Heat and Cold Inactivation of Toxoplasma gondii in Meat

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SUMMARY: Tissue cysts of Toxoplasma gondii, which may be found in sheep, swine, goats or chickens, are inactivated by the proper exposure to heat or cold. Though the literature provides some temperatures for the destruction of the parasite, the industry and the consumer may wish to utilize less severe exposure temperatures for longer time periods. Pork from pigs infected with approximately 10,000 T. gondii oocysts of each of 6 to 8 strains, for a total of 60,000 to 80,000 per pig and spiked with infected rat brains, was subjected to a sequence of times and temperatures for the heat inactivation of the parasite. A curve, Log (min) = 7.92 - 0.146(C), characterized the heat inactivation of T. gondii (r = -0.72). A curve, Square root of the time (hr) = 26.72 + 2.16 (C) characterized the cold inactivation of T. gondii (r = 0.77). These heat and cold treatments are less severe than those recommended for the inactivation of Trichinella spiralis in pork. The industry will be well served by additional research to develop a vaccine for cats, which are a definitive host, and by additional research to define the effects of processing agents and procedures on the inactivation of T. gondii.

INTRODUCTION: Human Toxoplasma infection is usually acquired by ingesting oocysts or tissue cysts of Toxoplasma gondii. Oocysts are associated with feline fecal material whereas tissue cysts are to be found in some birds but particularly in meat from pork and lamb. Dreesen and Lubroth (1981) suggested that handling or eating infected meat may in some instances be more important in the transmission of Toxoplasma than the direct infection of humans from the oocysts of cats. Oocysts do not appear to contribute to the direct contamination of meat but remain important in their role of infecting the slaughter animal from which meat and poultry are derived. Development of a cat vaccine, as recommended repeatedly by Leighty (1990), would be an extremely positive step in the control of that parasite in meat.

Presently the serologically determined incidence of T. gondii is about 21 to 95% in sheep (Malik et al., 1990; Dubey, 1990), 5.4% in finishing swine, 11.4% among sows/gilts according to Zimmermann et al. (1990), and 23% in swine according to Dubey et al. (1991), about 22% in dairy goats (Dubey and Adams, 1990) and practically zero for beef (Dubey, 1990) and broiler chickens. Some estimates of the incidence of T. gondii range as high as 30% in swine (Dubey, 1986) and range hens (Knapen et al., 1982). Regardless of the incidence of T. gondii in slaughter animals, it is recognized that meat may contain the parasite and therefore methodologies must be identified to ensure inactivation of any T. gondii that might be present. The newest technology available, irradiation, effectively inactivates T. gondii with 50 krad of exposure (Dubey et al., 1986). This approach, though effective, would be costly at three to eight dollars per carcass, and has not gained wide consumer acceptance or regulatory approval; however, approval has been granted for the irradiation of pork at up to 100 krad for the destruction of Trichinella spiralis. The most feasible approaches include inactivation by heat, curing and freezing.

A number of times/temperatures for the heat inactivation of T. gondii are provided in the

literature. Work (1968) demonstrated that cooking meat to an internal temperature of 70 C destroyed T. gondii. Sibalic (1973) recommended heating at 50 C for 30 min, and Ciembor (1981) reported that heating infected ground pork to 60 C did not destroy the infectivity of the parasite whereas heating to 65 C effectively did eliminate infectivity. In each of those studies the authors attempted to define a particular temperature which would ensure the destruction of T. gondii but did not provide a sequence of effective times and temperatures. Segments of the meat industry may wish to use lower temperatures for longer times than those published in these studies. T. gondii can also be inactivated by subjecting the infected meat to freezing for prescribed lengths of time. Grossklaus (1977) indicated T. gondii is destroyed by freezing at -18 C for 3 months or instantaneously at -30 C, Matuschka and Werner (1978) reported -20 for 24 h to be effective. Dubey (1988) found tissue cysts to be non-infective after 3 d at -12 C. Each of these reports provides a specific time and temperature at which T. gondii will be inactivated but as in the case of heat inactivation, the industry will benefit from a continuum of times and temperatures at which exposure to freezing will destroy the parasite. The present study addresses the time of exposure at hot or cold temperatures for the inactivation of T. gondii.

