



Thermal inactivation of *Bacillus anthracis* Sterne in irradiated ground beef heated in a water bath or cooked on commercial grills[☆]

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ABSTRACT

The thermal stability of heat-shocked and non-heat-shocked spores of the virulence-attenuated Sterne strain of *Bacillus anthracis* was evaluated at select temperatures in irradiated, raw ground beef (25% fat) heated in a water bath or cooked using two different commercial grills. For the former, 3-g portions of inoculated ground beef were packaged in bags that were completely immersed in a temperature-controlled circulating water bath held at 65 °C (149 °F), 70 °C (158 °F), 75 °C (167 °F), and 80 °C (176 °F) for a predetermined length of time. For the latter, formed ground beef patties (95-g each) were inoculated with spore stock A or B of the Sterne strain and then cooked on a commercial open-flame gas grill or on a commercial clamshell electric grill to achieve target internal temperatures of either 71.1 °C (160 °F), 82.2 °C (180 °F), or 93.3 °C (200 °F). Cooking ground beef patties on commercial grills, resulted in reductions of ca. 0.8 to 3.5 log₁₀ CFU/g for spore stocks A and B of *B. anthracis* Sterne after heating to 71.1 °C (160 °F), 82.2 °C (180 °F), or 93.3 °C (200 °F) on either the open-flame gas grill which required ca. 9.6 min to reach the target internal temperatures or on the clamshell electric grill which required ca. 4.0 min to reach the target internal temperatures. In comparison, our data using a water bath system and heating at 65° to 80 °C predict nearly 4 log reductions in spore levels for short times, ~½ min, depending possibly on the temperature. Thus, our data suggest that models based on heating ground beef in a water bath is not a good predictor of reductions of levels of spores of *B. anthracis* Sterne strain that would be obtained when cooking ground beef patties on commercial grills under conditions that may be typically used by consumers and/or retail establishments. Nevertheless, our data validated that cooking ground beef patties on a commercial grill at a temperature considered to be “well-done” and a temperature (71.1 °C; 160 °F) recommended by the USDA/FSIS, is effective at killing spores of *B. anthracis* Sterne.

Industrial relevance: Heating ground beef in a water bath or cooking ground beef patties on commercial grills under conditions simulating those that are used by consumers and/or that occur in retail food service establishments is effective at killing spores of *B. anthracis* Sterne.

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1. Introduction

Bacillus anthracis causes a disease known as anthrax, which is an often fatal bacterial infection that occurs when spores enter the body through abrasions in the skin or by inhalation or ingestion. Thus, the disease could be of the cutaneous, inhalation, or gastrointestinal forms (Friedlander, 1999). Symptoms of gastrointestinal infection or food-borne anthrax include nausea, fever, abdominal pain, diarrhea, ulceration, hemorrhage, edema, and ascites, as well as an eventual

fluid shift from the vascular compartment leading to shock and death of an individual within 2 to 3 days following consumption of a contaminated food (Sirisanthana & Brown, 2002). Therefore, contamination of food with *B. anthracis*, although rare, is a potentially significant public health hazard.

Since the American food chain relies on a centralized production and processing system for an ever-increasing and widespread distribution of food products (Sobel, Khan, & Swerdlow, 2002), our food supply is particularly vulnerable to deliberate contamination with threat agents. Although the incidence of *B. anthracis* in foods is rare and although medical advances have decreased its importance for both livestock and humans (Smith et al., 2001), following the October 2001 incident of terrorism involving the purposeful addition of *B. anthracis* spores to mail, this pathogen has been identified as a potential biological weapon that could be used by terrorists. An intentional contamination of food

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with *B. anthracis* spores could result in public panic, massive fatalities, and large economic losses. Thus, in a previous study we evaluated the fate of *B. anthracis* spores to germinate and/or survive in ground beef, a high-volume, minimally-processed food (Tamplin, Stewart, Phillips, Luchansky, & Kelley, 2008). Our results showed that *B. anthracis* was weakly inactivated at temperatures between 2 and 16 °C and at temperatures ≥ 45 °C, whereas growth was observed when ground beef was stored at temperatures ranging from 17 to 44 °C (Tamplin et al., 2008). Therefore, every effort should be made to guard against the introduction of this pathogen into foods, be it from natural contamination or from purposeful addition, and to validate the efficacy of existing processing technologies to eliminate it.

