DISSERTATION

# PREVALENCE AND CONTROL OF *LISTERIA, SALMONELLA* AND *ESCHERICHIA COLI* O157:H7 IN COLORADO RURAL HOUSEHOLDS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

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Fort Collins, Colorado

Summer 2009





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May 5th, 2009

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MAWILL R. RODRíGUEZ MARVAL ENTITLED PREVALENCE AND CONTROL OF *LISTERIA, SALMONELLA* AND *ESCHERICHIA COLI* O157:H7 IN COLORADO RURAL HOUSEHOLDS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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## ABSTRACT OF DISSERTATION


### PREVALENCE AND CONTROL OF *LISTERIA, SALMONELLA* AND *ESCHERICHIA COLI* O157:H7 IN COLORADO RURAL HOUSEHOLDS

The household environment has been linked to multiple outbreaks of foodborne illnesses, including listeriosis and salmonellosis. The food handling habits of consumers play a critical role in the food chain continuum, and need to be investigated to better prevent foodborne illnesses that originate at home. The objective of this work was to identify risk factors associated with prevalence of *Listeria, Salmonella* and *Escherichia coli* O157:H7 in the rural household environment, and to provide scientific data for the development of reheating instructions for frankfurters in the home setting.

To study risk factors associated with *Listeria, Salmonella* and *Escherichia coli* O157:H7 prevalence in rural Colorado households with or without ruminants, households were recruited, and samples from food and the environment, as well as behavioral data from the primary foods preparer in the house, were collected. *Listeria* was isolated from refrigerators, kitchen sinks, shoes soles, clothes washing machine and food samples, with higher prevalence in households with ruminants. No sample was found positive for *E. coli* O157:H7, and *Salmonella* was isolated from one refrigerator, one washing machine, one working glove, and two shoe samples. Results indicated that behavior related to handling and cooking of perishable foods affected the probability of household samples

testing positive for *Listeria*, regardless of presence of ruminants. Personal cleanliness habits were related to presence of *Listeria* on shoe soles, clothes washing machine, and working gloves. Shoes testing positive in households with ruminants were more frequently associated with multiple positive environmental samples compared to households without ruminants. Results indicated that consumer education on handling and storing perishable foods, and animal handling to prevent contamination of the household through shoes or clothes may reduce prevalence of *Listeria* in home environments.

Two studies evaluated reheating of frankfurters inoculated with *L. monocytogenes* with or without antimicrobials. In both cases, frankfurters were formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate and were inoculated with a ten-strain composite of *L. monocytogenes*. After inoculation, frankfurters were vacuum-packaged and stored under conditions simulating manufacturing/retail and consumer storage. In one study, after the appropriate storage time, frankfurters were placed in a bowl with water and treated in a household microwave oven. Exposure to high power for 75 s reduced pathogen levels (0.7±0.0 to 1.0±0.1 log CFU/cm$^2$) to below the detection limit (<-0.4 log CFU/cm$^2$) on frankfurters with lactate/diacetate. On frankfurters without lactate/diacetate, initial levels of *L. monocytogenes* (1.5±0.1 to 7.2±0.5 log CFU/cm$^2$) on untreated samples increased as storage in vacuum and aerobic packages progressed. For this formulation, the exposure to high power for 75 s produced reductions between >1.5 and 5.9 log CFU/cm$^2$. Depending on the treatment and storage time, the water used to reheat the frankfurters had viable *L. monocytogenes* counts of <-2.4 to 5.5±0.5 log CFU/ml. Results indicated that levels of *L. monocytogenes* contamination <3.7 log

CFU/cm$^2$, on frankfurters can be significantly ($P \geq 0.05$) reduced by microwave oven heating at high power for at least 75 s. Higher contamination levels, such as those found on frankfurters without lactate/diacetate and stored for a prolonged period of time, require longer exposure to microwave heating in order to render the product safe for consumption.

In the other study, inoculated frankfurters were treated with hot water after different storage periods to evaluate the destructiveness of different time and water-temperature combinations on *L. monocytogenes*. Treatments at 80°C (60, 120 s) and 94°C (30, 60 s) reduced pathogen counts on frankfurters with PL/SD to at/below the detection limit (<-0.4 log CFU/cm$^2$) from initial levels on control (immersed in 25°C water for 300 s) samples. For frankfurters without PL/SD, where pathogen numbers reached 6.1 log CFU/cm$^2$ on 60-day old vacuum-packaged product stored aerobically for 7 days, hot water treatments reduced counts by 1.0 (30 s/80°C) to >6.0 (120 s/94°C and 300 s/94°C) log CFU/cm$^2$. No survivors were detected in the heated water after any treatment (detection limit <-2.5 log CFU/ml). While low levels of *L. monocytogenes* on frankfurters can be inactivated with short exposure to hot water, increased contamination that may occur as the product ages needs longer times and/or higher temperature for inactivation.

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## ACKNOWLEDGEMENTS

*"When you want something, the whole Universe conspires to help you realize your desire."* Paulo Coelho in *The Alchemist*

I want to thank all the people that have made my desire possible.

To Dr. John Sofos for his patience and advice during countless revisions of my manuscripts, and to all my committee members, Dr. Patricia Kendall, Dr. Keith Belk and Dr. John Scanga.

Especial thanks to Dr. Ifigenia Geornaras, for all her support as a scientist, and especially as a friend.

To Dr. Jim zumBrunnen for all his help and patience with the statistical analysis.

To Mary Schoneder, Ruth Inglis-Wooo and Janine Reiss.

To all people that babysat Emanuel in these 4 years, especially Yorleny and Alberto. Your support, help, and especially your friendship are invaluable.

To all graduate students and personnel in the lab that help me through endless hours of work, especially Dr. Yohan Yoon, Dr. Ioanna Barmpalia, Catie Simpson, Gianna Durán, Alex Byelashov, Alexia Lianou and Alicia Valdéz.

To María, William, Melissa and Marcel, who has been a source of motivation beyond what words could explain.

This work is dedicated to my dear husband Harvey, who have had immense patience and love for me for the last 13 years, and have encourage and support me to go

for everything I have wanted. And to my son Emanuel, with the most beautiful brown eyes I have ever seen, for each smile, kiss and hug, even when mama was gone so much time. He and his sister Sophia, on her way, have helped me and inspired me in so many lovely ways to reach my goal.

*"Ask and it will be given to you; Seek and you will find; Knock and the door will be opened to you." Matthew 7:7*

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# CHAPTER I

# INTRODUCTION

*Listeria monocytogenes* has become a serious problem for the food industry and public health authorities after being identified as a foodborne pathogen more than 25 years ago (Schlech *et al.,* 1983). The pathogen is commonly present in a variety of foods, from fresh produce to ready-to-eat food (RTE) items (Gianfranceschi *et al.*, 2003). In addition, it is commonly found in the natural environment, such as in soil and decaying vegetation (Welshimer and Donker-Voet, 1971; Colburn et al., 1990). Due to its ubiquitous nature and resistance to various environmental stresses (Gandhi and Chikindas, 2007), food producers have encountered challenges in the control of *L. monocytogenes* in their facilities and products. Appropriate control of this microorganism is especially important in RTE food products, since those are designed and prepared to be consumed without any further cooking treatments by the consumers, as it is the case with certain cheeses and deli meats, and other meat and poultry products. Usually, contamination of these types of food items occurs after the lethality step in processing has been applied (Wenger *et al.,* 1990; Tompkin, 2002) as a consequence of cross-contamination through processing equipment, such as a slicer. Thus, proper equipment sanitation and hygiene, as well as monitoring of these activities, are of critical importance in the prevention of contamination of food products with *L. monocytogenes*, and are some of many actions

that industry and government agencies have taken to reduce the incidence of the pathogen in food and, therefore, the occurrence of foodborne listeriosis.

The Food Safety and Inspection Service (FSIS) of the United State Department of Agriculture (USDA) began testing for presence of *L. monocytogenes* in 1987, and has since declared it to be an adulterant when detected in RTE products such as hot dogs and luncheon meats, establishing a zero tolerance policy in this type of meat products (Shank *et al.,* 1996; USDA-FSIS, 1999a). The Pathogen Reduction, Hazard Analysis and Critical Control Point (HACCP) Systems Final Rule appeared in the Federal Register in July 1996 (USDA-FSIS, 1996) with the objective of reducing the incidence of *L. monocytogenes* and other foodborne pathogens in foods produced under USDA-FSIS inspection. After national outbreaks associated with RTE meat and poultry products (CDC 1990; 1991;1992;1993;1994;1995;1996;1997;1998), and several recalls related to the pathogen (USDA-FSIS, 1999b), USDA-FSIS issued a notice advising establishments to reassess their HACCP plans to ensure that they were adequately addressing *L. monocytogenes* (USDA-FSIS, 1999b). In addition, quantitative microbial risk assessments were conducted to determine the extent of consumer exposure to foodborne *L. monocytogenes* through RTE foods (USDHHS-FDA, 1999; USDHHS-FDA-CFSAN/USDA-FSIS, 2003). Also, in 2001, FSIS proposed several new requirements for the processing of RTE and other meat and poultry products (USDA-FSIS, 2001). The proposed food safety performance standards for all RTE and all partially heat-treated meat and poultry products set the levels of pathogen reduction as well as the limits on pathogen growth in order to produce not-adulterated products. Since October 2003, Control of *L. monocytogenes* in Ready-to-Eat Meat and Poultry Products Final Rule

(USDA-FSIS, 2003) requires that any establishment producing post-lethality exposed RTE product must meet the specific requirements of one of three alternative programs for addressing *L. monocytogenes*. Using Alternative 1, an establishment may control *L. monocytogenes* by applying a post-lethality treatment of the product *and* an antimicrobial agent that inhibits or limits the growth of the pathogen. Using Alternative 2, establishments may address *L. monocytogenes* control with a post-lethality treatment *or* an antimicrobial agent that inhibits or limits the growth of the pathogen. Lastly Alternative 3 allows establishments to control the pathogen in the post-lethality processing environment using only sanitation procedures, and is likely to result in plants being subject to a more frequent testing by FSIS than establishments using Alternative 1 or 2.

All these industry and government efforts have lead to a consistent reduction in the rate of RTE meat and poultry products contaminated with *L. monocytogenes* (USDA-FSIS, 2008d). Currently, USDA-FSIS conducts three different individual sampling programs (USDA-FSIS, 2008d) known as ALLRTE (which includes samples taken through 2004 from randomly selected establishments), RTE001 (started in 2005, where establishments are selected for sampling based on different risk factors for *L. monocytogenes*), and RLm (which includes sampling of products, product contact surfaces and environmental surfaces in conjunction with a comprehensive Food Safety Assessment). Combined data from these three sampling programs showed a steady decrease in the percentage of positive samples from 4.61% in 1990 to 0.43% in 2007 (USDA-FSIS, 2008d). There also has been a concurrent reduction in numbers of reported cases of listeriosis infection during this period of time; estimated annual

incidence rates decreased over 40% between 1989 and 1993. This was attributed to the increased efforts that industry and government directed towards prevention of contamination during food processing and handling, as well as an increased public awareness due to targeted educational efforts of the Centers for Disease Control and Prevention (CDC), the USDA and the FDA (Tapero *et al.,* 1995; Shank *et al.,* 1996; Swaminathan and Gerner-Smidt, 2007). By 2002, a minimum of 0.26 cases per 100,000 persons was reached, almost achieving the Healthy People 2010 objective of 0.24 that was established by the CDC, the FDA and the FSIS (USDA-FSIS, 2008d). However, no additional reduction in the incidence of listeriosis has been achieved since 2002; with 122 laboratory-confirmed cases of listeriosis, the reported incidence was 0.27 cases per 100,000 population in year 2007, the same that was reported in year 2001 (CDC, 2008). The *Listeria* Risk Assessment, published in 2003 (USDHHS-FDA-CFSAN/USDA-FSIS, 2003), estimated the likely impact of control strategies on the predicted number of listeriosis cases. Interestingly, in that assessment, two control measures which are directly in the hands of consumers (controlling home refrigerators operating temperature and limiting the storage time for deli meats) were estimated to potentially reduce the number of cases of listeriosis by 13.6 to >98% every year. It seems that new strategies are needed in order to go further in the prevention of this disease, and since, as described previously, the majority of actions taken have addressed the manufacturing and retail portions of the food production chain, the last link, -consumers and their households- may need to be addressed next. Food consumption data need to be generated to better understand the food handling practices for various segments of the population, and to measure the

impact of educational campaigns on consumers (ILSI Research Foundation/Risk Science Institute, 2005).

Consumers are responsible for storage and preparation of foods after products leave the manufacturing and retail facilities. Measures to assure safety of foods are designed in a way that specific conditions need to be met by consumers at home, such as storage time, refrigerator temperature, and appropriate reheating of certain foods according to directions found on product labels. However, beyond any direct regulatory control, there is no guarantee that the consumer will handle foods appropriately at home. Thus, safety measures designed and applied by producers should consider the worst case scenarios, such as temperature abuse and prolonged storage time. Literature suggests that *L. monocyotgenes* increase to high numbers on RTE meat and poultry products during storage under common household conditions (Lianou *et al.,* 2007).

In an evaluation of a production facility, Wenger *et al.* (1990) found that the most probable number (MPN) of *L. monocytogenes* in finished turkey franks at a retail establishment was less than 0.3/g, but an opened package from a listeriosis patient's refrigerator had a MPN >1100/g. It has also been suggested that cross-contamination of the kitchen environment and other foods may occur as a consequence of mishandling of exudates from contaminated frankfurter packages (Wang and Muriana, 1994). Thus, consumers should be motivated and educated in the prevention of foodborne illness by applying proper food handling techniques at home, and by controlling the potential risk factors associated with the presence of the pathogen in the household environment. Since most households differ, there may be unique factors to each one that may increase the risk of infection with this foodborne pathogen, such as the presence of animals that have

been identified as potential carriers of the organism. Some pets, like dogs, cats and birds, also have been reported as carriers (Iida *et al.*, 1991; Weber *et al.,* 1995), but of special concern are ruminant farm animals such as cattle, goats and sheep, which may be a reservoir and disseminators of *L. monocytogenes* (Nightingale *et al.,* 2004; 2005) and other pathogens such as *Salmonella* and *Escherichia coli* O157:H7 (Winfield and Groisman, 2003; Doane *et al.,* 2007). Thus, working in a farm environment within the household premises may represent a pathway for introduction and transfer of microbial contamination that may be initially present in the farm environment or animals. Thus, it is important to clarify the dynamics related to the behavior of farmers in order to identify the potential risk factors associated with this practice.

In addition to prevention of contamination of the food supply in the household, proper cooking and reheating of food is crucial in the prevention of listeriosis, especially for people with compromised immune systems. These groups of the population (i.e., pregnant women and their fetuses, the elderly, persons with HIV/AIDS, transplant organs recipients and patients under chemotherapy treatments, etc.) are more susceptible to infection with listeriosis, and even low numbers of pathogen cells can cause disease (Maijala *et al.,* 2001; Angelakopoulos *et al.,* 2002; ILSI Research Foundation/Risk Science Institute, 2005). Proper food handling and preparation is crucial for these people, and since they may be cared for in their own residence (Jarvis, 2001; Hayes *et al.,* 2003), preparation of the food is responsibility of the individual or a family member. Thus, proper cooking and reheating instructions for risky foods must be provided on the labels of such products.

The objectives of this dissertation were:

1. To identify risk factors among rural households that may potentially be related with an increased prevalence of *Listeria, Salmonella* and *E. coli* O157:H7 within the household environment.
2. To evaluate different combinations of power level and time for reheating of frankfurters in a domestic microwave oven for the inactivation of potential contamination of *L. monocytogenes* that may be present on frankfurters formulated with or without antimicrobials, which were stored under conditions simulating manufacturing/retail and household storage conditions.
3. To evaluate different water temperature and time combinations for the inactivation of potential contamination of *L. monocytogenes* that may be present on frankfurters formulated with or without antimicrobials, which were stored under conditions simulating manufacturing/retail and household storage conditions.

The main goal of the present work was to provide scientific data that can be used to develop recommendations for consumers on food handling and general household cleanliness that may help in the prevention of foodborne illnesses.