MATERIALS and METHODS: Seven of ten mixed breed 8-10 wk-old pigs that did not have detectable T. gondii antibodies in 1:25 dilutions of serum examined using the agglutination test (Dubey and Desmonts, 1987), were inoculated orally with a mixed inocula containing 1,000 10,000 oocysts of each of six strains. The remaining three pigs were used as controls. The pigs were killed between 84 and 144 days postinoculation (DPI), their tongues and hearts were added to muscle tissue and infected rodent brains and homogenized in a high speed food processor (Kotula et al., 1983). Tissues for heat treatment were processed using procedures described by Dubey et al. (1990). Twenty-gram bagged samples were equilibrated in a water bath for 2 min at 25 C and subsequently transferred to a constant temperature water bath where they were heat treated at 49, 52, 55, 58, 61, 64, or 67 C for 0.01, 3, 6, 12, 24, 48 or 96 min.

The treated sample was tempered for 2 min in the 25 C bath and then digested in HCl-pepsin using the method of Dubey and Beattie (1988). The resultant sedimented pellet was suspended in 2 ml of saline, mixed with 3 ml of antibiotic saline solution (2,000 U of penicillin and 200 ug of streptomycin/ml of saline) and injected subcutaneously into 5 Swiss-Webster albino female mice (1.00-1.25ml/mouse). Infected control and non infected control samples were handled in a manner similar to the treatment samples. Nine replicates were completed.

Inoculated mice were examined for T. gondii as described by Dubey and Beattie, (1988). Impression smears of lungs and brains of the mice that died were fixed in methanol, stained with Giemsa stain and examined microscopically. Blood was collected from each mouse 30 DPI. After serological examination was completed, mice were killed and the brain of each mouse was examined for T. gondii tissue cysts. Two squash-smears were made from 1 x 3 mm pieces of cerebrum of each mouse and examined microscopically without staining. After examination, the coverslip was removed and the slide was fixed in methanol and stained with Giemsa stain for permanent records. Serum of each mouse was examined for T. gondii antibodies in the dire

agglutination test as described by Dubey and Desmonts (1987). Sera were screened at 1:25 and 1:100 dilutions. Brains of seropositive mice without visible tissue cysts were subinoculated into mice or fed to cats as described by Dubey and Beattie (1988).

Infected and control pork samples for the cold temperature treatments were obtained from twenty-one mixed breed pigs which were infected as described above for the heat treated pork. The bagged 20 g samples were subjected to temperatures of -1 to -171 C for exposure times of 1 sec to 67 days. The treated samples were bioassayed in the same manner as the heat treated samples. The eleven replicates in this portion of the experiment resulted in the bioassay of about 4,000 mice.

Data were analyzed statistically (Snedecor and Cochran, 1972) to obtain a linear regression equation and the 99% upper confidence limit for the time at each temperature for the inactivation of T. gondii.

RESULTS and DISCUSSION: T. gondii tachyzoites were found in impression smears of lungs of mice that died between 10 and 28 DPI. T. gondii cysts were found in brains of all serologically positive mice that survived. Some T. gondii survived up to 3 min at 64 C but none at 67 C. Of 45 mice inoculated in 9 replicates, the numbers of T. gondii-positive mice were: 42, 37, 21 and 0 at 3, 6, 12 and 24 min, respectively, at 49 C; 29, 7, 0, 1 and 0 at 0.01, 3, 6, 12 and 24 min, respectively, at 52 C; 6 and 0 at 0.01 and 3-96 min, respectively, at 55 C; 2, 5, and 0 at 0.01, 3 and 6-96 min respectively, at 58 C; 0 at 0.01-96 min at 61 C; 0, 1, and 0 at 0.01, 3, and 6-96 min, respectively, at 64 C; and 0 for 0.01-96 min at 67 C.

