Effects of High Pressure Processing on Infectivity of *Toxoplasma gondii* Oocysts for Mice

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hosts, such as fiddler crabs, are infected by trematode stages that swim from snails. Birds become infected with trematode adults when they forage on prey, like fiddler crabs, that are infected with trematode metacercariae. After the trematodes mature in the birds, they pass their eggs with the birds' excreta and these eggs may then infect nearby snails. The distribution of birds' feces and, therefore, infections to snails, is probably higher in roosting areas such as mangroves. The highest prevalence of trematodes occurred at the mangrove nursery site, suggesting that this area provided roosting or foraging habitats for birds. This is consistent with the study by Smith (2001), which found direct associations between bird abundance at dead mangrove perches and trematode prevalence in caged snails. However, Smith (2001) found no such association between birds and parasitism in free-ranging snails. In contrast, we found that snail movement did not fully obscure associations between habitat and parasitism. This could occur if *B. minima* moves less or lives shorter than *C. scalariformis* or could be a result of larger distances between sampling sites (our sites were >50 m apart while Smith's were >10 m apart).

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Effects of High Pressure Processing on Infectivity of *Toxoplasma gondii* Oocysts for Mice

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ABSTRACT: High pressure processing (HPP) has been shown to be an effective non-thermal method of eliminating non-spore forming bacteria from a variety of food products. The shelf-life of the products is extended and the sensory features of the food are not or only minimally effected by HPP. The present study examined the effects of HPP using a commercial scale unit on the viability of *Toxoplasma gondii* oocysts. Oocysts were exposed from 100 to 550 MPa for 1 min in the HPP unit and then HPP treated oocysts were orally fed to groups of mice. Oocysts treated with 550 MPa or less did not develop structural alterations when viewed with light microscopy. Oocysts treated with 550 MPa, 480 MPa, 400 Mpa, or 340 MPa were rendered noninfectious for mice. Mice fed oocysts treated with no or 100 to 270 MPa became infected and most developed acute toxoplasmosis and were killed or died 7 to 10 days after infection. These results suggest that HPP technology may be useful in the removal of *T. gondii* oocysts from food products.

Toxoplasma gondii is an important parasite of humans and other warm-blooded animals. There are about 1,500,000 cases of toxoplasmosis in the United States each yr and about 15% of those infected have clinical signs (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). Toxoplasmosis is also a frequent and fatal complication in patients with AIDS or those that receive organ transplantation (Soave, 2001). The annual economic impact of toxoplasmosis in the human population in the United States is about $7.7 billion (Buzby and Roberts, 1996). It is not yet possible to determine if tissue cysts in meat or oocysts from cats are the main source of human infection in the United States.

High pressure processing (HPP) has been shown to be an effective non-thermal means of eliminating non-spore forming bacteria from a variety of food products (see Tewari et al., 1999). The shelf-life of the products is extended and the sensory features of the food are not or only minimally effected by HPP. Other advantages of HPP over tradi-

tional thermal processing include reduced processing times; minimal heat damage; retention of freshness, flavor, texture, and color; no vitamin C loss; no undesirable changes in food during pressure-shift freezing due to reduced crystal size and multiple ice-phase forms; and minimal undesirable functionality alterations (see Tewari et al., 1999).

Little has been done with parasites and HPP. Ohnishi et al. (1992, 1994) determined that pressures of greater than 200 MPa (1 MPa = 145 psi = 10 bar = 9.87 atm) killed 8-wk-old *Trichinella spiralis* larvae. Gamble et al. (1998) determined 55 to 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 HPP effective in killing *A. simplex* in salmon but that the pressures and exposure times needed to reach 100% killing caused a significant change in the color of treated salmon fillets. Slifko et al. (2000) examined the effects of 550 MPa on *Cryptosporidium parvum* oocysts in apple and orange juice. They determined that a 1 min exposure at 550 MPa was 100% effective in decreasing infectivity of oocysts for cell cultures. The present study was done to determine the effects of HPP on oral infectivity of treated *T. gondii* oocysts for mice.

A *T. gondii* naïve cat was fed tissues from a naturally infected chicken from New England containing tissue cysts of *T. gondii* (Dubey et al., 2003). The cat was housed and infected in a cat colony at the United States Department of Agriculture, Animal Parasite Diseases Laboratory, Beltsville, Maryland (Dubey, 1995). Feces containing unsporulated oocysts were collected, sporulated and purified using Sheather's sugar solution, and the suspension of purified oocysts sent on cool packs to the Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia. Oocysts were counted in a hemocytometer and dose of oocysts administered to mice (Table I) were based on visual counts.

Groups of 2 each, female, CD-1 mice were used to determine the effects of high hydrostatic pressure treatment on the infectivity of *T. gondii* oocysts. All mice were orally fed control or HHP treated oocysts using animal feeding needles. Impression smears were made from the mesentric lymph nodes or lungs of mice that died and were examined unstained by light microscopy for tachyzoites. Five to 8 wk post-inoculation (PI), all surviving mice were bled from the retro-orbital plexus, serum collected, and assayed for antibodies to *T. gondii* in a modified direct agglutination assay (Dubey and Desmonts, 1987). The brains from all mice were examined for *T. gondii* tissue cysts by squash preparations. Oocysts exposed to various HPP treatments were examined using an Olympus BX60 microscope equipped with differential contrast optics.