# CHAPTER II

# LITERATURE REVIEW

## Food safety and consumers

Food safety must be addressed along the food chain, from the farm to the consumer's household. Use of safe food handling practices at home, and consumer awareness about foodborne pathogens and their control, could reduce the number of foodborne illnesses (Albrecht, 1995; Chung-Tung *et al.*, 2005). The safety measures taken by consumers play a critical role in the prevention of foodborne illnesses because they constitute the final step in the food preparation process, and the domestic kitchen is considered to be "the final line of defense" (Redmond and Griffith, 2003). Between 1970 and 1999, up to 87% of all reported foodborne disease outbreaks in Europe, Australia, New Zealand, the United States, and Canada, were associated with foods prepared or consumed in the home (Redmond and Griffith, 2003). In addition, foodborne diseases originating in private homes are three times more frequent than those from food consumed in cafeterias (Redmond and Griffith, 2003), which is in agreement with the fact that factors such as temperature control, food wrapping, cleaning and disinfection procedures seem to be better controlled in these establishments. Furthermore, Centers for Disease Control and Prevention reports (CDC, 1990; 1991; 1992; 1993; 1994; 1995; 1996; 1997; 1998; 1999; 2000; 2001; 2002; 2003; 2004a; 2005) suggest that, between 1990 and 2005, 18 different listeriosis outbreaks were identified. Out of those, 10

outbreaks were related to food consumed or prepared in private homes. These numbers emphasize the importance of proper food handling and storage in the household. Thus, the household environment should be taken into account when considering strategies for prevention and control of foodborne illnesses.

Evidence indicates that most consumers think about food safety as a responsibility of some other entity. According to a survey by Cates *et al.* (2006), 75% of respondents believed that food manufacturers and restaurants have a lot of responsibility for ensuring the safety of the U.S. food supply. Likewise, 60% of respondents believed that food manufacturers have a lot of control over ensuring the safety of the U.S. food supply. In addition, consumers and farmers were viewed as not having a lot of control. Another study by Bruhn and Schutz (1999) showed that 69% of consumers were somewhat confident in the safety of food purchased at the supermarket. Furthermore, according to Chung-Tung *et al.* (2005), only 17% of U.S. consumers thought homes were the sites in which food safety problems were most likely to occur. Roseane *et al.* (2005) reported that consumers identify food processing plants and restaurants as the most likely locations for food safety problems to occur. This sense of security may lead consumers to believe that they do not need to follow safety measures at home.

Yang *et al.* (2006) conducted a consumer phase risk assessment for *L. monocytogenes* in deli meats. They used a one-dimensional Monte Carlo simulation to model variability in growth and cross-contamination of *L. monocytogenes* during food storage and preparation of deli meats at home. In contrast to what most consumers believed, their results indicated that with an approximate 0.3% of the servings contaminated with $>10^4$ CFU/g of *L. monocytogenes* at the time of consumption, home

food-handling practices can increase the mean mortality from consumption of deli meats by as much as $10^6$ times. These finding stress the importance of appropriate home food handling, especially of higher risk foods, like deli meats and frankfurters (USDA-FSIS, 2003).

Even though consumers' behavior at home has been recognized as very important with respect to food safety, they remain the least studied link in the food chain, and information available about the consumer has been considered to be largely anecdotal. A considerable amount of food preparation and handling occurs in the domestic environment, so research and consumer education regarding the risk of unsafe food-handling practices is an essential element for prevention of foodborne disease (Williamson *et al.,* 1992; Käferstein, 1997). Some studies have reported that it is very common to find home food-safety practices that may lead to illness. For example, Trepka *et al.* (2007) found that a high percentage of people in high risk groups (pregnant women specifically) do not follow food safety recommendations such as reheating of hot dogs before consumption or use of a food thermometer. Kosa *et al.* (2007b) found that 29% of consumers use smell as a factor in deciding whether to eat a refrigerated food product. Furthermore, the results of that study indicated that consumers do not understand the meaning of the different types of date labeling, which may lead to considering some food products as safe for longer times than those recommended. According to Altekruse *et al.* (1999), about one fifth of consumers do not wash their hands with soap after handling raw meat or chicken. A similar proportion of participants reported not washing cutting surfaces with soap/bleach after using them for cutting raw meat or chicken. This emphasizes the importance of and the need for research that address consumer practices.

Increasing consumer's awareness of major foodborne pathogens is a potentially useful way to promote safer food handling practices (Chung-Tung *et al.* 2005). Microbial food contamination can often be controlled in the home through a combination of careful storage, preparation, and cooking procedures (Smallwood, 1989).

In order to successfully inform and educate consumers about food safety at home, it is necessary to understand what is important for them, what their motivations are, and what are their current habits and practices at home. The Ajzen-Fishbein theory argues that individuals make rational decisions about health behavior when they are aware of associated health problems, have some knowledge concerning these problems, and have some judgment as to the level of risk involved in not changing their behavior (McIntosh *et al.* 1994). This means that, in order to take actions regarding food safety, the consumer should know that they are at risk for foodborne illness at home if they do not follow appropriate food safety practices, and they need a motivation for change. McCurdy *et al.* (2005) conducted focus groups among consumers that reported cooking meat. Participants were asked about their motivations to use a food thermometer to check for doneness of meat items, and the most frequently mentioned motivational reason was avoidance of foodborne illness, especially when cooking for children or elderly persons. Participants felt that the strongest motivation was their own experience with foodborne illness in the past.

In the last 20 years, most efforts to control *L. monocytogenes* have targeted the food processing industry. Gombas *et al.* (2003) reported a survey of *L. monocytogenes* in ready-to-eat (RTE) foods. Their results showed a prevalence of 1.82%, and the majority of the positive samples were contaminated at levels of <10 CFU/g. In addition, according

to CDC (2007), there has been a reduction of approximately 34% in the laboratory confirmed cases of *L. monocytogenes* infection from 1996-98 to year 2006. However, most of the decline in this rate occurred before 2006. In 2006, the incidence of *Listeria* infections remained higher than at its lowest point in 2002, with 138 cases, which represents an incidence of 0.31 cases per 100,000 population. These numbers point to the need for further measures to prevent foodborne listeriosis infection. Listeriosis still occurs, and since food processors seem to be doing a good job in controlling the incidence and the levels of contamination in their products, consumer habits are an obvious next target.

Consumers are beyond the scope of action for regulations, which makes motivation, education and awareness the only instruments to reach them and control listeriosis incidence at the last step of the food chain. Knowledge and awareness of *Listeria* among U.S. consumers can be considered low, and it is affected by several factors, such as age, gender, and demographic characteristics. Altekruse *et al.* (1996) conducted a survey to estimate consumer knowledge of foodborne microbial hazards and food-handling practices. Their results showed that, by 1993 only 9.6% of participants were aware of *Listeria and* only 1.1% were aware of a common food vehicle for the transmission of the pathogen. By 2001, 32% of U.S. consumers said they had heard of *Listeria* according to Chung-Tung *et al.* (2005). In that study, researchers found that awareness of health problems related to eating sprouts, drinking unpasteurized juices, or mercury in some fish was associated with a larger probability of having heard of *Listeria.* Consumers which were more likely to be aware of the pathogen were those that perceive pathogen contamination as a serious food safety problem, perceive that they had a higher

likelihood of getting sick from unsafe practices, had a household member with sickness possibly caused by eating contaminated food, or who were the main meal preparer in their households. More recently, Cates *et al.* (2006) reported a 43.8% awareness and knowledge of *Listeria* among U.S. consumers. However, over two-thirds of consumers who reported awareness of *Listeria* were unable to identify a food vehicle. Some consumers identified raw meat (17%) and poultry (3%) as likely food vehicles for this pathogen. Only five percent identified fruits or vegetables, seafood, cheese, milk and processed meats as likely food vehicles for *Listeria.* In order to control the pathogen by taking actions at home that minimize its incidence, consumers must know where to look, and what foods they need to be careful about.

***L. monocytogenes* and the home environment**

*L. monocytogenes* is a Gram-positive, motile, rod-shaped bacterium which is pathogenic to animals and humans, causing the zoonotic disease known as listeriosis, a commonly fatal infection of the bloodstream and central nervous system (Low and Donachie, 1997; Acheson, 2000; Schlech *et al.,* 2005). This disease has long been considered to be invasive and, as such, affecting only susceptible population groups (e.g., immunocompromised people, newborn children and fetuses, etc.). However, in recent years, a new non-invasive form of listeriosis that causes febrile gastroenteritis in immunocompetent people has increased the public health significance of *L. monocytogenes* (Heitmann *et al.*, 1997; Aureli *et al.*, 2000; Hof, 2001; Carrique-Mas *et al.*, 2003; Ooi and Lorber, 2005; Schlech *et al.*, 2005). The organism is widespread in the environment and has been recovered from vegetation, soils, animal feces, silage, water, dust (Welshimer and Donker-Voet, 1971), meat and meat products, sauces, vegetables,

dairy products, fish and fish products and environmental samples from food processing plants (Colburn *et al.,* 1990; MacGowan *et al.,* 1994; Gianfranceschi *et al.,* 2003), and farms (Fenlon *et al.,* 1996; Nightingale *et al.,* 2004; 2005). Its ubiquitous character makes all environments a potential source for contamination with this pathogen. However, it is now recognized that nearly all cases of human listeriosis are foodborne (Painter and Slutsker, 2007). The pathogen is commonly isolated from foods kept under refrigeration temperatures (Cox *et al.*, 1989; Chunhua and Muriana, 1994; Jackson *et al.*, 1993; Sergelidis *et al.*, 1997; Wallace *et al.,* 2003; Azevedo *et al.,* 2005); this fact represents a problem when foods contaminated with this pathogen are consumed without further thermal treatment, as it is the case with delicatessen meats, soft cheeses, and other RTE foods.

The home environment constitutes a potential source of bacterial contamination, particularly the kitchen and the bathroom, which may serve as reservoirs of microorganisms (Kagan *et al.*, 2002). It has been suggested that once pathogens which cause intestinal disease enter the domestic environment, they can be transmitted between surfaces, people and their food supply (Curtis *et al.*, 2003). Duggan and Phillips (1998) suggested that contamination with *L. monocytogenes* can be disseminated widely in kitchens. These authors isolated *Listeria* from kitchen dishcloths and refrigerators in 73% (22/30) of the houses they sampled. A correlation was found between contamination levels of dishcloths and refrigerator salad compartments. The primary source of contamination was unknown. However, these results indicated that cross-contamination may have occurred. Prevention of cross-contamination is considered one of the most important food-handling behaviors for control of *L. monocytogenes* (Hillers *et al.,* 2003).

In a study of vulnerable population groups, cross-contamination was implicated as a risk factor in 39% of foodborne diseases in England and Wales (Redmond *et al.,* 2004). In another study, Mattick *et al.* (2003) reported on the potential survival and cross-contamination of foodborne pathogens in the kitchen environment, funding, that it is possible to contaminate clean dishes from dishcloths and it is even possible, but rare, to contaminate food from dishes. For these reasons, it is important to control contamination of the household environment with pathogens. This is critically important when any member of the household is immunocompromised. In order to control pathogen contamination, it is necessary to know its ecology, how it enters the home, and how it spreads through different surfaces and environments within the house.

It has been observed that when contaminated cloths were applied to surfaces, organisms were transferred to the surface and hands of the user in numbers that may lead to infection and illness (Scott and Bloomfield, 1993; Mattick *et al.,* 2003), stressing the importance of cross-contamination in the spread of organisms in the kitchen. *L. monocytogenes* has been isolated from multiple places within the house environment. Cox *et al.* (1989) isolated *L. monocytogenes* from 6 out of 35 dishcloths and 1 out 35 refrigerators they sampled. In a study of *Listeria* species in the domestic environment, Beumer *et al.* (1996) recovered *L. monocytogenes* from vegetable compartments of refrigerator, kitchen sink, washing-up brush, dishcloth, toothbrush, and bathroom, with counts ranging from $10^2$ to $10^4$ CFU/object. In a similar study, Duggan and Phillips (1998) isolated *L. monocytogenes* from dishcloth, toothbrush and the salad compartment of the refrigerator. More recently, Wagner *et al.* (2007) conducted a study where they isolated *L. monocytogenes* from 1.1% of dust samples. In addition, they detected a cross-

contamination scenario, where in a household of elderly individuals, three food items, the kitchen and a fecal sample were positive for a genetically indistinguishable pulsotype. Controlling or minimizing cross-contamination at home could reduce the prevalence of foodborne illness (Scott, 2000).

Another potential source of *L. monocytogenes* contamination for the home environment is the asymptomatic carriage of the pathogen by one or more members of the household. A study by Schuchat *et al.* (1993) suggested that in homes where a case of invasive listeriosis had been diagnosed, carriage among household contacts might represent transmission between household members, through either food handling or direct person-to-person transmission. More studies are necessary to determine if this transmission and contamination of the household environment is possible when no cases of listeriosis have been diagnosed in the home.

Asymptomatic human carriage of *L. monocytogenes* has been reported previously (Luppi *et al.,* 1988; Grif *et al.,* 2001; 2003), and can occur not only in healthy people, but also among persons in high risk groups for listeriosis, appearing to be seasonal (MacGowan *et al.,* 1991; 1994). According to Lamont and Postlethwaite (1986), pregnancy, which is considered a risk condition for listeriosis, does not affect the fecal carriage rate of *L. monocytogenes,* and as many as 44% of pregnant women could be asymptomatic carriers of the pathogen and have normal pregnancies. MacGowan *et al.* (1991) reported that 2.5% of renal transplant and haemodialysis patients were asymptomatic carriers of *Listeria,* including *L. monocytogenes, L. innocua* and *L. welshimeri.* In another study, Luppi *et al.* (1988) detected 10 strains of *Listeria* spp. in 513 fecal specimens from asymptomatic humans. Grif *et al.* (2001) reported a 0.8% rate

of fecal carriage for healthy individuals. More recently, Stepanović *et al.* (2007) reported a rate of 0.1% (1/958) vaginal carriage of *L. monocytogenes* among women of reproductive age. Prevalence of *L. monocytogenes* appears to be low, ranging from 2.7% in healthy pregnant women, to 77% among laboratory workers who handled the pathogen (Sauders *et al.,* 2005). All these results stress the fact that, even when *Listeria* carriage may vary among different populations and the prevalence is generally low in human stools, asymptomatic carriage of this pathogen does occur. If *Listeria* can cross contaminate the household environment (Wagner *et al.,* 2007) as other bacteria do (Curtis *et al.,* 2003), then the potential of the household being a source for listeriosis is real, and should be addressed.

Environmental contamination with *L. monocytogenes* may be persistent over time. The pathogen can survive several years in the food processing environment. Several studies have shown that some strains of *L. monocytogenes* can become established in a food-processing facility and remain members of the resident flora for months or even years (Unnerstad *et al.*, 1996; Tompkin, 2002; Lunden *et al.*, 2003). This may also be the case in the household environment. Thus, it is very important to prevent initial contamination.

Animal pets also can constitute a source for *Listeria* contamination within the household. This microorganism has been isolated from rats, pigs and dogs (Embil *et al.,* 1984; Iida *et al.,* 1991), so there is potential for contamination of the household from these animals, especially if they are allowed inside the house. Consumers that own pets and other animals should be informed about correct handling to avoid contamination of the household environment.

Other reasons that stress the importance of the household environment as a potential source for *L. monocytogenes* contamination include the increased number of high risk people who receive health care at home, for example the elderly. According to Jarvis (2001), since 1950, the number of persons >65 years of age in the United States has tripled, from 12.2 million to 36 million, and it is expected that by 2035, the population of persons > 65 years of age will exceed 80 million. Other immunocompromised populations also have increased. In a survey by Chung-Tung *et al.* (2005), 12% of consumers said they had one or more health conditions, including liver disease, diabetes, reduced gastric acidity, HIV, AIDS, a weakened immune system, or were under chemotherapy or radiation therapy, and it is estimated that high risk individuals comprise approximately 25% of the United Stated population (Smith and Fratamico, 2000). In addition, health-care is often delivered at home, is unregulated, and may be provided by family members. Under such circumstances, the avoidance of household environment contamination with opportunistic pathogens such as *L. monocytogenes* is critical.