A thermal death curve for the complete inactivation of all T. gondii cysts in pork predicted from these data is shown in Figure 1. The equation for the linear regression of the log heating time (min) on the end-point temperatures (C) was Log(min) = 7.918 -0.146(C). The correlation for that relationship was r = -0.717. The log of the time was plotted to better describe the geometric function of the response of the tissue cysts to different exposure durations at the selected temperatures. From the equation of log heating time regressed on temperature, the thermal death times at 67, 61, 55 and 49 C are 1, 6, 44 and 366 sec, respectively. The predicted 99% upper confidence limits in Figure 1 indicate thermal death times of 3, 12, 94 and 1,116 sec for exposure at 67, 61, 55 and 49 C, respectively. In addition to the dwell time at temperature, the model experiment required an average of 2 min for come-up and 1.5 min for come-down time.

A freeze death curve for the inactivation of T. gondii tissue cysts in pork is presented in Figure 2. The curve is based on the presence or absence of infective T. gondii in pork exposed to temperatures from -12.2 to -1 C for time periods of 17 hr to 33.6 days. The data for colder temperatures and longer times were not utilized because the T. gondii were not infective after such exposure. Three spurious bioassay positive samples were found at -34.4 and -37.5 C, but were not used in the analysis because their use would have resulted in a less conservative analysis. Those 3 positive mice are not readily explained since all samples between -31.9 and -15 C were negative. The least squares linear regression analysis resulted in the equation: Square root of the time (hr) = 26.72 + 2.16 temperature (C). The correlation coefficient was

r = 0.77. When the equation was solved for 0 time, a temperature of -12.37 C was obtained as the theoretical temperature at which T. gondii would be inactivated instantaneously.

Cooking presently is the most effective control measure for the prevention of human infection with T. gondii as well as other meat-borne pathogens. The fact that T. gondii was shown by this research to be more readily inactivated by heat than Trichinella spiralis is indeed fortunate because the cooking temperatures and times recommended for T. spiralis are generally well publicized. Thankfully, the freezing times and temperatures for inactivation of T. gondii are also less stringent than those for the inactivation of T. spiralis. Raw or undercooked meat products may pose a potential hazard, particularly in non-pork meat products which historically have not been cooked to the "well" degree of doneness.

The infectivity of T. gondii can also be eliminated by exposure to procedures used in curing of meats. Undoubtedly, moisture content, salts, and pH in combination with other parameters influence the inactivation of the parasite. Slowakiewicz and Starzyk (1970) demonstrated the parasite could survive slow drying at 18 to 20 C for up to 10 days. Grossklaus (1977) reported T. gondii does not survive normal meat processing and cooking, however, so many different meat curing and processing procedures are in use, it may be difficult to accept such an all inclusive statement without research which identifies the effects of individual or combinations of the ingredients and practices utilized in the curing and processing of meat products. Presently, T. gondii appears more sensitive to curing ingredients and processes than T. spiralis and since cured meat products are processed in a manner to ensure the destruction of T. spiralis, one assumes T. gondii is also destroyed. Since the major portion of pork is sold as cured or processed meat, such research appears very desirable to ensure the safety of existing and new processing technologies.

The meat industry has the possibility of using the pooled digestion technique to exempt pork from required treatment to inactivate T. spiralis but a similar testing program is not available for T. gondii. If rapid detection methods could be developed for meat, the regulatory agencies could allow the use of such methods for T. gondii in accredited laboratories. Since we do not presently have adequate data on the effectiveness of the various curing/processing procedures and ingredients, the scientific community should be encouraged to study such alternative approaches. Possibly the time has come for regulatory agencies to use serological testing for T. gondii on individual animals or on herds. This testing would identify pockets of infection so that production practices can be improved where necessary to minimize infection with T. gondii. Destruction of the parasite, once it is in the meat of a nation's food supply, can be considered only a temporary measure. Hazard analysis shows that domestic cats remain a major vector for toxoplasmosis. Common sense dictates vaccines must be developed to minimize the major role of the cat in the life cycle of T. gondii, and to reduce or eliminate tissue cysts in meat.