At present, there is a general lack of information concerning the effectiveness of interventions that would eliminate *B. anthracis* spores in food. The use of heat to achieve a specific lethality is the most important critical control point used to assure the microbiological safety of processed foods, and it plays a major role in preventing foodborne disease. Numerous studies have been published, before and after 2001, on the resistance of spores of *B. anthracis* and/or its potential surrogates to heating in a variety of mediums, including distilled water, physiological saline, synthetic media, skim milk, phosphate buffer (pH 7.0 or pH 4.5), and orange juice (Francis, 1956; Montville, Dengrove, De Siano, Bonnet, & Schaffner, 2005; Murray, 1931; Novak, Call, Tomasula, & Luchansky, 2005; Schneiter & Kolb, 1945; Stein & Rodgers, 1945). From these studies, it is clear that *B. anthracis* spores are not unusually heat resistant compared with other *Bacillus* species and/or other *B. anthracis* strains; D values, that being the time to achieve a 90% or 1-log₁₀ reduction, for *B. anthracis* strains Sterne, Vollum, and Pasteur in buffer, milk, or orange juice ranged from <1 min at 90 °C to >200 min at 70 °C (Montville et al., 2005). Of note, spores of the avirulent Sterne strain of *B. anthracis* exhibited similar heat resistance to that of other *Bacillus* species, including virulent strains of *B. anthracis* (Montville et al., 2005).

Our search of the literature found no studies on the heat resistance of *B. anthracis* in solid foods. There are, however, numerous studies published on the fate of other spore-forming food borne pathogens in response to thermal challenge in a solid food. For example, Byrne, Dunne, and Bolton (2006) reported D values of 32.1 and 2.0 min at 85 °C and 95 °C, respectively, for a three-strain cocktail of *Bacillus cereus* spores (strains DS 4313, DSM 626, and NCTC 07464) inoculated into pork luncheon roll. Faille, Lebre, Gavine, and Maingonnat (1997) also evaluated the heat resistance of *B. cereus* spores (strains LMG 6923, CUETM 93/60, CUETM 93/61, and CUETM 93/62) in mechanically separated poultry meat and reported D-values that ranged from 3.3 to 209 min at 80 °C, 5 to 89 min at 85 °C, and 2 to 17 min at 90 °C. As another example, Juneja, Eblen, Marmer, Williams, Palumbo, & Miller (1995) explored the possibility of predicting the heat resistance of non-proteolytic *Clostridium botulinum* spores in turkey slurry (50% in H₂O) from data obtained in phosphate buffer (0.1 M, pH 7.0), and found increased thermal resistance in the slurry compared to buffer. As reported by others, the thermal resistance of spores of *Bacillus* species can also be appreciably increased by food components such as protein and fat (Behringer & Kessler, 1992; Shehata, Khalafalla, El-Magdoub, & Hofi, 1977). Thus, it would be misleading to predict the thermal-death-time (TDT) values of *B. anthracis* spores in ground beef from data obtained in buffer, milk, or any other liquid substrate. Accordingly, the work reported herein was undertaken to provide a quantitative and comparative assessment of the heat resistance of spores of *B. anthracis* Sterne in ground beef heated in a water bath and cooked on commercial grills. Based on the D-values for *B. anthracis* strains in buffer (pH 7.0 or pH 4.5), pasteurized 2% fat milk, or pasteurized orange juice reported by Montville et al. (2005), the attenuated Sterne strain was selected as a suitable surrogate for virulent *B. anthracis* strains, including the Ames strain. The results presented herein could be used to establish cooking temperatures and times that would minimize the potential danger of *B. anthracis* infections from the gastrointestinal form of anthrax arising

from its intentional addition to foods such as ground beef that are produced in high volume and subsequently cooked and consumed by a significant portion of the population.