### *L. monocytogenes* in the farm environment and ruminants

*L. monocytogenes* has been identified as a major infectious agent causing neurological syndromes and uterine infections in bovine, sheep and goats (Rebhun and deLahunta, 1982; Jemmi and Stephan, 2006). This pathogen can cause encephalitis, which was first described as "circling disease" (Gill, 1931); the clinical signs of infection are a consequence of the lesions in the brain stem and/or medulla oblongata (Rebhun and deLahunta, 1982; Hamir and Moser, 1998). The uterine infection can result in spontaneous abortion, stillbirth or infection of the newborn (Low and Donachie, 1997).

Animals carrying *L. monocytogenes* can lead to direct contamination of milk as a consequence of listeric mastitis, encephalitis, or *Listeria*-related abortion (García *et al.*, 1996). Thus, animal feces and the farm environment are important sources of raw milk and meat contamination by *L. monocytogenes* (Arimi *et al.*, 1997; Nightingale *et al.*, 2005; Jemmi and Stephan, 2006).

Most cases of animal listeriosis are associated with contaminated feedstuffs (Arimi *et al.*, 1997). Since *Listeria* is widely distributed in soil, water and vegetation, it is very likely for this microorganism to be detected in silage (Wagner *et al.*, 2005). Thus, emphasis must be placed in avoiding its multiplication within the bale, since this can be a common source of infection for different animals within the farm environment. There are other studies that have pointed to silage as the source of *L. monocytogenes* in ruminants. Fenlon *et al.* (1996) conducted a study on farms, and tested different environments, feed and feces of ruminant and non-ruminant animals. They found a relationship between contamination of feed and excretion of *L. monocytogenes*. Furthermore, excretion of *L. monocytogenes* by cattle continued after two months of discontinued feeding of contaminated silage. These findings show that ruminants are both targets and vehicles of infection (Low and Donachie, 1997; Nappi *et al.*, 2005), and in cases where the household environment is near the farm, it may be possible that members of the household, who had contact with the farm environment and animals, can introduce this pathogen into the house environment and their food supply. More research is necessary to determine possible pathways for the contamination of the household from the farm environment. Nightingale *et al.* (2004) conducted a case–control study on the incidence of *L. monocytogenes* on farms with and without previously reported cases of listeriosis.

Their results indicated a higher overall prevalence of the pathogen (27.3%) in case farms compared to controls (14.6%). They also found differences in the prevalence of *L. monocytogenes* on bovine farms (22.2%) compared to small ruminant (goat and sheep) farms (16.8%). In another survey carried out by Pritchard *et al.* (1995) the researchers detected *Listeria* in environmental samples from 90.5% (19 out of 21) of the dairy plants that they tested. According to Oliver *et al.* (2005), in dairy farms the incidence of *L. monocytogenes* in bulk tank milk can vary from 1.0 to 12.6% of the samples. The incidence of *L. monocytogenes* in the feces of dairy cows has been reported to be 9.6% (Husu, 1990). In another study, Nightingale *et al.* (2005) tested feces and feed samples from multiple cattle and small ruminant (goats and sheep) farms, finding a seasonal effect on the shedding of *L. monocytogenes.* During winter, 62.5% of the cattle tested positive for *L. monocytogenes,* whereas only 7.5% were positive during the summer. These findings were consistent with those of MacGowan *et al.* (1994), where the percentage of specimens which contained *Listeria* spp. varied with the time of the year, 3.2% positive in August and September and 1.0% and 1.7% in June and July, respectively.

Other studies point to the farm environment as the source of the pathogen that may later cross-contaminate other environments. In a cheese producing farm, Wagner *et al.* (2005) reported a case where *L. monocytogenes* was possibly transmitted from contaminated feeds to the milk supply and from there to the working surfaces of the cheese-making facility and humans. In that study, *L. monocytogenes* was detected two months after the outbreak on the boots and in the feces of a worker. As a consequence, a cross-contamination cycle was established between the worker and the cheese processing environment. The study of the farm is relevant since several outbreaks of listeriosis have

been traced back to the farm environment or the primary production facility as the source of contamination. For example, in 2001, an outbreak of febrile gastroenteritis involving 48 people in Gaevleborg, Sweden, was linked to on-farm manufactured dairy products (Carrique-Mas *et al.,* 2003).

Some studies have reported that sheep are more susceptible to infection with *L. monocytogenes* than cattle (Low and Donachie, 1997; Wagner *et al.*, 2005). This susceptibility seems to be related to the type of feed consumed. Silage-fed sheep are more susceptible to infection, not only because silage may be a source of *L. monocytogenes,* but also because silage may reduce the number of lymphocytes in the serum, which may affect their immune system (Grønstol, 1980; Nicolas, 1983; García *et al.*, 1996). This fact is especially important during the winter months, when the availability of fresh pastures is reduced and animals are fed more silage. Sheep can shed the pathogen for 3 months after an outbreak, which is a potential risk of contamination for other areas of the farm, the home environment and the food supply (Nightingale *et al.*, 2004).

Some strains of *L. monocytogenes* may be persistent in the farm environment. Ho *et al.* (2007) used molecular subtyping to identify specific strains of the pathogen that were present in milk farms and the adjacent processing facility. According to their results, the same ribotype was isolated on one occasion from both the farm environment and the processing facility, suggesting cross-contamination between these two environments. These authors also found that the subtypes that can be persistent on the farm are different from those found in the nearby dairy processing facility. However, there are no data available regarding the potential cross-contamination scenario between the farm and the household environment, which may constitute a potential route of contamination for both

sporadic and outbreak cases.

The farm environment has been reported as a source of *L. monocytogenes* isolates that have been linked to human illness (Arimi *et al.,* 1997; Fugett *et al.*, 2007). Fugett *et al.* (2007) used pulsed-field gel electrophoresis (PFGE) to analyze temporally matched *L. monocytogenes* isolates from different sources, and found that seven matched isolates from listeriosis outbreaks in Los Angeles and Switzerland occurred among isolates from farms. These results stressed the importance of the farm environment as a source of listeriosis infection. There is a need for research that can help in elucidating the routes that allow *L. monocytogenes* to be disseminated from the farm environment to its human hosts.

**_Salmonella_ in the farm and the household environment**

*Salmonella* is a Gram-negative bacterium of considerable animal and public health concern (Huston *et al.*, 2002a). This ubiquitous microorganism is known to cause important disease in many species of animals (Huston *et al.*, 2002b) which can carry and pass it in their feces. Salmonellosis is a zoonotic disease. Baby chicks and ducklings are especially likely to pass salmonellosis to people. Other animal pets are also a source of contamination for the household and its members. *Salmonella* has been isolated from dogs, cats, birds, horses (Pelzer, 1989) and reptiles like lizards, snakes, and turtles (Strohl *et al.,* 2004). Also, direct and indirect exposure to amphibians has been reported to be associated with human salmonellosis (Srikantiah *et al.,* 2004).

Cattle are an important source of *Salmonella,* where infection can result in clinical disease, but most cases are sub-clinical. *Salmonella* serotypes are associated with a variety of febrile diseases in food animals characterized by enterocolitis, bacteremia or

abortion (Andrews and Bäumler, 2005). Small ruminants such as sheep and goats, as well as pigs, also are potential carriers of *Salmonella* (Alvseike and Skjerve, 2002; Hendriksen, *et al.,* 2004). Cattle infected with *Salmonella* may shed, continuously or intermittently, large numbers of the organism in their feces, with sub-clinical shedding of the organism being more common than clinical disease (Richardson, 1975; Smith, 1996). Several factors may affect cattle shedding of this pathogen. Fossler *et al.* (2005) found an association between administration of antimicrobials within 14 days of sampling and a significantly decreased probability for *Salmonella* shedding. These authors also found a lack of association of other cattle-level factors such as stage of lactation, parity, and pre-weaned calf age with *Salmonella* shedding, which indicated that few factors at the cattle-level can be used for *Salmonella* control and that efforts may be more effectively directed at herd-level factors, such as pen management systems.

Apparently healthy cattle may introduce *Salmonella* into abattoirs and processing plants. Intestinal carriage or an infected organ may result in contamination of equipment surfaces or workers hands, thereby leading to contamination of carcasses and processed foods. Thus, carriage of *Salmonella* serotypes by apparently healthy food animals is directly responsible for its subsequent introduction into derived food products (Andrews and Bäumler, 2005). Food animals infected with *Salmonella* represent a substantial risk for people if they make their way into the food supply; this risk is reflected in the fact that several cases of human salmonellosis have been traced back to dairy farms (Holmberg *et al.*, 1984).

Members of *Salmonella enterica* subsp. I account for more than 99% of human cases of disease and are associated with three distinct clinical syndromes: typhoid fever,

bacteremia and enterocolitis (Andrews and Bäumler, 2005). Foodborne transmission accounts for approximately 90% of human salmonellosis in the U.S. (CDC, 2004b). Data from the USDA-FSIS 2007 Pathogen Reduction, Hazard Analysis and Critical Control Point verification testing program, showed that 2006 was the year with the lowest percentage of chickens and other raw products testing positive for *Salmonella* (USDA-FSIS, 2007). However, according to CDC (2007) data, the estimated incidence of *Salmonella* for the year 2006 did not decrease significantly compared with the baseline of 1996-1998. Furthermore, although *Salmonella* incidence did not decrease significantly overall, the incidence of *S.* Typhimurium decreased significantly but in contrast, significant increases in incidence compared with baseline occurred for *S.* Enteriditis, *S.* Newport, and *S.* Javiana. These data indicated that, in order to control foodborne salmonellosis, it is necessary not only to reduce salmonellosis in the animal population by good animal husbandry practices, but it is also necessary to educate the public in the proper handling, preparation, and storage of foods (Pelzer, 1989).

Interestingly, and in contrast to the relative low level of awareness of *L. monocytogenes,* most U.S. consumers have some knowledge about *Salmonella.* According to Altekruse *et al.* (1996), by 1992, 80.2% of consumers were aware of this pathogen; Cates *et al.* (2006) reported 93.9% awareness and knowledge of *Salmonella.* However, there is a consistently high number of salmonellosis cases every year in the U.S., with about 45,000 reported cases in 2004 (CDC, 2004b). Thus, it is not a problem of lack of consumer knowledge, but it may be still closely related to the final consumer and the household environment. Speirs *et al.* (1995) suggested that public awareness of hygiene alone may not be enough to prevent foodborne illness and that an increased

understanding leading to improved practices at home is needed.

The household environment has become a major source of contamination for *Salmonella*, especially in small children. Jones *et al.* (2006) carried out a case-control study addressing salmonellosis in infants. Compared to adults, infants have limited diets and environmental exposures; however, they are exposed to sources of contamination with *Salmonella* which are common among older populations. The authors suggested that many of the risk factors identified in the study were potentially modifiable through targeted preventive education and behavioral change among the caretakers of infants. Haddock and Malilay (1986) conducted a study on risk factors for infant salmonellosis in Guam, and found a particularly high incidence of this disease among infants. These authors reported that fifty percent of all cases of known age were less than 1 year old, giving an incidence for this age group of 4,348 per 100,000 population. Even when these authors were not able to identify any discrete risk factor, their findings suggested that aerosols created by vacuuming, sweeping and other household activities were an important source of salmonellosis infection in infants. Similar results were reported by Schutze *et al.* (1999) when they conducted a study on children 4 years of age or younger with confirmed *Salmonella* infection. These authors found *Salmonella* positive samples from vacuum cleaners and dirt surrounding the front door more often than for other environmental samples, such as countertops or refrigerators, supporting the hypothesis that infants and young children may come into contact with *Salmonella* either through aerosolization or by having direct contact with contaminated soil or debris. Haddock and Nocon (1994) found that vacuum cleaners used in homes of infants with confirmed *Salmonellosis* infection were more likely to contain *Salmonella* bacteria than those used

in control households. These authors hypothesized that dust blown through open windows or dirt tracked into homes on footwear were possible mechanisms for spread of this microorganism inside the house. In another study, Haysom and Sharp (2003) recovered *Salmonella* from vacuum cleaner dust in three different households. These authors also inoculated autoclaved vacuum dust with *Salmonella,* and were able to recover the microorganism 65 days after inoculation. An interesting finding of that study is that total viable counts of vacuum cleaner dust collected from households were significantly higher in samples from rural than urban environments and in households where pets were present. Enterobacteriaceae also were more numerous in rural than in urban households. These authors concluded that a major source of bacterial contamination for the household in rural areas were livestock, manure and soil that could be introduced into the domestic environment on footwear, the feet of pets and currents of air. These findings and the fact that a high proportion of cases of *Salmonella* infection are reported in children 5 years old or younger (Haddock and Malilay, 1986; Haysom and Sharp, 2003), make the study of the household environment a priority in the search for control measures.

Once *Salmonella* enters the household environment, it can be transferred between different surfaces and household members. Gorman *et al.* (2002) studied the kitchen environment of a group of 25 consumers before and after they prepared roasted chicken. Although that study reported a small number of *Salmonella*-infected chickens (2/25), there was 100% cross-contamination with other sites in the kitchen after preparation and cooking of those chickens (using their own choice of whole chicken product and their own preparation method) that were contaminated. Those sites included the counter-top

and dishcloth.

Rice *et al.* (2003) found that contamination of the household with *Salmonella* increases with increased exposure to the organism due to occupational activities, such as farming with known salmonellosis in cattle. In that study, the authors tested vacuum bag samples for the presence of *Salmonella* and found positive results when one or more members of a household had direct contact with livestock, even if there were not known recent cases of salmonellosis. In a case-control study, Baker *et al.* (2007) found an increased risk of *Salmonella* infection associated with contact with sheep carcasses. Increased risk also was associated with household contact with dogs or sheep, and household occupational contact with live animals or carcasses. Hendriksen *et al.* (2004) reported on a case of salmonellosis on a Dutch farm due to *S.* Typhimurium DT104A where the same strain was isolated from a diseased pig, a calf, and a child. This showed the potential importance of the household environment in the transmission of *Salmonella*, especially if the home is closely linked to a farm environment. Furthermore, contrary to most consumers' perception, it appears that a significant proportion of infectious intestinal disease, including salmonellosis, is contracted in the home (Gillespie *et al.*, 2001; Bloomfield, 2003).

Other places within the home where *Salmonella* can be found, and as a consequence become potential sources of contamination, include toilet bowls, refrigerators, kitchen sinks and countertops (Haddock and Robert, 1994; Schutze *et al.*, 1999; Finley *et al.*, 2006). In a case-control study, Parry *et al.* (2005) found that dishcloths were twice as likely to be contaminated with *Salmonella* (10%) in households with sporadic cases of salmonellosis than those in households without reported cases of

salmonellosis. In another study, Barker and Bloomfield (2000) reported the survival and environmental spread of *Salmonella* from domestic toilets in homes where a family member contracted salmonellosis. These authors found that the pathogen can persist in the biofilm material found under the recess of the toilet bowl rim. It also became incorporated into the scaly biofilm adhering to the toilet bowl surface below the water line. Furthermore, *Salmonella* Enteriditis persisted in one toilet for four weeks after the affected individual recovered from the symptoms of the infection, which constitutes a potential source of contamination for other sites within the household and its members. Education regarding sanitation, food safety and general household cleanliness may be useful in reducing the cases of salmonellosis (Gillespie *et al.,* 2001).

***Escherichia coli* O157:H7 in the farm and the household environment**

*Escherichia coli* is a ubiquitous organism that lives commensally in the gastrointestinal tract of most mammals (Brabban *et al.*, 2004), and occurs in fecal material at levels of millions per gram. This microorganism was long considered harmless to health and was used simply as an indicator of fecal contamination in food and water (Foster, 1986; Winfield and Groisman, 2003). However, some strains of some *E. coli* serotypes are human pathogens. *E. coli* O157:H7 is the best known member of this group. The organism can cause severe illnesses, including bloody diarrhea which can progress to hemolytic uremic syndrome (HUS), a serious disease of the urinary tract that is characterized by kidney dysfunction and hemolytic anemia (Bell *et al.,* 1997; Dundas *et al.,* 1999; Tsai, 2003; Zheng and Sadler, 2008).