CONCLUSIONS: Procedures for the cold and heat inactivation of Toxoplasma gondii in meat are now available, however, this technology must be transferred to the meat industry and the consumer. Additional research is needed to determine the effectiveness of curing processes

and ingredients on the inactivation of the parasite. We need to develop a vaccine for cats to remove that major source of infection of slaughter animals with Toxoplasma gondii.

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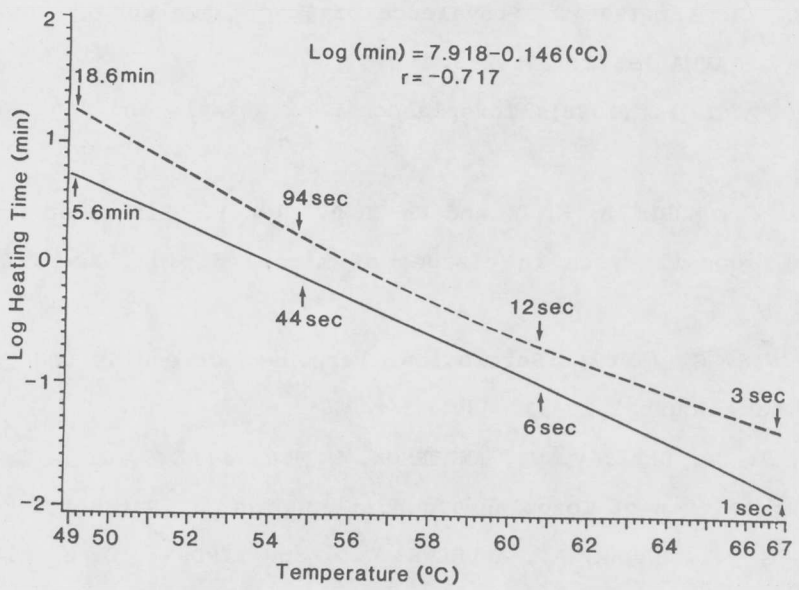

FIGURE 1. Linear regression (solid line) and the 99% upper confidence limits (dotted line) of the time required at each temperature for the inactivation of Toxoplasma gondii. Three and one-half minutes representing come-up and come-down times must be added to the times obtained from the equation on the curves.

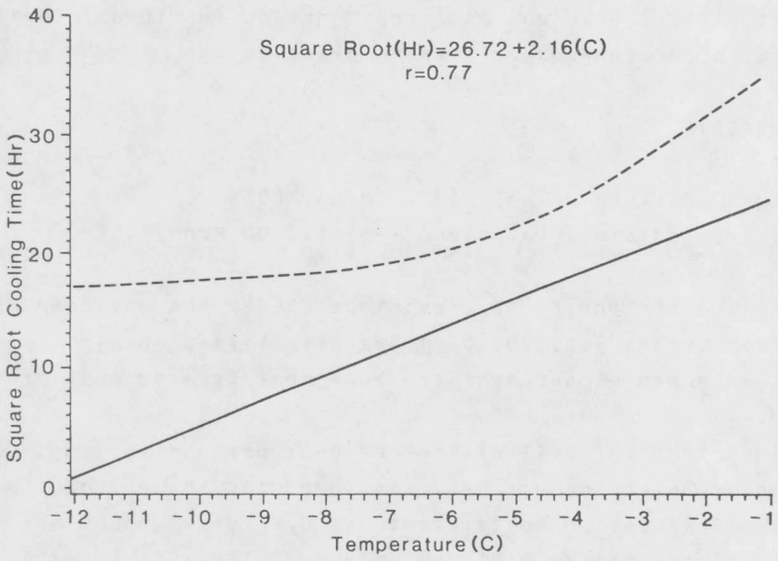

FIGURE 2. The least squares linear regression of freezing times and temperatures for the inactivation of Toxoplasma gondii (solid line) and the 99% upper confidence interval for individual values (dotted line).