2. Materials and methods

2.1. Bacterial strains and ground beef

Two different spore stocks of the avirulent Sterne strain of *B. anthracis* were used in this study to address the potential for variability among sources of spores/strains. One of the spore stocks, designated as spore stock A, was obtained from the United States Department of Agriculture, Food Safety and Inspection Service (USDA/FSIS) Microbial Outbreaks and Special Projects Laboratory (MOSPL) in Athens, Georgia. The MOSPL spore stock was originally obtained from Dr. Bruce Harper of the U.S. Army Dugway Proving Grounds (Dugway, UT). The other spore stock, designated as spore stock B, was originally obtained from Drs. Jeff Karns and Michael Perdue ((Perdue, Karns, Higgins, & van Kessel, 2003); USDA, Agricultural Research Service, Beltsville Area Research Center, Beltsville, MD). The spores were prepared essentially as described by Novak et al. (2005) and were stored in 20% ethanol (vol/vol) in dH₂O at 4 °C. Spore population densities were determined by plating serial dilutions in 0.1% (wt/vol) peptone water, with and without prior heat shock (75 °C for 20 min), onto brain heart infusion (BHI) agar plates. It is worth mentioning that heat shocking activates the spores to germinate. Therefore, higher numbers are likely to be enumerated on recovery medium. Data were recorded as colony forming units (CFU)/ml after incubating the plates at 37 °C for 24 h. Fresh raw ground beef (75% $\pm$ 1% lean) was purchased at a local grocery store and was prepared, irradiated, and stored essentially as described by Porto-Fett, Juneja, Tamplin, and Luchansky (2009).

2.2. Thermal inactivation of *B. anthracis* Sterne in irradiated ground beef heated in a temperature-controlled water bath

Fifty gram portions of irradiated ground beef were inoculated with an appropriate dilution of non-heat-shocked *B. anthracis* Sterne stock A spores to achieve a target level of ca. 7.0 log₁₀ spores/g. The inoculated ground beef samples were mixed and aseptically weighed into 3-g portions as described (Juneja, Marks, & Mohr, 2003; Porto-Fett et al., 2009). Unheated ground beef inoculated with non-heat-shocked spores served as a control. Thermal inactivation was conducted by placing the inoculated samples in a basket that was completely submerged in a temperature-controlled, circulating water bath (Excal, Model Ex-251HT, NESLAB Instruments Inc., Newington, NH) stabilized at 65 °C (149 °F), 70 °C (158 °F), 75 °C (167 °F), or 80 °C (176 °F) $\pm$ 0.5 °C (0.4 °F) as previously described (Lindsay & Murrell, 1985; Porto-Fett et al., 2009). The temperature of the water was monitored using a type K thermocouple (Omega Engineering Inc., Stamford, CT) connected to a temperature data logger (Dickson SP150, Addison, IL). Total heating times ranged from 180 min at 65 °C (149 °F) to 5 min at 80 °C (176 °F). After removal from the water bath, samples were immediately plunged into an ice–water bath and analyzed within 30 min. For each of three trials, duplicate samples were analyzed at each sampling interval. The data are reported as the average log₁₀ CFU/g for all trials and replicates.

2.3. Thermal inactivation of *B. anthracis* Sterne in irradiated ground beef cooked on commercial grills

Irradiated ground beef was transferred aseptically to a sterile stainless steel food-processing bowl and inoculated with spores that were heat-shocked in sterile 0.1% peptone water at 80 °C for 10 min or with non-heat-shocked spores of spore stock A or B of *B. anthracis* Sterne to achieve a target level of ca. 6.0 log₁₀ spores/g of meat. Thereafter, the inoculated ground beef was mechanically mixed

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