One major reservoir for *E. coli* 0157:H7 is cattle, and transmission from cattle to humans occurs via contaminated food or water (Hussein and Sakuma, 2005; Smith and

Fratamico, 2005). This pathogen can colonize the bovine intestinal tract, thus providing a source for fecal contamination of meat during slaughter (Foster, 1986; Vanselow *et al.*, 2005). In the dairy farm environment, *E. coli* has been isolated from milk tanks at rates that vary from 0.8 to 3.8 % (Oliver *et al.*, 2005). Several outbreaks involving this pathogen have been traced back to cattle, involving direct or indirect contact with the animals, or consumption of food contaminated with cattle waste (Griffin and Tauxe, 1991; Feng, 1995). In 2000, two cases of *E. coli* 0157:H7 infection occurred in children after visiting an inner city open farm. Verocytotoxin-producing *E. coli* O157:H7 strains were isolated from fecal samples from cows, horses, pigs, sheep, goats and from compost samples which had been processed for 3 months (Chapman *et al.* 2000). In another case of animal-to-human transmission, 51 people were confirmed or suspected of infection with *E. coli* O157:H7 after a visit to a dairy farm (Crump *et al.*, 2002). The results of this study showed that 13% (28/216) of cattle on the farm were colonized with *E. coli* O157:H7 which had the same distinct pulse-field gel electrophoresis pattern as that found in isolates from the patients. In addition, the pathogen also was recovered from surfaces that were accessible to the public, which shows how this pathogen can be transferred from the animals to other environments and surfaces. Between 2000 and 2005, 25 outbreaks of *E. coli* O157 in the United States were associated with animal contact in public settings (Steinmuller *et al.*, 2006). These results emphasize the importance of public education regarding good hygiene practices, especially hand washing, which was found in this study to be a protective behavior. The severity of the disease and the high costs associated with it stress the necessity for development of control measures directed toward avoiding contamination of foodstuffs, the household environment, and, ultimately,

the consumer. It has been estimated by Frenzen *et al.* (2005) that the cost of illnesses caused by *E. coli* O157:H7 in the U.S. for 2003 was $405 million.

Between 1982 and 2002, CDC received 350 reports of outbreaks due to *E. coli* O157:H7, representing 8,598 cases, 1,493 (17%) hospitalizations, 354 (4%) HUS cases and 40 (0.5%) deaths. Transmission route for 183 outbreaks (52%) was foodborne, 74 (21%) unknown, 50 (14%) person-to-person, 31 (9%) waterborne, 11 (3%) animal contact, and 1 (0.3%) laboratory-related. The food vehicle for 75 (41%) foodborne outbreaks was ground beef, and for 38 (21%) outbreaks was produce (Rangel *et al.,* 2005). More recently, it has been found that produce may be a common vehicle for infection with *E. coli* O157:H7, and several outbreaks have been traced back to fresh produce (Feng *et al.,* 1995; Hilborn *et al.,* 1999; Rangel *et al.,* 2005).

Most studies regarding the ecology of *E. coli* O157:H7 have addressed the farm environment (especially dairy farms) and ruminants as a reservoir of the pathogen (Dewell *et al.,* 2005; Oliver *et al.,* 2005; Doane *et al.,* 2007), and the meat manufacturing chains (Doyle and Schoeni, 1987; Marshall *et al.,* 2005) as the place where most cross-contamination episodes take place. However, limited research has been conducted on the potential role of the household in the spread of this microorganism and how to control it at the home level, even when it has been reported that the rate of household transmission for *E. coli* O157:H7 is about 4% (Parry and Salmon, 1998).

Considering the severity of the illness that this pathogen produces, and that the patient may excrete the organism and become a risk to the wider community (Parry and Salmon, 1998), it is important to control this pathogen at home. According to Scott (2001), there is a need for control measures that help in the prevention of contamination

of the household environment, especially regarding food, hands and environmental hygiene, since primary prevention is critical when infections are more difficult to control by the use of antibiotics.

**Control of *L. monocytogenes* contamination in RTE foods at the consumer level**

Contamination of the plant processing and household environments with *L. monocytogenes* has been reported in the past by numerous researchers (Cox *et al.*, 1989; Pritchard *et al.*, 1995; Sergelidis *et al.*, 1997). Several explanations about how contamination occurs have been proposed. Bloomfield and Scott (1997) conducted a study about cross-contamination and infection in the domestic environment. Their findings suggested that surfaces, hands, cloths and floors can serve as reservoirs and/or disseminators of a series of pathogens. Thus, if *L. monocytogenes* is present in the household environment, it may contaminate the food supply and vice versa. *L. monocytogenes* can enter the household environment via contaminated food products, such as RTE meats and vegetables. During storage, handling, and preparation of these foods, a constant risk exists of cross-contamination of hands and food contact surfaces in the kitchen (Scott, 2001).

Since *L. monocytogenes* is effectively controlled by heat treatments (Doyle *et al.*, 2001; Porto *et al.*, 2004), most of the residual contamination found on food products is due to post processing cross-contamination, and is usually present at low levels (Gombas *et al.*, 2003; Reij and Aantrekker, 2004). In the industrial environment, cross-contamination usually occurs after lethality steps have taken place, for example during peeling or slicing (Wang and Muriana, 1994; Reij and Aantrekker, 2004). In the household environment, cross-contamination between raw and prepared meals as well as

poor refrigeration techniques and sanitation may be principal causes of food contamination with *L. monocytogenes*. Residual levels of contamination should be low, but in case of abuse or consumption by immunocompromised people, even low levels of contamination may be of concern (Hayes *et al.*, 2003). Therefore, besides the usual dietary recommendations, immunocompromised people should follow recommendations for treating RTE foods before consumption to control residual contamination. Education of the consumer should be especially targeted to persons in the high risk groups and their caregivers in order to avoid infection with *L. monocytogenes*.

In contrast to the usually closely monitored conditions under which food products are kept at manufacturing facilities and retail stores, household conditions are beyond the control of any authority and consumers do not always pay the necessary attention to how food should be handled and stored after it is purchased. According to Godwing and Coppins (2005), only few consumers have a thermometer in their refrigerator, and those who have one, do not check it on a regular basis. In addition, these authors reported that consumers can abuse their refrigeration-requiring food during transport and prior to storage. This shows the importance of studying domestic consumer practices and of including temperature-abusing conditions when studies are designed to simulate household handling of foods.

As the final step of the food chain, consumers are in charge of the final treatments that are applied to food before consumption. Cooking and reheating methods such as microwave oven heating, steaming, grilling and boiling are commonly used in meal preparation. Some of these methods have been reported in the literature as being effective in killing *L. monocytogenes* in foods (Hollywood *et al.*, 1991; Porto *et al.*, 2004). One of

the most popular of these methods is the microwave oven, which is near the top of home appliances most appreciated by the public (Magaziner and Patinkin, 1989; Osepchuk, 2002).

Microwave energy in used in the food industry in various processes; for example tempering of frozen foods, which involves raising the temperature from approximately 0°C to approximately 27°C (Schiffmann, 2001) to permit mechanical processing such as slicing, dicing, grinding, pressing and molding of the product, which may be meat, fish, butter, vegetables or fruits. Microwave ovens also are used industrially for cooking of bacon, processing potato chips, and drying of pasta (Osepchuk, 2002; Berteli and Marsaioli, 2005), prevention of mold growth, conditioning of wheat, baking, poultry cooking, sherry making, chewing gum manufacture, pasteurization of beer, and other uses (Osepchuk, 2002).

Another potential use of microwave energy in the industry is the in-package pasteurization of ready to eat meats for elimination of *L. monocytogenes.* According to Huang (2005), an on-off control mechanism was able to maintain the surface temperature of beef frankfurters near the set points of 78, 80 or 85°C, achieving a 7-log reduction of *L. monocytogenes* in inoculated frankfurters, by the use of a 600W nominally rated microwave oven within 12-15 minutes.

Microwave ovens have been reported to be effective in killing foodborne pathogens. Gundavarapu *et al.* (1995) reported complete inactivation of *L. monocytogenes* ($5x10^5$ CFU/g) inoculated on shrimp with 2 minutes holding after microwaving for 168, 84, 62, and 48s at 240, 400, 560, and 800W, respectively. Kaya *et al.* (2003) used microwave heating to kill *L. monocytogenes* inoculated in milk ($1.5x10^9$

CFU/ml). In that case, the pathogen was not recovered from milk after heating for 50 or 60s at full power (550W).

In another study, Pucciarelli and Benassi (2005) used a microwave oven for inactivation of *Salmonella* Enteriditis on raw poultry. These authors found that *Salmonella* destruction at medium power level had a more uniform destruction rate compared to treatments at high power. After 110 sec at high power, the pathogen was non-detectable in the samples.

Daşdağ *et al.* (1995) inoculated lamb and quail meat with *L. monocytogenes* ($10^9$ *Listeria*/g), and treated them in a microwave oven at 550W. The authors used different sizes of samples, and adjusted the times for the treatments accordingly. Complete elimination of *L. monocytogenes* was achieved after 7, 14, 21, 28, 35 and 42 sec for samples of 5, 10, 15, 20, 25, and 30 g of weight, respectively.

Huang *et al.* (1993) used a microwave oven to cook catfish fillets inoculated with *L. monocytogenes* and *Aeromonas hydrophila* ($10^6$ cell/cm$^2$). The fish fillets were heated in the microwave oven to final temperatures of 55, 60, and 70°C. When heated to 70°C as the end point temperature, no *L. monocytogenes* or *A. hydrophila* were recovered from fish samples if they were covered while heated. These authors concluded that, due to uneven heating of food during microwave cooking, it is often difficult to assure that adequate thermal process has been applied and they recommended covering the food during cooking to entrap heat.

Lund *et al.* (1989) inoculated *L. monocytogenes* into the stuffing of chicken and onto the surface of chicken to a level of approximately $10^7$ *Listeria*/g of stuffing and between $10^6$-$10^7$/g of skin. They then cooked the birds in a 650W microwave oven,

following the manufacturer instructions for cooking (22min/kg), followed by 20 min of standing time. Results of that study showed the importance of standing time after microwave cooking to allow the heating to be more evenly distributed by conduction. After full cooking time, but before standing time, *L. monocytogenes* was recovered by enrichment, but after the standing time, no cells of the pathogen could be isolated, indicating a lethality of over $10^6$ log.

Hollywood *et al.* (1991) also showed the importance of standing time after microwave cooking. In their study, minced beef (500g) was cooked in a microwave oven to rare (37min), medium (39min) or well done (41min). They observed much greater reductions in counts after the samples were allowed to stand for 30 min, wrapped in aluminum foil.

A domestic microwave oven is a multimode cavity in which electromagnetic waves form a resonant pattern. When food is present inside the oven, the energy of the electromagnetic waves is transferred to the water molecules, ions, and other food components, making them vibrate in their attempt to align their electric charge with that of the electromagnetic field, raising the food temperature (Zhang and Datta, 2001). This cooking technique is very popular and easy to use in the household environment because it is fast and economical. However, there is strong concern over possible pathogen survival in contaminated food cooked in microwave ovens (Kaya *et al.*, 2003). Several cases of foodborne illness have been related to food processed in microwave ovens. Barnes *et al.* (1989) reported a case of listeriosis in a cancer patient, where *L. monocytogenes* was recovered from an opened package of turkey frankfurters and other foods in the patient's refrigerator. The pathogen also was recovered from two unopened

packages of the same type of frankfurters from a local store. The patient reported that she had eaten one turkey frank daily, after reheating it in the microwave oven.

Gessner and Beller (1994) reported on an outbreak of gastroenteritis due to *Salmonella* Typhimurium in roast pig that was prepared far before consumption. These authors conducted a case-control and a retrospective cohort study on the case. They found that thirty people reheated the meat before eating it, 10 used microwave ovens, and 20 used conventional ovens for reheating. All 10 persons who ate meat that had been reheated in a microwave oven became ill, compared with none of 20 who ate meat reheated in a conventional oven or skillet. While in that case poor food-handling techniques probably allowed *Salmonella* to proliferate in the meat, these authors concluded that probably due to more uniform heating and longer times used during conventional reheating, the conventional methods provided a protective effect compared with reheating it in a microwave oven.

In 1995, another outbreak of *Salmonella* infection was attributed to microwave oven cooking (Evans *et al.,* 1995). In that case, six persons assisting in a dinner in a private home became sick after eating a dish made from cold boiled rice, raw shell eggs, grated raw carrots, cheese and commercial curry powder. Those ingredients were reported to have been mixed and cooked in a domestic 500W microwave oven with a rotating turntable on full power for 5 min.

Levre' and Valentini (1998) suggested that infection hazard linked to microwave cooked food can be avoided by following adequate procedures concerning heating time, temperature and post-heating holding time. Furthermore, these authors suggested that a standardization of microwave ovens for domestic use is desirable, making it easier to give

users correct instructions for their use. It has been also suggested that since core temperatures of foods cooked in a microwave are not consistently associated with sterilization, food preparers would need to follow cooking times carefully and determine temperatures at multiple locations to determine whether food prepared in a microwave oven is safe to eat (Gessner and Beller, 1994). It is necessary then to provide consumers with specific instructions for safe reheating of foods in the microwave oven in the household (Lacroix *et al.*, 2003).

Most studies on cooking of foodstuffs in household microwave ovens have been conducted using whole pieces of meat, such as birds, roasts and fish (Huang *et al.*, 1993; Daşdağ *et al.*, 1995). Research has been also conducted on the use of microwave ovens for reheating of cook-chilled foods, such as that of Sheeran *et al.* (1989). That study focused on the ability of microwave oven heating to eliminate *Listeria* ($10^6$ recoverable organisms/g) from cook-chill food obtained from retail outlets when the reheating instructions provided on the packages were followed. Results showed that 81% (22/27) of the dishes tested yielded large numbers of viable *Listeria* after heating, while from 19% (5/27) no organisms were recovered.

A similar study by Dealler *et al.* (1990) used uninoculated cooked and chilled/frozen meals from supermarkets that were designed for microwave reheating. These authors found that the outside of the food was most consistently hot and would commonly be about 100°C, whereas the core was often much lower, stressing that microwave heating may be ineffective at killing bacteria present throughout the food, and that the poor temperatures reached at the core of some of the meals account for survival of bacteria in the food. Regarding chilled leftovers and small meals, limited or no

information is available about the safety of microwave oven use in reheating those types of food. This is especially important when those foods are contaminated with foodborne pathogens such as *L. monocytogenes.*

Microwave oven heating is fast and convenient, characteristics reflected in its shorter heating and cooling times compared to conventional heating (Giese, 1992; Kaya *et al.,* 2003). However, the non-uniform heating of the food inside the microwave oven is a notorious problem, which may cause inadequate heating of foods (Suga *et al.,* 2007). Non-uniform heating produces hot and cold spots within the same piece of food, with reported temperature differences of up to 63.9°C (Ramaswamy and Pillet-Will, 1992). In order to allow temperature uniformity, standing time after heating and use of reduced power for longer times have been recommended (Lund *et al.,* 1989; Fakhouri and Ramaswamy, 1993; Göksoy *et al.,* 1999). It has been also suggested that non-uniform heating and overheating in microwave ovens may be minimized by cycling microwave power. Such cycling is generally done as a simple on-off operation, turning the oven on at full power for some time during the cycle and turning it off during the rest of the cycle (Chamchong and Datta, 1999).

Several factors can affect reheating efficiency (related to temperatures achieved) when using a microwave oven. Some factors are related to the microwave oven and some are related to the food itself. Product parameters include mass, chemical composition (especially salts, fat, protein, and water), density, size and shape (Anantheswaran and Ramaswamy, 2001). Higher fat content improved heating rate as well as temperature uniformity, while protein had the opposite effect (Fakhouri and Ramaswamy, 1993; Riveni and Anantheswaran, 2001). Protein content affects microwave heating largely

through binding of available water and salts, thus reducing the dielectric activity (Heddlesson *et al.,* 1996). Oven parameters that affect microwave heating efficiency include placement inside the oven, size of the cavity, power, frequency and turntables (Anantheswaran and Ramaswamy, 2001).

