewari et al., 1999).

Little has been published on the effects of HPP on parasites in food. Ohnishi et al. (1992, 1994) determined that pressures of greater than 200 MPa kill 8-wk-old *Trichinella spiralis* larvae. Gamble et al. (1998) determined 55–60 MPa did not kill all *T. spiralis* larvae in pork tenderloin or diaphragm. Treatment at 200 MPa for 10 min at temperatures between 0 and 15 C kills *Anisakis simplex* larvae, with a lack of motility being used as an indicator of larval death (Molina-Garcia and Sanz, 2002). Dong et al. (2003) found that times and pressures required to kill 100% *A. simplex* larvae in king salmon and arrowtooth flounder were 30–60 sec at 414 MPa, 90–180 sec at 276 MPa, and 180 sec at 207 MPa. Slifko et al. (2000) examined the effects of 550 MPa on *Cryptosporidium parvum* oocysts in apple and orange juice. They determined that a 60-sec exposure at 550 MPa was 100% effective in decreasing infectivity of oocysts for cell cultures.

The brains from 6 mice having tissue cysts of the VEG strain of *T. gondii* (Dubey et al., 1996) were shipped by overnight carrier from the Animal Parasitic Diseases Laboratory, Beltsville, Maryland, to the Center for Molecular Medicine, Virginia Tech, Blacksburg, Virginia. The brains were mixed with ground pork until a 60-g sample was present. Brains and pork were mixed manually with a spatula for approximately 5 min. The ground pork had been purchased commercially and had been frozen at –80 C for 2 days to kill any *T. gondii* tissue cysts that might have been present. Four grams of the pork and mouse brain mixture

were placed in plastic bags and used for each treatment. Four grams of the source ground pork was not mixed with mouse brains and was used as a negative control. The bags were compressed to force out air and to obtain a uniform thickness of 3–4 mm. The bags were then sealed with a sealing machine. The bags were placed in additional bags and vacuum-sealed. The vacuum-sealed bags containing pork–mouse brain mixture were used for HPP. Two days elapsed between killing of *T. gondii* mice infected with the VEG strain, treatment with HPP, and mouse bioassay; during this time, materials were kept cold (4–10 C).

Table I shows the pressures and exposure times examined in the HPP study. The bags were placed into a commercial HPP unit (Quintus Food Press QFP 35 L-600 model, Flow International Corporation [Avure Technologies], Kent, Washington) with a 7XS-6000-intensifier pump and a maximum operating pressure of 600 MPa. The HPP unit was installed and operated at Virginia Tech's Department of Food Science and Technology.

Samples of HPP-treated and control ground pork were shipped to the Animal Parasitic Diseases Laboratory, Beltsville, Maryland, on cold packs by overnight express courier. Samples were digested in acid-pepsin solution and bioassayed in groups of 4 mice as described (Dubey, 1998). Mice were inoculated subcutaneously in the dorsal scapular region with digested ground pork. During the study, impression smears were made from the brains or lungs of any mice that died and were examined unstained by light microscopy for tachyzoites. At 6 wk post-inoculation, all surviving mice were bled from the retro-orbital plexus. The serum was collected and examined for antibodies to *T. gondii* in a modified direct agglutination assay (MAT) (Dubey and Desmonts, 1987). The mice were killed 43 day postinoculation, and brain squashes of all mice were examined for tissue cysts, irrespective of serologic data (Dubey and Beattie, 1988). Mice were considered negative if they had a negative MAT and no tissue cysts were seen in their brains. Mice were considered positive when *T. gondii* was demonstrable in tissues.

None of the mice inoculated with noninfected control ground pork became infected with *T. gondii* (Table I). All mice inoculated with non-

TABLE I. Efficacy of high-pressure processing of ground pork containing *Toxoplasma gondii* tissue cysts on the infectivity of tissue cysts for mice.