Oocysts were placed in Hanks' balanced salt solution (HBSS) (Experiment 1), or distilled water (Experiments 2–4) in sealable leak proof bags. The bags were manually compressed to force out air and then sealed with a sealing machine. The bags were placed in additional bags and vacuum-sealed. The vacuum-sealed bags were used for HPP. All studies were done in a Quintus food press QFP 35L–600 (Flow International Corporation, Kent, Washington). Mice were each fed 5×10^4 to 4×10^6 HPP treated oocysts (Table I). Control samples were handled in the same manner but placed in the treatment tank of the HPP unit and not exposed to HPP treatment.

No alterations were seen in the structure of sporozoites or sporocysts in oocysts exposed to any of the HPP treatments in any of the experiments. The walls of oocysts exposed to HPP treatment also appeared normal.

All control mice in all experiments developed acute toxoplasmosis and all died or were killed when ill except for 1 mouse in experiment 1. This mouse was *T. gondii* brain tissue cyst positive when examined 8 wk PI. Pressures of 340 MPa or above were effective in rendering oocysts nonviable. Treatment of oocysts using HPP at 100 MPa, 140 MPa, 200 Mpa, or 270 MPa for 1 min did not cause a reduction in infectivity for mice (Table I).

Pressures of 550 MPa are routinely reached by commercial HPP machines. The current study indicates that HPP at 340 MPa for 1 min can be used to inactivate *T. gondii* oocysts. As mentioned previously, little work has been done on HPP and parasite inactivation. The results of our study are similar to those reported by Slifko et al. (2000) for *C. parvum* oocysts in apple and orange juice and 550 MPa for 1 min.

TABLE I. Experimental protocol and results of high-pressure processing on the infectivity of sporulated *Toxoplasma gondii* oocysts for mice.

Pressure (in MPa)	Exposure time*	No. oocysts fed/mouse	No. fed/No. infected†
Experiment 1			
550	60	5×10^4	2/0
480	60	5×10^4	2/0
400	60	5×10^4	2/0
340	180	5×10^4	2/0
340	120	5×10^4	2/0
340	90	5×10^4	2/0
340	60	5×10^4	2/0
0	0	5×10^4	2/2
Experiment 2			
550	60	5×10^5	2/0
480	60	5×10^5	2/0
400	60	5×10^5	2/0
340	60	5×10^5	2/0
0	0	5×10^5	2/2
Experiment 3			
270	60	5×10^4	2/2
200	60	5×10^4	2/2
140	60	5×10^4	2/2
100	60	5×10^4	2/2
0	0	5×10^4	2/2
Experiment 4			
550	60	4×10^6	2/0
550	60	2×10^6	2/0
550	60	1×10^6	2/0
550	60	5×10^5	2/0
0	0	5×10^5	2/2

* Exposure time in sec.
† Number of mice fed oocysts/number of mice positive for *T. gondii.*

High pressure is able to inactivate or destroy prokaryotic and eukaryotic cells because it affects biochemical molecules required for metabolism. Timson and Short (1965) reported that proteins are denatured under hydrostatic pressures. Pressure induces a decrease of protein volume as well as denaturation. As widely recognized, conformational changes of protein by pressure strongly depend on the volume effect caused by hydration (Ishizaki et al., 1995). Pressure has a volume decreasing effect and disrupts hydrophobic interactions (Balny and Masson, 1993). High pressure can result in the denaturation of proteins and their gelatinization. High pressure denaturation of several proteins, ovalbumin, bovine serum albumin, and beta-lactoglobulin, was assessed by spectrofluorometry, specific rotation analysis, and differential scanning calorimetry and compared with heat and chemical denaturation. In all cases, the denaturation caused by high pressure was similar to that caused by the cleavage of hydrogen bonds with urea or guanidine hydrochloride. The studies showed that hydrogen bonds holding together alpha-helical structures broke down, resulting in an unfolding of the protein chains (Hayakawa et al., 1996). Recent research has demonstrated that pressure levels above 100–200 MPa often induce on proteins the dissociation of oligomeric structure into their subunits, partial unfolding and denaturation of monomeric structures, protein aggregation, and protein gelation (Mozhaev et al., 1996; Heremans et al., 1997). The pressure from which these modifications appear, depend on the treated samples, temperature, and protein concentration. Research on the effects of high pressure on enzyme activities have clearly demonstrated that high pressure can affect the three dimensional structure of enzyme molecules leading to their irreversible inactivation. Numerous data have been elaborated on the kinetic inactivation parameters and kinetic model fitting pressurized isolated enzymes (Lemos et al., 1999).

Biomembranes have also been identified as a site affected by pressure

(Hoover et al., 1989). Biological membranes are composed by a bilayer of phospholipids with embedded functional proteins that, among others, play an important role in transporting ions and other substances across the membrane. It has been observed that lipid bilayers undergo phase transitions under pressure. Physiological phase corresponds to a liquid-crystalline phase, in which the hydrocarbon chains of the lipid bilayers are conformationally disordered (San Martin et al., 2002).

Coccidial oocysts have been detected in shellfish (see Fayer et al., 2004) and a variety of fresh produce (Ho et al., 2000; Dillingham et al., 2002; Doller et al., 2002). Studies on the efficacy of HPP on inactivating coccidial oocysts in fresh shellfish and on produce are logical avenues of research in preventing human food borne infections with coccidial parasites.

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