Additionally, the effect of microwaves on microorganisms present in foods is influenced by the intrinsic characteristics of the products being processed, such as pH, humidity, oxidation-reduction potential, antibodies present, biological structures, chemical composition, amount and geometry of the food (Zhou *et al.,* 1995; Zhang and Datta, 2001). Also, factors such as temperature, humidity, ambient gases, frequency and intensity of radiation, time of exposure, position of the foods in relation to the effective radiation field, among others have an effect on microbial destruction (Zhang and Datta, 2001). In addition, chemical composition of the microorganism to be irradiated, its stage of development (vegetative cell, spore or development phase, wet or dry, etc.) and its initial amount are important factors that affect microbial destruction during microwave heating (Valsechi *et al.,* 2004). The heat resistance of microorganisms is greatest at their optimum growth pH. As expected, this is true also for bacterial species heated by microwave energy (Heddlesson, *et al.,* 1996).

Power transfer in the microwave oven is also influenced by type or shape of sample container. For example, tall cylindrical or square containers are not suitable for rapid and uniform heat distribution because more energy is absorbed near the surface or edge of food when microwaves are generated at 240 MHz compared to a 915 MHz (Choi *et al.,* 1993).

Microwave-oven heating has been reported effective in *L. monocytogenes*

inactivation. Heddleson *et al.,* (1996) used a microwave oven (700 W, 2450 MHz) to heat beef broth, cream sauce, pudding, liquid whole egg and milk. They found that destruction of *L. monocytogenes* strains heated to 60°C was significantly lower in cream sauce than in milk and pudding. They also found significantly lower reductions for beef broth compared to cream sauce, probably due to differences in food composition. According to their results, sodium content affected the uniformity of temperatures achieved within the food products and on the resultant bacterial destruction. Foods of higher sodium content, such as beef broth, typically exhibited surface heating and non-uniform temperature distribution which often resulted in a smaller reduction in the bacterial population.

Another method of cooking commonly used in the home is boiling water. An informal survey (Porto *et al.*, 2004) found that 72% of the people interviewed reheated frankfurters before eating, and thirty-three percent of those people preferred boiling over other methods. Steaming has been also evaluated for control of *L. monocytogenes.* Murphy *et al.* (2005) used pressurized and ambient steam to treat sliced bologna before and after packaging; a reduction of up to 2.5 log of *L. monocytogenes* was obtained. That study was designed for testing of industrial and retail use of steaming, where packaging materials and bigger amounts of product were involved in contrast to the household setting. Steaming may be used at home for reheating of frankfurters and for cooking of other meals. Even though all of these methods are very popular at the household level, no information is available about their effectiveness for killing pathogens that may be present on foods in the household. There is not uniformity in the directions provided by manufacturers for reheating of these products, and there are no scientific data available that validate the efficacy of such recommendation in destroying potential contamination

of *L. monocytogenes*. Research is needed in order to provide manufacturers and consumers with appropriate reheating instructions that can assure food safety at the consumer level.

## CHAPTER III

### Risk factors associated with *Listeria, Salmonella* and *Escherichia coli* O157:H7 prevalence in rural households of Colorado with and without ruminant animals


### ABSTRACT

Ruminants may be a reservoir for *Listeria, Salmonella* and *Escherichia coli* O157:H7 and a potential source of household contamination. However, there are no available data on how these pathogens could be introduced in the household environment. Understanding consumer behavior may help in development of education materials for preventive measures to further reduce the risk of infection with these microorganisms. This study evaluated consumer behaviors in rural households, with or without ruminants, associated with cleaning, food handling and storage, which may be related with increased prevalence of *Listeria, Salmonella* and *E. coli* O157:H7 in the household environment. The study was completed over a three year period, with samples collected during years one and three. Rural Colorado households (28 with and 26 without ruminants) were recruited, and samples (food, environmental, and human and animal feces) were collected four times (at 2-3 weeks intervals), and tested for *Listeria*, *Salmonella* and *E. coli* O157:H7 presence. Participants answered surveys regarding household cleaning habits, food handling (storage, preparation and preferences), and animal handling. No sample

tested positive for *E. coli* O157:H7. *Salmonella* was isolated only from households with ruminants (one refrigerator, one washing machine, one working glove, and two shoe samples). *Listeria* spp. was isolated from all types of samples with higher, but not significant ($P > 0.05$) prevalence in households with ruminants. *L. monocytogenes* was isolated mainly from food samples. Seven indices were developed from survey information, and were statistically analyzed for relationships with the outcome of a sample positive for *Listeria* as the dependent variable. Behavior related to handling and cooking of perishable foods affected ($P < 0.05$) the probability of households testing positive for *Listeria*, regardless of presence of ruminants. Personal cleanliness habits were related to presence of *Listeria* on shoe soles, clothes washing machine, and working gloves. Shoes testing positive in ruminant households were more frequently associated with multiple positive environmental samples, when compared to households without ruminants. Consumer education on handling and storing perishable foods, and animal handling to prevent contamination of the household through shoes or clothes may reduce prevalence of *Listeria* in home environments.

## INTRODUCTION

The home environment constitutes a potential source of bacterial contamination, particularly the kitchen and the bathroom, which may serve as reservoirs of microorganisms (Kagan *et al.*, 2002). It has been reported that once pathogens which cause intestinal disease enter the domestic environment, they can be transmitted between surfaces, people and their food supply (Curtis *et al.*, 2003). For example, several studies have found *Listeria* in different places within the kitchen and the home in general, such as vegetable compartments of refrigerators, kitchen sinks, dishcloths, washing-up brushes,

toothbrushes, and the bathroom (Beumer *et al.*, 1996; Duggan and Phillips, 1998; Wagner *et al.*, 2007). Duggan and Phillips (1998) suggested that contamination with *Listeria monocytogenes* can be disseminated widely in kitchens.

Another potential source of *L. monocytogenes* contamination for the home environment is the asymptomatic carriage of the pathogen by one or more members of the household (Schuchat *et al.*, 1993). Asymptomatic human carriage of *L. monocytogenes* has been reported previously (Luppi *et al.*, 1988; Grif *et al.*, 2001; 2003) and can occur not only in healthy people, but also among persons in high risk groups for listeriosis (MacGowan *et al.*, 1991; 1994).

Another characteristic supporting the importance of controlling *Listeria* prevalence in the household environment is the increased number of persons at high risk for foodborne illness that are cared for at home, for example the elderly. Since 1950, the number of persons > 65 years of age in the United States has tripled, from 12.2 million to 36 million, and it is estimated that by year 2035, the population of persons 65 years of age will exceed 80 million (Jarvis, 2001).

*L. monocytogenes* has been identified as a major infectious agent causing neurological syndromes and uterine infections in bovine, sheep and goats (Rebhun and deLahunta, 1982; Jemmi and Stephan, 2006). This microorganism can cause animal encephalitis, which was first described as "circling disease" (Gill, 1931). Animals carrying *L. monocytogenes* can lead to direct contamination of milk as a consequence of listeric mastitis, encephalitis, or *Listeria*-related abortion (García *et al.*, 1996). Thus, animal feces and the farm environment are important sources of raw milk and meat contamination by *L. monocytogenes* (Nightingale *et al.*, 2005; Jemmi and Stephan, 2006).

In addition, the farm environment, and especially ruminants, have been reported as a source of *L. monocytogenes* isolates that have been linked to human illness (Fugett *et al.*, 2007). Other species of *Listeria* that are generally recognized as non-pathogenic, such as *L. innocua*, have also been identified as the cause of bacteremia and death (Perrin *et al.*, 2003). Other foodborne pathogens associated with ruminants and the farm environment includes *Salmonella* and *Escherichia coli.*O157:H7. *Salmonella* has been isolated from different locations in the household environment, including vacuum cleaners, refrigerators and kitchen countertops (Haddock and Robert, 1994; Schutze *et al.*, 1999). However, little information is available on how these pathogens are introduced into the household environment and on the potential of household contamination as a source of infection.

Thus, the objective o-f this study was to evaluate consumer behaviors in households, with or without ruminants, associated with cleaning, food handling and storage, and hygiene practices after animal care, which may be related with increased prevalence of *Listeria*, *Salmonella* and *E. coli* O157:H7 in the household environment.

## MATERIALS AND METHODS

**Recruiting of participants and behavioral data collection.** Rural households with and without ruminant animals were recruited from the surrounding area of Fort Collins, CO. Approval for recruiting and participation of human subjects was received from the Human Research Committee of Colorado State University (CSU, Appendix 1). Participants were recruited by researchers from the Department of Food Science and Human Nutrition of CSU. Researchers contacted the CSU Larimer and Weld county Extension 4-H programs, which provided their members with a recruiting flier (Appendix

2) via electronic mail. Also, announcements and fliers were made available at local 4-H club monthly meetings. The recruiting flier also was distributed within the CSU campus and Veterinary Teaching Hospital. In addition, the researchers contacted the Weld County Chapter of the Farm Families of America (FFA) organization and sent the recruitment flier to be copied and distributed to its members. Other recruiting methods included posting an announcement on the CSU Today website, under Research Studies, visits by the recruitment coordinator to rural farms to personally ask if they would like to participate, and contacting local veterinarians by telephone and sending the recruitment flier via e-mail to post at their facilities and/or distribute to their clientele. Interested families contacted researchers directly by telephone to sign up. Each participant household received a monetary compensation of $65 in years 1 and 3 for their time and samples collected.

To qualify for the study, participants needed to reside in rural households (outside city limits). Households with at least one child under 14 years of age were preferred. The primary person responsible for cooking food in the home needed to be willing to be interview audio-taped by a researcher, and able to participate in the study over a 3 year time period. Participants needed to be willing to participate in the following activities: complete survey/interview, allow research assistant to collect household environmental and food samples for test purposes, and provide 3 fecal samples to be tested for *Listeria.* Households were classified as with and without ruminant animals on their premises. Households without ruminants were required not to have contact with ruminant animals during the sample collection period.

Each household was visited four times, at 2-4 week intervals, between February and July. The primary household food preparer was asked to complete the Household Survey (42 and 47 questions for households without [Appendix 3] and with [Appendix 4] ruminants, respectively), and the Food Handling and Eating Preferences Questionnaire (4 questions, Appendix 5). For households with ruminants, the participant also was asked to complete the Farmer/Rancher Survey (19 questions, Appendix 6). Questions included in these instruments were previously tested and validated for reliability (Kendall *et al.*, 2004). These instruments were mailed in advance to participants and gathered by a researcher during the first household visit. During that visit, the researcher conducted the Interview with the Primary Food Preparer in the household (70 questions, Appendix 7). In addition, the researcher completed a visual kitchen audit, filled out the Kitchen Safety Checklist, and obtained a measurement of the refrigerator temperature (Appendix 8). Food and environmental samples also were collected (procedure follows below); the participant was provided with a commode specimen collection system (Cardinal Manufacturers INC, Streetboro, OH, USA) for stool sample collection, and follow-up visits were scheduled. Follow-up visits (visits 2, 3 and 4) consisted only of microbiological sample collection. The complete protocol for behavioral data and sample collection was completed in its entirety during year-1 and year-3 of the study.

**Microbiological sample collection.** During each visit, 3 food samples, 5 environmental samples and, in the case of farm households, a ruminant fecal sample were collected. In addition, during visits 2, 3 and 4, a stool sample from any member of the household also was collected (year-1 only). Food samples included leftovers (preferably from a home-made meal), dairy products (preferably non-pasteurized milk), deli meats

and cut fruit and/or vegetables. Environmental samples were taken from the refrigerator (handles and one shelf, preferably the meat drawer), kitchen sink (faucet and drain), clothes washing machine (rim), shoe soles and the floor underneath (if this was not carpet), kitchen countertop or sink next to clothes washing machine (faucet and drain), and/or gloves used for farming activities. Food samples were collected with a sterilized metal spoon and placed in a sterile 15 × 23 cm bag (Whirl-pak, Nasco, Modesto, CA, USA). Environmental samples were obtained with a moist sponge (10 ml of 1% peptone water, Hydrasponge™, 3M Microbiology, Saint Paul, MN, USA) by swabbing. All food and environmental samples were collected by the participants, after proper instruction. Stool samples from any household member (1 per visit for visits 2, 3 and 4) were collected from each participant household in the commode specimen collection system. Ruminant fecal samples were collected with a sterilized tongue depressor and transferred to a 15 × 23 cm Whirl-pak bag. All samples were transported to the laboratory in coolers with ice packs, and analyzed within 24 hours of collection.

**Microbiological analyses of samples.** All samples were analyzed for presence of *Listeria,* using the USDA-FSIS Microbiology Laboratory Guidebook procedures (USDA-FSIS, 2008b), with some modifications. For environmental samples, 90 ml of Universal Preenrichment Broth (UPB, Difco, Becton Dickinson, Sparks, MD, USA) were added to each premoistened sponge in its bag, massaged for 2 min (Masticator, IUL Instruments, Barcelona, Spain), and incubated at 35°C for 22-24 h. Then, 1 ml of UPB was transferred to 9 ml of Fraser Broth (FB, Difco) and incubated at 35°C for 22-24 h. After incubation, FB was streaked on PALCAM agar plates (Difco) and incubated for 48±2 h at 30°C. Colonies on PALCAM agar plates with morphology typical of *Listeria* (black or dark

gray round colonies surrounded by blackened media) were selected and isolated for further biochemical analyses for differentiation between *L. monocytogenes* and other *L.* species following procedures of the USDA-FSIS Microbiology Laboratory Guidebook (USDA-FSIS, 2008b) and the USFDA Bacteriological Analytical Manual (USDHHS-FDA-CFSAN, 2003). Suspected colonies were confirmed as *Listeria* based on Gram Stain (Gram-positive short rods under the microscope), motility (umbrella motility in soft agar after incubation at 25°C for 7 days), catalase activity (negative reaction) and oxidase activity (positive reaction). *L. monocytogenes* was differentiated from other *Listeria* species by hemolysis of sheep blood agar and fermentation of rhamnose, xylose and mannitol (USDHHS-FDA-CFSAN, 2003).

For food and fecal samples, 225 ml of UPB were added to 25 g of sample in a 15 × 23 cm Whirl-pack bag, and then incubated at 35°C for 22-24 h. Then, 1 ml of UPB was transferred to 9 ml FB and incubated at 35°C for 22-24 h. After incubation, FB was streaked on PALCAM agar plates and incubated at 30°C for 48±2 h. After incubation, typical *Listeria* colonies, if any, were selected for biochemical analysis and confirmation, as describe above. All isolates consistent with *L. monocytogenes* by the biochemical tests were confirmed by use of PCR and serotyped (Zhang and Knabel, 2005) at the Ohio Agricultural Research and Development Center (The Ohio State University, Wooster, OH, USA). DNA sequences of the *intC* gene were obtained for all *L. monocytogenes* isolates, and then were assembled, proofread, and aligned using Seqman and Megalign (DNAStar, Lasergene, Madison, WI, USA).

Environmental samples (sponge swabs from refrigerators, kitchen sinks, kitchen countertops, washing machines, shoes and gloves) also were tested for *Salmonella* and

*Escherichia coli* O157:H7 presence. For *Salmonella* testing, the USDA-FSIS Microbiology Laboratory Guidebook (2008c) protocols were followed, with some modifications. One ml from the UPB enrichment was transferred to 9 ml of Tetrathionate Broth (TTB, Difco) and incubated at 35°C for 22-24 h. After incubation, a loopfull of TTB was streaked on Brilliant Green Sulfa agar (BGS, Difco) and Xylose Lysine Tergitol$^{TM}$4 agar (XLT4, Difco) plates. Plates were incubated at 35°C and were first examined at 18-24 h and later after 48 h for *Salmonella* suspect colonies. Suspect colonies were selected for biochemical confirmation with API 20E strips of biochemical tests and database (bioMérieux sa, Marcy-l'Etoile, France). Serogrouping and serotyping analyses were completed at the Veterinary Diagnostic Laboratory, Veterinary Teaching Hospital, Colorado State University, Fort Collins, CO, USA.