Treatment (MPa)	Exposure time (sec)*	No. of mice inoculated/ No. of mice positive
400	30	4/0
400	60	4/0
400	90	4/0
300	30	4/0
300	60	4/0
300	90	4/0
200	30	4/4
200	60	4/4
200	90	4/4
100	30	4/4
100	60	4/4
100	90	4/4
0	NA	4/4
0†	NA	4/0

* NA = not applicable.

† Negative control sample did not contain tissue cysts.

pressure-treated *T. gondii* tissue cyst containing ground pork became infected. Treatment of *T. gondii* containing ground pork with HPP at 300 or 400 MPa for 30 sec or longer completely eliminated infectivity for mice (Table I). Treatment of *T. gondii* containing ground pork with HPP at 100 or 200 MPa for 90 sec did not eliminate infectivity for mice (Table I).

The results of this study indicate that HPP can be used to render meat products free of viable *T. gondii* tissue cysts. Humans and other hosts can also become infected with *T. gondii* by ingesting oocysts in contaminated food or water. Oocysts treated with HPP for 60 sec at 550, 480, 400, or 340 MPa for 60 sec were rendered noninfectious for mice (Lindsay et al., 2005). Oocysts treated for 60 sec with HPP at 100, 140, 200, or 270 MPa for 60 sec were infectious for mice (Lindsay et al., 2005).

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Use of *Trichomonas vaginalis* Clinical Isolates to Evaluate Correlation of Gene Expression and Metronidazole Resistance

J. R. Mead[†], M. Fernandez[†], P. A. Romagnoli[†], and W. E. Secor[‡], *Veterans Affairs Medical Center, Decatur, Georgia 30033; [†]Emory University School of Medicine, Department of Pediatrics, Atlanta, Georgia 30022; [‡]Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30341; e-mail: jmead@emory.edu

ABSTRACT: We investigated whether variations in gene expression of enzymes associated with anaerobic resistance of laboratory-derived strains of *Trichomonas vaginalis* could be detected in a group of 28 clinical isolates with variations in metronidazole sensitivity. We compared isolates by real-time PCR because this method allows for highly sensitive quantification of mRNA and for evaluation of several genes simultaneously. We found that PFOR gene A mRNA levels were highly correlated with PFOR gene B levels, as well as the D subunit of malic enzyme and ferredoxin. Ferredoxin mRNA expression was also significantly correlated with that of malic enzyme and hydrogenase. However, when we evaluated relationships between these enzymes and resistance to metronidazole, we found no significant correlations between aerobic or anaerobic in vitro sensitivity to drug and mRNA levels of any of the enzymes tested. Similarly, using a Student's *t*-test, no significant differences in enzyme mRNA levels were observed between isolates separated by metronidazole resistance or susceptibility. The lack of correlation between gene expression and resistance or susceptibility could be the result of differences in expression at the protein level or because other biochemical pathways or genes are involved in the resistance observed in clinical settings.

Trichomonas vaginalis is a parasitic protozoan that causes the sexually transmitted disease known as trichomoniasis. Estimated annual incidence of trichomoniasis is over 8 million cases in North America and more than 170 million infections worldwide (Petrin et al., 1998). *Trichomonas vaginalis* infections in women range from an asymptomatic carrier state to an acute, inflammatory disease of the genital tract and have been associated with preterm labor and associated low birth weights. In males, *T. vaginalis* can cause urethritis and prostatitis. In addition, trichomoniasis is also associated with increased HIV transmission (Sorvillo and Kerndt, 1998; Buve et al., 2001; Chesson et al., 2004).

The 5-nitroimidazoles, especially metronidazole, are the most widely used antimicrobial agents for the treatment of trichomoniasis. However, metronidazole resistance is an increasingly recognized problem in treating patients infected with trichomoniasis (Sobel et al., 1999). Metronidazole enters the parasite by passive diffusion as a nonlethal prodrug. Once inside the cell, metronidazole is reduced, resulting in the generation of nitro radicals, which lead to DNA damage and cell death. *Trichomonas vaginalis* cells use pyruvate-ferredoxin oxidoreductase (PFOR) and ferredoxin-linked enzymes to metabolize pyruvate to acetate via