To test for *E. coli* O157:H7 presence in environmental samples, the USDA-FSIS protocol was followed, with some modifications (USDA-FSIS, 2008a). In summary, 1ml of the UPB enrichment was transferred to 9 ml of modified *E. coli* broth (mEC, Difco) and incubated at 35°C for 22-24 h. After incubation, mEC was streaked on sorbitol MacConkey agar plates (Difco) supplemented with cefixime and potassium tellurite (SMAC-CT, Invitrogen Dynal, Oslo, Norway), and incubated at 35°C for 22-24 h. Then suspect colonies, if any, were tested for agglutination with O157 test latex (Thermo Fisher Scientific, Remel Products, Lenexa, KS, USA). Colonies with positive agglutination were tested with API 20E strips, and subjected to PCR analysis (Hu *et al.*, 1999) for confirmation.

**Statistical analysis:** Answers from surveys, interview and questionnaires (Appendices 3-8) were coded on a scale from 0 to 5, with 0 being the least desirable

behavior and 5 being the most desirable behavior. All data was uploaded into Microsoft Excel® files and imported into SAS/STAT® (SAS Institute, 2007). Seven indices were developed by grouping questions related to a common construct from the behavioral data collection instruments. The PROC CORR function of SAS/STAT® was used to calculate Cronbach Alpha coefficient to test for internal reliability of each index (Cronbach, 1951; Cortina, 1993). A Cronbach Alpha coefficient of al least 0.5 was considered acceptable for relatedness of the questions, based on the validation for reliability previously performed on the instruments (Kendall *et al.,* 2004) and other literature reviewed (Cortina, 1993). The indices included Perishable Food Handling and Cooking index (PFHCI), Pathogen Awareness index (PAI), Personal Cleanliness index (PCI), Kitchen and Household Cleanliness index (KHCI), Inside Cross-contamination index (ICCI), Outside Cross-contamination index (OCCI), and Risky Foods Preferences index (RFPI). Households were considered positive for *Listeria* if at least one sample was positive for this microorganism. Logistic regression analysis with the Glimmix® procedure of SAS/STAT® (SAS Institute, 2007) was used to determine potential relationships between indices and the probability of a household testing positive for *Listeria.*

Prevalence of the pathogen was divided in overall prevalence (OP), which included all samples within a household, except for animal and human fecal samples; food prevalence (FP), kitchen prevalence (KP), which included all samples from refrigerators, kitchen countertops and kitchen sink, and non-kitchen environmental prevalence (NKEP), which included samples from shoes, farm gloves and clothes washing machine. Differences were considered statistically significant at the $P < 0.05$ level.

## RESULTS AND DISCUSSION

**Household demographics.** Table 3.1 presents the demographic characteristics of the households recruited. A total of 54 rural households were initially recruited, 28 with and 26 without ruminant animals. Ruminant animals on the household premises included cattle (12 houses), goats (13 houses), sheep (9 houses), llamas (5 houses) and/or alpacas (1 house). Some households, including those classified as non-ruminant, may have had other animals such as cats, dogs, horses, pigs, chickens and other birds. Two households with ruminants (ID 1106 and 1118) decided not to participate in the second period of sample collection (year-3), but their data from the first sample collection period (year-1) was used in the analysis. Two households (ID 1215 and 1226) initially classified as non-ruminant acquired animals after the first sampling period ended but before the second one started, thus, they were considered ruminant households for the year-3 sample collection period (ID changed to1129 and 1130 in year-3 collection, respectively). One household initially classified as ruminant (ID 1116) sold all their animals after the first sample collection period was completed but before the second one started, thus, it was considered a non-ruminant household for the year-3 sample collection period (ID changed to 1228 in year-3 collection).

**Overall *Listeria* prevalence.** Tables 3.2 and 3.3 describe the different samples from which *Listeria* spp. and *L. monocytogenes* were recovered in households with and without ruminants, respectively. The prevalence values are presented in Table 3.4.

*Listeria* spp. was recovered from all types of samples collected, except from human stools and swabs from utility sinks. *L. monocytogenes* prevalence was very low (0.5 to 1.9%, Table 3.4), and most of the samples that tested positive for the pathogen

correspond to foods (7/13 in households with ruminants and 6/14 in households without ruminants, Tables 3.2 and 3.3). The majority of the isolates belonged to serotypes 1/2a and 4b (Tables 3.2 and 3.3), which along with serotype 1/2b, are responsible for 95% of human cases of listeriosis infection (Graves *et al.,* 2007). Most of the positive samples were cheeses and meats and meat products, which are known vehicles for the pathogen. This indicates that food purchase behavior may have an effect on prevalence of *L. monocytogenes* in the household environment (Wagner *et al.,* 2007).

Due to the low prevalence of *Listeria* spp. and *L. monocytogenes* in the different types of samples, individual prevalence were grouped according to the origin of the sample within the household, and the results by household type and collection year are presented in Table 3.5. There was no significant effect ($P \geq 0.05$) of ruminant presence or collection year on any of the individual prevalence of *Listeria* (Table 3.4). However, there was a clear trend for the grouped prevalence of *Listeria* spp. to be numerically higher in ruminant households than in households without ruminants (Table 3.5), indicating a potential higher exposure of these households to *Listeria.* The lack of statistical significance may be due to the small sample size and the small number of samples that were positive for *Listeria.*

***Listeria* prevalence in human stools.** Because no stool samples were found positive during the year-1 sample collection, this collection was discontinued for year-3 (Table 3.4). Household members that provided a stool sample were between 11 months and 69 years of age. *Listeria* was not recovered from any of the samples collected, probably due to limited numbers of sample collected, long sample collection intervals (2-4 weeks), different persons within the same household providing the sample, and the

short length of *Listeria* fecal shedding periods in humans, which have been reported to last no more than 4 days (Angelakopoulos *et al.,* 2002; Grif *et al.,* 2003). It has been previously reported that the prevalence of *Listeria* in human stools is low (from < 1 to 3.4%) among healthy individuals (Lamont and Poslethwaite, 1986; Luppi *et al.,* 1988; Grif *et al.,* 2001; 2003; Sauders *et al.,* 2005).

***Listeria* prevalence in the feces of ruminants.** *Listeria* spp. was isolated from fecal samples (Table 3.2) of cows (12 positives out of 70 samples, 17.1%), sheep (8 positives out of 46 samples, 17.4%) and goats (2 positives out of 58 samples, 3.4%). A combined sample of goats and sheep feces also was positive. Three samples were positive for *L. monocytogenes,* one from cows, one from goats and one from sheep (Table 3.2). None of the fecal samples from alpacas or llamas was positive for any *Listeria*. Ivanek *et al.* (2007) reported on the dynamics of pathogen fecal shedding, specifically *L. monocytogenes*, and their results indicated that fecal shedding is subtype specific and can vary from 2 to 92%. These authors also found that there was a considerable day-to-day variability in fecal shedding of the pathogen, and suggested that fecal samples should be collected at least daily in order to calculate the true prevalence within a herd of cattle. This may explain the low number of fecal samples found positive for *L. monocytogenes* in this study, since samples were collected every two to four weeks. Nonetheless, overall, 17.1% (12/70) of the samples from cattle were positive for *Listeria* spp., as well as 9.6% (10/104) of the samples from goats and sheep. However, none of the houses with ruminants reported to have had a case of listeriosis within the 12 month period before sample collection began, indicating asymptomatic carriage of *Listeria* by these animals, and potentially spreading of contamination to the household environment.

Several studies have found that ruminant animals may be asymptomatic carriers of *Listeria,* and that may be a reservoir and source of contamination for other animals (Low and Donachie, 1997; Nightingale *et al.*, 2004; 2005; Nappi *et al.*, 2005), as well as humans and food manufacturing environments. Wagner *et al.* (2005) reported a case in a cheese producing farm where *L. monocytogenes* was possibly transmitted from contaminated animal feeds to the milk supply and from there to the working surfaces of the cheese-making facility and humans. In that study, *L. monocytogenes* was detected two months after the outbreak on the boots and in the feces of a worker. As a consequence, a cross-contamination cycle between the worker and the cheese processing environment was established. This may also have been the case in this study, since there were several cases where samples collected from shoes tested positive at the same time that some other samples from the inside of the household environment also tested positive (kitchen sinks, washing machines and refrigerators). Shoes were more likely contaminated while used to work with the animals (Wagner *et al.,* 2005).

In household 1102, which had cows, the animal feces sample tested positive for *Listeria* spp. twice during year-1 sample collection and then all four times during year 3 sample collections (Table 3.2). Swabs from shoes and the washing machine also tested positive at the same time, indicating a potential scenario of cross-contamination and /or re-contamination between the animals and the household.

In another case, household 1109, which had goats on the property, had samples from the kitchen sink testing positive for *Listeria* spp. during all four visits in year-3 collection, and one shoe sample also was positive (Table 3.2). These results may be

indicative of not only potential cross-contamination events, but also of re-contamination or persistence of *Listeria* within the household environment.

Feces from cows in household 1120 tested positive in year-1 (*Listeria* spp.) and again in year-3 (*Listeria* spp. and *L. monocytogenes,* Table 3.2). In addition, during year-3, multiple food, refrigerator and shoe samples were positive for both *Listeria* spp. and the pathogenic *L. monocytogenes* in another potential cross-contamination scenario where the most likely source may have been the animal feces. The isolates confirmed as *L. monocytogenes* were not identical by DNA sequencing of the *inlC* gene. These results point to ruminant animals as an important source of contamination and re-contamination for the household environment, and to a potentially higher exposure of the household members to the microorganism when compared to households without ruminants.

***Listeria* prevalence in the kitchen environment.** *Listeria* spp. and *L. monocytogenes* were isolated from all sampling sites within the kitchen (Tables 3.2 and 3.3), and with the exception of the kitchen countertop, the overall number of positive samples (Kitchen Environment Prevalence, KEP, Tables 3.3 and 3.4) was higher in houses with ruminants that in those without, but not statistically different ($P \geq 0.05$). The total KEP for *Listeria* spp. was 2.6 and 1.4% for households with and without ruminants on premises, respectively (Table 3.5). As it was the case with samples involving animals and shoes, several cases of possible cross-contamination, re-contamination and/or persistent contamination occurred within the kitchen environment of several houses. Household 1103 had the kitchen sink and two different food samples testing positive at the same time with *L. monocytogenes* strains serotype 4b that were genetically identical by DNA sequence of the *inlC* gene (Table 3.2). Household 1109 had multiple foods,

kitchen sink and refrigerator samples that were positive for *Listeria* spp. during year-1 sampling (Table 3.2). Household 1127 had two food samples and a refrigerator swab that were positive for the same serotype of *L. monocytogenes* during the same visit. The two strains from the food samples were genetically identical by DNA sequence of the *inlC* gene. Household 1128 had two food samples and a refrigerator sample that tested positive, and one of the food samples was positive for *L. monocytogenes*. Finally, household 1220 had a refrigerator swab and a food sample that were positive for different strains of *L. monocytogenes* during year-1 and year-3 sample collection, respectively, indicating re-contamination of the kitchen environment between the two sample collection periods. Even when it is not possible to establish the origin of contamination, it is clear that cross-contamination and/or re-contamination occurred within the kitchen environment, which has been reported before (Redmond *et al.*, 2004). Wagner *et al.* (2007) reported a case where the same isolate was recovered from the kitchen sink, three different food samples, and stools of household members. In this study, cases of potential cross-contamination/re-contamination were more likely to occur in households with ruminants, with multiple samples testing positive at the same time (Table 3.2 and 3.3). Samples positive for *Listeria* in non-ruminant households tended to be isolated and sporadic (single samples from a given household testing positive for a given visit, Table 3.6).

***Listeria* prevalence in non-kitchen environmental samples.** No statistical difference ($P \geq 0.05$) was found for the prevalence of *Listeria* in samples collected from the Non-Kitchen Environment (NKEP, Tables 3.2 and 3.3) between households with and without ruminants, or between collection years. In the non-kitchen environment, *L. monocytogenes* was isolated only from one shoe sole and one washing machine (Table

3.3). Both samples were collected from households without ruminant animals. The prevalence of *Listeria* was numerically higher in all types of samples collected from households with ruminants (total NKPE was 2.8 and 0.9% for households with and without ruminants on premises, respectively). Interestingly, the NKEP was affected ($P < 0.05$) only by the interaction between ruminant presence and collection year. Since, as described above, there was no significant effect of these two factors individually, and the NKEP remained constant for households without ruminants, the interaction was significant due to the increase of positive samples recovered from ruminant households in year-3 when compared to year-1. The NKEP comprises the individual prevalence from washing machines, shoes, utility sinks and farm gloves; out of those individual prevalence, the only one that significantly increased from year-1 to year-3, was the prevalence on shoe soles (only from households with ruminant animals), directly affecting the grouped NKEP from this type of houses. The results show a trend of higher prevalence of *Listeria* in households with ruminant animals on their premises, indicating an increased exposure to the microorganism and a potentially higher risk for listeriosis infection to the household members. Thus, families in households on ruminant farms should be educated about the potentially increased risk and exposure, and the preventive measures they can apply during and after farming activities, to reduce the threat if infection with special attention to personal cleanliness habits such as handwashing and change of clothing and shoes after animal care. This type of education campaign may help preventing cross-contamination, re-contamination and persistent contamination of the household environment and food supply.

**Risk factors associated with increased *Listeria* prevalence.** Table 3.7 shows the Cronbach Coefficient Alpha (Cronbach, 1951) for each of the indices used in the analysis, which is an indication of the relatedness of the questions used to develop each of the indices. High relatedness between questions is desirable since it indicates that variance in the responses is due to individual differences between the subjects providing the answers (Cortina, 1993). The values of the coefficients, ranging from 0.500 to 0.823 (Cortina, 1993), along with previous validation of the questions (Kendall *et al.,* 2004), indicated high correlation or relatedness of the questions within each index, making the indices used an appropriate measurement of the behavior they were designed to describe. Table 3.8 shows the β coefficients for the model that correlates prevalence of *Listeria* in the households with the indices, and it measures the degree of association between the probability of any given household having a positive sample and the value of a particular index (Ott and Longnecker, 2001); exp (β) is the odds ratio of any given household having a positive sample when a specific index changes by one unit. Beta coefficients are negative, meaning that as the mean value of the index increases (therefore indicating more desirable behaviors) the predicted prevalence will be reduced. This indicates that, as expected, households that apply the more desirable behaviors will have a decrease in the prevalence of *Listeria* in the environment. This is because the higher values in the scales for each question were assigned to better behaviors.

Due to the low prevalence of all *Listeria* spp. in general, and especially *L. monocytogenes, Listeria* spp. (referred as *Listeria*) prevalence was considered for analysis of risk factors. In any case, the detection of any *Listeria* spp. within the household environment may be a cause for concern, since *Listeria* in general is used as

hygiene indicator in all stages of the food processing chain (Jemmi and Stephan, 2006). Generally only two species of the genus *Listeria* are considered to be pathogenic, *L. monocytogenes* in humans and *L. ivanovii* in other mammals (Gasanov *et al.*, 2005). *L. ivanovii* specifically affects ruminants, causing septicemia and abortion, but not meningo-encephalitis (Domínguez-Bernal *et al.*, 2006). However, some studies indicate that originally non-pathogenic species of *Listeria* may have acquired pathogenicity genes from *L. monocytogenes.* A study by Lan *et al.* (2000) suggested that *L. innocua* lineage I acquired the *gtcA* gene (which is essential for the incorporation of galactose and glucose into the teichoic acids of the cell wall) via lateral transfer from *L. monocytogenes* serotype 4b. Johnson *et al.* (2004) reported on a *L. innocua* strain that carries the *Listeria* pathogenicity island 1, and also expresses at least two of the virulence cluster genes, *hly* and *plcA,* which encode the *L. monocytogenes* listeriolysin O, and inositol-specific phospholipase C, respectively. In another study by Perrin *et al.* (2003), actual pathogenicity of *L. innocua* in humans was reported; it was the case of a 62-year-old woman who died after a severe septic shock and multiple-organ dysfunction caused by a hemolytic strain of *L. innocua* serovar 6a.

Additionally, the detection of *Listeria* spp., rather than *L. monocytogenes* in any given sample, does not necessarily assure that the pathogenic species was not present. In general, *Listeria* cells can be rapidly out-grown by competitor microorganisms (Gasanov *et al.*, 2005) that are likely to be present in food and environmental samples, and other species of *Listeria* may share the same ecological niches in the environment with *L. monocytogenes* (including food, vegetation and soil) (Lan *et al.*, 2000). More specifically, *L. innocua* can grow faster than *L. monocytogenes* at temperatures less than 40°C (Petran

and Swanson, 1993). Even some strains of *L. monocytogenes* lineage 2 may outcompete lineage 1 strains in some enrichment broths (Bruhn *et al.,* 2005). Thus, the presence of any *Listeria* spp. may serve as an indicator of the general contamination status of the household, and the results from the risk evaluation can be applied to the prevention of contamination by any of the species.

The Overall Prevalence (OP) of *Listeria* was affected ($P < 0.05$) only by the Perishable Food Handling and Cooking Index (PFHCI). This suggests that the way people handle and cook perishable foods at home is very important in the prevention of *Listeria* contamination. In addition 25% (8/31) of households were positive for *Listeria* due to a single food sample being found positive for the microorganism. From data in Table 3.5, it can be calculated that 40 and 29% (8 out of 20 and 12 out of 41) of the positive samples in the OP category came from food samples, in households without and with ruminants, respectively. More specifically, 53.8% (7/13) and 42.9% (6/14) of the samples positive for *L. monocytogenes* were food samples in households with and without ruminants, respectively. Thus, perishable foods may be associated with a potential source for the pathogen in the household, stressing the importance of carefully handling foods commonly contaminated with *Listeria.*

The Personal Cleanliness Index (PCI) was related ($P < 0.05$) to the NKEP (Non-Kitchen Environmental Prevalence). Behaviors included in this index were associated with personal hygiene, especially after farming activities and before entering the house (Table 3.8). Practices that should be followed after farming chores include changing of footwear and clothing, to avoid tracking of soil, dirt and manure into the house. This was found to be especially important for households with ruminants, where prevalence of

*Listeria* on shoes was more than twice that on shoes from non-ruminant houses (5.6 and 1.5%, respectively).

***E. coli* O157:H7 and *Salmonella* prevalence.** No sample was found positive for *E. coli* O157:H7 in this study. *Salmonella* was isolated from samples obtained from refrigerators (1/421 samples, household 1121), work gloves (1/34 samples, household 1109), washing machines (1/419 samples, household 1102), and shoes (2/422 samples, households 1113 and household 1102). All positive samples for *Salmonella* were recovered from households with ruminants. With the exception of one household (1113), all *Salmonella* positive samples were collected in houses that also were positive for *Listeria* (1102, 1121, 1109), in some cases both in year-1 and again in year-3 (Table 3.2). Household 1102 had cows, and households 1113, 1121 and 1109 had goats. Households 1102, 1121 and 1109 had multiple samples positive for *Listeria* in both years 1 and 3 (Table 3.2). These results supported the theory of potential cross-contamination, re-contamination and/or persistence discussed earlier.

In other studies, *Salmonella* has been isolated from dogs, cats, birds, horses (Pelzer, 1989) and reptiles like lizards, snakes, and turtles (Strohl *et al.,* 2004). Cattle are considered an important source of *Salmonella,* where infection can result in clinical disease, but most cases are sub-clinical. Small ruminants, such as sheep and goats, also have been reported as potential carriers of *Salmonella* (Alvseike and Skjerve, 2002; Hendriksen, *et al.,* 2004). Cattle infected with *Salmonella* may shed, continuously or intermittently, large numbers of the organism in their feces, with sub-clinical shedding of the organism being more common than clinical disease (Richardson, 1975; Smith, 1996). The results presented here may be an indication of a severe cross-contamination problem

within the household environment and from the animals on the premises, which may eventually lead to salmonellosis infection in household members. That ruminant houses were more likely to have positive shoe samples, in addition to an increased chance of multiple samples being positive at the same time as the shoes, indicated that it is highly likely that contamination of the household may have occurred from shoes that tracked dirt inside the house from animal pens.

An association between dust contamination with *Salmonella* and salmonellosis infection, especially in young children, was previously reported (Haddock and Malilay, 1986). This is especially relevant to the households that participated in this study, since most of them had children. Haddock and Malilay (1986) conducted a study on risk factors for infant salmonellosis in Guam, and found a particularly high incidence of this disease among children. Even when these authors were not able to identify any discrete risk factor, their findings suggested that aerosols created by vacuuming, sweeping and other household activities were an important source of salmonellosis infection in children. Similar results were reported by Schutze *et al.* (1999) when they conducted a study on children age 4 and younger with confirmed *Salmonella* infection. These authors found *Salmonella* positive samples from vacuum cleaners and dirt surrounding the front door more often than from other environmental samples, such as countertops or refrigerators, supporting the hypothesis that infants and young children may come into contact with *Salmonella* either through aerosolization or by having direct contact with contaminated soil or debris. Haddock and Nocon (1994) found that vacuum cleaners used in homes of infants with confirmed *Salmonellosis* infection were more likely to contain *Salmonella* bacteria than those used in control households. These authors hypothesized that dust

blown through open windows or dirt tracked into homes on footwear were possible mechanisms for spread of this microorganism inside the house. In another study, Haysom and Sharp (2003) recovered *Salmonella* from vacuum cleaner dust in three different households. These authors also inoculated autoclaved vacuum dust with *Salmonella,* and were able to recover the microorganism 65 days after inoculation. An interesting finding of that study is that total viable counts of vacuum cleaner dust collected from households were significantly higher in samples from rural than urban environments and in households where pets were present. These authors concluded that major sources of bacterial contamination in rural areas were livestock, manure and soil that could be introduced into the domestic environment on footwear, the feet of pets and currents of air. These findings and the fact that a high proportion of cases of *Salmonella* infection are reported in children 5 years old or younger (Haddock and Malilay, 1986; Haysom and Sharp, 2003), make the study of the household environment a priority in the search for control measures for prevention of this disease. Results presented in here stress the importance of education of household members, especially in rural households with ruminant animals, on appropriate hygiene habits for cleaning of clothes and farming shoes after animal care and before entering the house.

## LIMITATIONS

One limitation of this study is the small sample size, which may be the reason that statistically significant differences or effects were not detected, even when the differences in trends were clear between houses with and without ruminant animals on their premises. Another limitation is the bias that is inevitable when working with human subject and self-reported behavior. It is possible to have recall bias, when the individual reporting a

specific behavior may not correctly remember the details, and also human subjects when given options (which was the case in most data collection instruments used in this study) tend to report the behavior they think is the best, rather than indicating their actual behavior is. Differences between self-reported and current behavior have been observed previously (Redmond and Griffith, 2003; Morarji Dharod *et al.*, 2007). Another bias that may have affected the results, especially the behavioral data, is the overrepresentation of households within the most educated categories, for both types of households, with and without ruminants (Table 3.1).

Also the results of this study are limited to a specific geographical area with its specific conditions, such a climate, which may have affected prevalence of *Listeria*.

## CONCLUSIONS

Households with ruminant animals tend to have higher prevalence of *Listeria* and *Salmonella* in the environment, leading to higher exposure of household members to these pathogens, and potentially increasing their risk of infection. Results point to foods as a potential important source of *L. monocytogenes* for the household environment. Results also suggest that cross-contamination, re-contamination and/or persistent contamination may have occurred in some cases with both microorganisms. Handling of perishable foods and personal cleanliness immediately after farm animal care plays an important role as potential routes for contamination. Education on better cleanliness habits regarding shoes, clothing and hand washing after animal handling may reduce the risk of contamination to those households.

Table 3.1. Demographic characteristics of participating households.

| Highest level of education completed by any adult household member | Ruminants households *n*=28 | | Non-ruminant households *n*=26 | |
|---|---|---|---|---|
| | Number | % | Number | % |
| Some high school | 0 | 0.0 | 0 | 0.0 |
| High school graduate | 0 | 0.0 | 1 | 3.7 |
| Some college/ technical school | 7 | 25.9 | 3 | 11.1 |
| 4-yr college degree | 14 | 51.9 | 3 | 11.1 |
| Post-graduate studies | 6 | 27.2 | 20 | 74.1 |
| House age | | | | |
| < 5 years | 4 | 14.8 | 3 | 11.1 |
| 5-14 years | 6 | 22.2 | 6 | 22.2 |
| 15-24 years | 5 | 18.5 | 3 | 11.1 |
| >25 years | 12 | 44.4 | 15 | 55.6 |

Table 3.2 Samples positive for *Listeria* in Colorado rural households with ruminant animals.

| ID | Year | Visit | Samples positive for (description of sample [serotype]) *Listeria* spp. | *Listeria monocytogenes* |
|---|---|---|---|---|
| 1101 | 3 | 4 | animal feces (cows) | |
| 1102 | 1 | 1 | animal feces (cows) | |
| | | 3 | animal feces (cows) | |
| | 3 | 1 | animal feces (cows), shoes, washing machine | |
| | | 2 | animal feces (cows) | |
| | | 3 | animal feces (cows) | |
| | | 4 | animal feces (cows) | |
| 1103 | 1 | 1 | food (chipped beef , cottage cheese), kitchen sink | food[1] (chipped beef [4b and other[a]], cottage cheese[1] [1/2a and 4b]), kitchen sink[1] [4b] |
| 1104 | 1 | 2 | animal feces (goats) | animal feces (goats [4b]) |
| | | 4 | animal feces (goats) | |
| 1109 | 1 | 1 | food (cheddar cheese), kitchen sink, washing machine | |
| | | 2 | fridge | |
| | | 3 | kitchen sink | |
| | 3 | 1 | kitchen sink | |
| | | 2 | kitchen sink | |
| | | 3 | kitchen sink | |
| | | 4 | kitchen sink, shoes soles | |
| 1110 | 1 | 1 | kitchen sink | |
| | | 2 | animal feces (cows) | |
| 1112 | 3 | 1 | animal feces (sheep) | |
| 1114 | 1 | 2 | food (lunch meat) | food (lunch meat [atypical[b]]) |
| 1117 | 1 | 3 | food (turkey) | |
| | 3 | 2 | food (deli chicken breast) | |
| | | 3 | shoes soles | |
| 1119 | 3 | 3 | shoes soles | |
| 1120 | 1 | 3 | animal feces (cows), refrigerator | |
| | 3 | 1 | food (round steak) | |
| | | 2 | shoes, animal feces (cows), food (pork sausage) | animal feces (cows [1/2a]), food (pork sausage [1/2a]) |
| | | 3 | animal feces (cows), refrigerator | |
| | | 4 | shoes soles | |

[a]Other serotype different from 1/2a and 4b

[b]A 350 bp band was amplified from the *inlB* gene instead of the 500 bp band expected for *L. monocytogenes*

[1,2,3]Isolates with the same superscript number were genetically identical by DNA sequencing of the *inlC* gene

Table 3.2 (Continuation) Samples positive for *Listeria* in Colorado rural households with ruminant animals.

| ID | Year | Visit | Samples positive for(description of sample [serotype]) *Listeria* spp. | *Listeria monocytogenes* |
|---|---|---|---|---|
| 1121 | 1 | 1 | animal feces (sheep) | |
| | | 2 | animal feces (sheep) | |
| | | 3 | animal feces (sheep) | |
| | 3 | 1 | shoes soles | |
| | | 2 | animal feces (sheep) | |
| | | 3 | animal feces (sheep) | |
| | | 4 | animal feces (sheep), shoes soles, refrigerator | animal feces (sheep [1/2a]) |
| 1123 | 3 | 1 | farming gloves | |
| 1124 | 3 | 1 | shoes soles | |
| 1126 | 3 | 1 | shoes soles | |
| | | 2 | animal feces (cows) | |
| 1127 | 1 | 2 | food (queso fresco, lettuce) | food (queso fresco[2] [1/2a], lettuce[2] [1/2a]) |
| | | 3 | refrigerator | refrigerator [1/2a] |
| 1128 | 1 | 1 | refrigerator, food (sliced ham) | food (sliced ham [other[a]]) |
| | | 3 | food (ham) | |
| | 3 | 4 | animal feces (sheep) | animal feces (sheep [4b and other[a]]) |
| 1130 | 3 | 3 | shoes soles | |
| | | 4 | shoes soles | |

[a]Other serotype different from 1/2a and 4b

[b]A 350 bp band was amplified from the *inlB* gene instead of the 500 bp band expected for *L. monocytogenes*

[1,2,3]Isolates with the same superscript number were genetically identical by DNA sequencing of the *inlC* gene

Table 3.3 Samples positive for *Listeria* in Colorado rural households without ruminant animals

| ID | Year | Visit | Samples positive for (description of sample [serotype]) *Listeria* spp. | *Listeria monocytogenes* |
|---|---|---|---|---|
| 1201 | 3 | 1 | food (bacon) | food (bacon [1/2a]) |
| 1202 | 1 | 1 | food (sliced cheese) | food (sliced cheese [other[a]]) |
| | | 4 | kitchen countertop | kitchen countertop [4b] |
| 1205 | 1 | 2 | food (roast beef ) | food (roast beef [other[a]]) |
| 1207 | 1 | 2 | shoes soles | |
| 1208 | 1 | 4 | food (lettuce) | food (lettuce [4b]) |
| | 3 | 3 | food (bacon) | |
| 1217 | 1 | 1 | refrigerator | |
| | 3 | 2 | kitchen sink | |
| | | 3 | kitchen sink, shoes soles | kitchen sink[4] [4b] |
| | | 4 | kitchen sink | kitchen sink[4] [4b] |
| 1218 | 1 | 4 | washing machine | washing machine [4b] |
| 1220 | 1 | 4 | refrigerator | [5]refrigerator [other[a]] |
| | 3 | 1 | food (salmon spread) | [5]food (salmon spread [1/2a]) |
| 1221 | 1 | 3 | shoes soles | shoes soles [4b] |
| 1222 | 1 | 4 | food (taco) | food (taco [1/2 and other[a]]) |
| 1225 | 3 | 2 | food (mushrooms) | |
| 1227 | 1 | 3 | refrigerator | refrigerator [4b and other[a]] |
| 1228 | 3 | 4 | kitchen sink | kitchen sink [atypical[b]] |

[a]Other serotype different from 1/2a and 4b

[b]A 350 bp band was amplified from the *inlB* gene instead of the 500 bp band expected for *L. monocytogenes*

[1,2,3]Isolates with the same superscript number were genetically identical by DNA sequencing of the *inlC* gene

Table 3.4. Number of samples positive for *Listeria* (%) in households by type of samples, collection year, and presence of ruminants

| Type of sample | Year 1 | | | | | | Year 3 | | | | | | Total (Year 1 + Year 3) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ruminants | | | No ruminants | | | Ruminants | | | No ruminants | | | Ruminants | | | No ruminants | | |
| | *n* | *L.* spp | *L. m.* | *n* | *L.* spp | *L. m.* | *n* | *L.* spp | *L. m.* | *n* | *L.* spp | *L. m.* | *n* | *L.* spp | *L. m.* | *n* | *L.* spp | *L. m.* |
| Food | 336 | 9 (2.7) | 6 (1.8) | 309 | 4 (1.3) | 4 (1.3) | 322 | 3 (0.9) | 1 (0.3) | 304 | 4 (1.3) | 2 (0.7) | 658 | 12 (1.8) | 7 (1.1) | 613 | 8 (1.3) | 6 (1.0) |
| Refrigerator | 112 | 4 (3.6) | 1 (0.9) | 103 | 3 (2.9) | 2 (1.9) | 106 | 2 (1.9) | 0 (0.0) | 100 | 0 (0.0) | 0 (0.0) | 218 | 6 (2.8) | 1 (0.5) | 203 | 3 (1.5) | 2 (1.0) |
| Kitchen sink | 112 | 4 (3.6) | 1 (0.9) | 103 | 0 (0.0) | 0 (0.0) | 106 | 4 (3.8) | 0 (0.0) | 100 | 4 (4.0) | 3 (1.3) | 218 | 8 (3.7) | 1 (0.5) | 203 | 4 (2.0) | 3 (1.5) |
| Kitchen counter | 60 | 0 (0.0) | 0 (0.0) | 87 | 1 (1.1) | 1 (1.1) | 53 | 0 (0.0) | 0 (0.0) | 74 | 0 (0.0) | 0 (0.0) | 113 | 0 (0.0) | 0 (0.0) | 161 | 1 (0.6) | 1 (0.6) |
| Washing machine | 112 | 1 (0.9) | 0 (0.0) | 103 | 1 (1.0) | 1 (1.0) | 106 | 1 (0.9) | 0 (0.0) | 98 | 0 (0.0) | 0 (0.0) | 218 | 2 (0.9) | 0 (0.0) | 201 | 1 (0.5) | 1 (0.5) |
| Shoe soles | 112 | 0 (0.0) | 0 (0.0) | 103 | 2 (1.9) | 1 (1.0) | 103 | 12 (11.7) | 0 (0.0) | 98 | 1 (1.0) | 0 (0.0) | 215 | 12 (5.6) | 0 (0.0) | 201 | 3 (1.5) | 1 (0.5) |
| Utility sink | 37 | 0 (0.0) | 0 (0.0) | 15 | 0 (0.0) | 0 (0.0) | 38 | 0 (0.0) | 0 (0.0) | 17 | 0 (0.0) | 0 (0.0) | 75 | 0 (0.0) | 0 (0.0) | 32 | 0 (0.0) | 0 (0.0) |
| Farm gloves | 16 | 0 (0.0) | 0 (0.0) | * | * | 0 (0.0) | 14 | 1 (7.1) | 0 (0.0) | 7 | 0 (0.0) | 0 (0.0) | 30 | 1 (3.3) | 0 (0.0) | 4 | 0 (0.0) | 0 (0.0) |
| Human stools | 77 | 0 (0.0) | 0 (0.0) | 73 | 0 (0.0) | 0 (0.0) | * | * | * | * | * | * | 77 | 0 (0.0) | 0 (0.0) | 73 | 0 (0.0) | 0 (0.0) |
| Ruminant feces | 107 | 9 (8.4) | 1 (0.9) | n/a | n/a | n/a | 97 | 13 (13.4) | 3 (3.09) | n/a | n/a | n/a | 204 | 22 (10.8) | 4 (2.0) | n/a | n/a | n/a |

*L.* spp: *Listeria* species.
*L. m.: Listeria monocytogenes.*
n/a: not applicable.
*n:* number of samples collected.
* no sample of this type was collected for this sampling period.

Table 3.5. Grouped prevalence of *Listeria* by household type and collection year [number of positive samples/total number of samples collected (%)]

| Year | Ruminants | Overall (OP) | Food (FP) | Kitchen environment (KEP) | Non-kitchen environment (NKEP) |
|---|---|---|---|---|---|
| 1 | Yes | 18/897[a] (2.0) | 9/336[a] (2.7) | 8/284[a] (2.8) | 1/277[a] (0.4) |
| | No | 11/823[a] (1.3) | 4/309[a] (1.3) | 4/293[a] (1.4) | 3/221[a] (1.4) |
| 3 | Yes | 23/848[a] (2.7) | 3/322[a] (0.9) | 6/265[a] (2.3) | 14/261[a] (5.4) |
| | No | 9/797[a] (1.1) | 4/304[a] (1.31) | 4/274[a] (1.5) | 1/219[a] (0.5) |
| **Total 1+3** | **Yes** | **41/1745 (2.3)** | **12/658 (1.8)** | **14/529 (2.6)** | **15/538 (2.8)** |
| | **No** | **20/1618 (1.2)** | **8/613 (1.3)** | **8/567 (1.4)** | **4/438 (0.9)** |

OP: includes all samples except for human and animal feces.
FP: includes all food samples.
KEP: includes refrigerator, kitchen sink and kitchen countertop samples.
NKEP: includes shoes soles, washing machine, utility sink and farming gloves samples.

Table 3.6. Households with samples positive for *Listeria* by year and household type [number of households in each category (households ID number)].

| | At least one positive sample | | | Two positive samples | | | Three or more positive samples | | |
|---|---|---|---|---|---|---|---|---|---|
| Ruminants | Year 1 | Year 3 | Both years | Year 1 | Year 3 | Both years | Year 1 | Year 3 | Both years |
| Yes | 11 (1102, 1103, 1104, 1109, 1110, 1114, 1117, 1120, 1121, 1127, 1128) | 13 (1101, 1102, 1109, 1112, 1117, 1119, 1120, 1121, 1123, 1124, 1126, 1128, 1130) | 6 (1102, 1109, 1117, 1120, 1121, 1128) | 4 (1102, 1104, 1110, 1120) | 3 (1117, 1126, 1130) | 0 | 5 (1103, 1109, 1121, 1127, 1128) | 4 (1102, 1109, 1120, 1121) | 2 (1109, 1121) |
| No | 10 (1202, 1205, 1207, 1208, 1217, 1218, 1220, 1221, 1222, 1227) | 6 (1201, 1208, 1217, 1220, 1225, 1228) | 3 (1208, 1217, 1220) | 1 (1202) | 0 | 0 | 0 | 1 (1217) | 0 |

Table 3.7. Cronbach Coefficient Alpha values for the behavior indices.

| Index[a] | Cronbach Coefficient Alpha[b] |
|---|---|
| Perishable food handling and cooking index (PFHCI) | 0.747 |
| Pathogen awareness index (PAI) | 0.659 |
| Personal cleanliness index (PCI) | 0.679 |
| Kitchen and household cleanliness index (KHCI) | 0.796 |
| Inside crosscontamination index (ICCI) | 0.787 |
| Outside crosscontamination index (OCCI) | 0.823 |
| Risky foods procurement index (RFPI) | 0.500 |

[a]Each index comprises a series of question from the different instruments used (Appendices 1-6), and is calculated as the average for the answers given by participants.
[b]Cronbach Coefficient Alpha the internal consistency reliability of the test, and is a function of the extent to which questions in each index have high communalities (Cronbach, 1951; Cortina, 1993).

Table 3.8. Behaviors associated with increased *Listeria* prevalence in the rural household.

| | Covariate effect | $\beta^a$ | p-value | exp $(\beta)^b$ |
|---|---|---|---|---|
| Overall prevalence | Perishable food handling and cooking index (PFHCI)<br>Associated behaviors:<br>Refrigeration of leftover foods within two hours of preparation<br>How full is the refrigerator?<br>Refrigerator temperature<br>Use of thermometer for cooking of whole chicken, ground beef, steaks and roasts<br>Coverage of leftovers inside fridge<br>Presence of visible spoiled food, odors, spills and/or dripping inside the fridge | -0.9064 | 0.0288 | 0.4040 |
| Non-kitchen environment prevalence | Personal cleanliness index (PCI)<br>Associated behaviors:<br>Hand wash after farming/pet activities<br>Boots change after farming activities<br>Clothes change after farming activities<br>Location, frequency and technique of hand wash after farming activities<br>Use of an automatic dryer for clothes | -1.0450 | 0.0337 | 0.3517 |

[a]β: measures the degree of association between the probability of any given household having a positive sample and the value of a particular index (Ott and Longnecker, 2001).
[b]exp (β): the odds ratio of any given household having a positive sample when an specific index changes one unit.

## CHAPTER IV

## Microwave oven heating for inactivation of *Listeria monocytogenes* on frankfurters before consumption


### ABSTRACT

Microwave oven heating, using different power/time combinations, was evaluated for inactivation of *Listeria monocytogenes* on inoculated and stored frankfurters. Frankfurters formulated without or with 1.5% potassium lactate and 0.1% sodium diacetate were inoculated with *L. monocytogenes* (1.9±0.2 log CFU/cm$^2$), vacuum-packaged, and stored at 4°C to simulate storage before purchase by consumers. At storage days 18, 36 and 54, packages were opened and placed at 7°C, simulating aerobic storage in a household refrigerator. At 0, 3 and 7 days of aerobic storage, two frankfurters were placed in a bowl with water (250 ml) and treated in a household microwave oven (1100 Watts, 2450 MHz) at high (1100 W) power for 30, 45, 60 or 75 s, or medium (550 W) power for 60 or 75 s. Frankfurters, and the water in which samples were heated, were analyzed for total microbial counts (tryptic soy agar with 0.6% yeast extract) and *L. monocytogenes* populations (PALCAM agar). Exposure to high power for 75 s reduced pathogen levels (counts on control 0.7±0.0 to 1.0±0.1 log CFU/cm$^2$) to below the detection limit (< -0.4 log CFU/cm$^2$) on frankfurters with lactate/diacetate, even after 54 days of vacuum-packaged storage followed by 7 days of aerobic storage. For frankfurters

without lactate/diacetate, high power for 75 s caused reductions between > 1.5 and 5.9 log CFU/cm$^2$ from control levels (no microwave heating) of 1.5±0.1 to 7.2±0.5 log CFU/cm$^2$. Depending on treatment and storage time, the water used to reheat the frankfurters had viable *L. monocytogenes* counts of < -2.4 to 5.5±0.5 log CFU/ml. Results indicated that frankfurters should be reheated in a microwave oven at high power (1100 W) for 75 s to inactivate up to 3.7 log CFU/cm$^2$ of *L. monocytogenes* contamination.

## INTRODUCTION

Being the last step in the food continuum, consumers are responsible for the final treatments that may be applied to food before consumption. Cooking and reheating methods, such as microwave oven heating, steaming, grilling and boiling, are commonly used in meal preparation. Due to speed and convenience, microwave ovens are popular for heating food. However, these appliances are known to provide non-uniform heating (Suga *et al.*, 2007) which may produce hot and cold spots within the same food item, with reported temperature differences of up to 63.9°C (Ramaswamy and Pillet-Will, 1992). As a result of the uneven distribution of heat, there is concern of possible pathogen survival in contaminated food cooked in microwave ovens (Kaya *et al.*, 2003), and several cases of foodborne illness have been linked to food processed in this type of home appliance (Barnes *et al.*, 1989; Gesner and Beller, 1994; Evans *et al.*, 1995).

Levre' and Valentini (1998) suggested that microbial hazards linked to microwave-cooked food may be avoided by heating for adequate time to an appropriate temperature, and holding for the appropriate length of time after heating. Since internal temperatures of foods cooked in a microwave oven are lower than those needed for sterilization, food preparers need to follow cooking instructions carefully and measure temperatures at

multiple locations to determine whether food prepared in a microwave oven is safe to eat (Gessner and Beller, 1994; Evans *et al.*, 1995). Therefore, it is important to provide consumers with specific instructions for reheating of foodstuffs in the microwave oven (Nott and Hall, 2005; Swain *et al.*, 2006).

Studies have been conducted to evaluate the effectiveness of microwave oven heating for the control of pathogens, including *L. monocytogenes*, immediately before consumption (Lund *et al.*, 1989; Sheeran *et al.*, 1989; Hollywood *et al.*, 1991; Heddleson *et al.*, 1996; Gundavarapu *et al*,. 1995; Pucciarelli and Benassi, 2005). Most of these studies, however, have focused on heating of frozen meals and cooking pieces of meat or fish fillets and whole birds. Regarding chilled leftovers and ready-to-eat (RTE) meats, like frankfurters and deli cuts, limited or no information is available about the effectiveness of microwave ovens in enhancing the microbial safety of such foods. Appropriate reheating recommendations for these products are critical, especially for high risk populations, such as the elderly and the immunocompromised, since these individuals are more susceptible to listeriosis infection.

Most commercial brands of frankfurters do not provide instructions or guidelines for reheating on their labels, even though it is known that frankfurters may potentially be contaminated with *L. monocytogenes,* and that this type of meat product can support growth of the pathogen under conditions such as those encountered at retail facilities and home refrigerators (Chunhua and Muriana, 1991; Wallace *et al.*, 2003). In the few cases that instructions are provided, they consist mainly of a recommended time (between 30 and 210 s) of microwave heating at high power. However, maximum power output is specific for each appliance, and varies by model and manufacturer. Thus, high power

may translate into anything between 500 to 1100 W for household microwave oven units. Therefore, the objective of this study was to evaluate different power and time combinations of microwave oven heating for inactivation of *L. monocytogenes* on inoculated and stored frankfurters.

## MATERIALS AND METHODS

**Preparation of frankfurters.** Frankfurters were prepared with a mixture of 60% pork (pork shoulder, 70-72% lean) and 40% beef (beef chuck, 76-78% lean). After grinding, meat was combined with ice, sodium chloride, dextrose, corn syrup solids, sodium nitrite, sodium erythorbate, polyphosphate and spices (Samelis *et al.,* 2002). Frankfurters were formulated with or without 1.5% potassium lactate (Purac Purasal® HiPure P, Lincolnshire, IL, USA) and 0.1% sodium diacetate (Niacet Corporation, Niagara Falls, NY, USA). The meat and other ingredients were emulsified and cooked according to the procedure described by Byelashov *et al.* (2008). After cooking, frankfurters were refrigerated (4°C) overnight, and then peeled and inoculated.

**Inoculation of frankfurters and storage.** The inoculum consisted of a mixture of 10 strains of *L. monocytogenes* habituated in autoclave-sterilized frankfurter homogenate in water (10%, w/v), as described by Lianou *et al.* (2007). Each frankfurter was inoculated (Byelashov *et al.* 2008) with 0.2 ml of the 10-strain mixture, appropriately diluted in autoclave-sterilized frankfurter extract, to achieve a target level of 2 log CFU/cm$^2$. Inoculated samples were kept at 4°C for 15 min to allow for cell attachment. Inoculated samples (six per package) were placed in zip-top type bags (Zip Vak 15.2×20.3 cm, nylon/EVA copolymer, Winpak Winnipeg, MB, Canada), vacuum-sealed (LVII Super, Hollymatic Corp., Countryside, IL, USA), and stored at 4°C for up to 54

days, simulating manufacturer and/or retail storage. On days 18, 36 and 54, the zip-lock of packages was opened to release the vacuum-seal and then reclosed and stored at 7°C for up to 7 days, simulating aerobic, home storage conditions.

**Microwave oven treatments.** Microwave oven treatments were applied to frankfurters on days 0, 3 and 7 of aerobic storage (7°C). A household microwave oven (Amana, model Radarange AMC5143AAW, Newton, IA, USA) equipped with a turntable and with a maximum power output (default high power setting) of 1100 Watts at 2450 MHz was used. The following treatments were evaluated: high power (1100 W) for 30, 45, 60 or 75 s, medium power (50% of high power = 550 W) for 60 or 75 s, and no microwave oven treatment (control). Each microwave oven treatment was applied to two frankfurters placed in a microwave-safe dish (22 cm diameter, 4 cm deep), to which 250 ml of sterile distilled water was added. The dish was covered with a shallow (22 cm diameter) microwave-safe dish and placed into the microwave oven. Treatments were applied by choosing the selected combination of power and time, using the control panel of the microwave oven. After each microwave oven treatment, the dish was taken out of the microwave, the cover plate was removed and the frankfurters were allowed to stand in the water for 2 min (standing time) for temperature equilibration and potential reduction of cold and hot spots. The temperature of the frankfurters and water in the dish was measured before treatment, immediately after treatment, and after the 2 min of standing time, using an infrared thermometer (Oakton® TempTestr® IR, Vernon Hills, IL, USA). For the control treatment, two frankfurters were placed in water (21±2°C) for 2 min. Microbiological analysis of frankfurter samples and water used for reheating followed.

**Microbiological analyses**. Immediately following the standing time after microwave treatment, frankfurters were transferred to a Whirl-Pak® bag (15x23 cm, Nasco, Modesto, CA, USA) containing 50 ml of maximum recovery diluent (MRD; 0.85% NaCl and 0.1% peptone) and were vertically shaken 30 times (Barmpalia *et al.*, 2004). Serial dilutions of the rinsate were prepared in 0.1% buffered peptone water (Difco, Becton Dickinson, Sparks, MD, USA) and were surface-plated on PALCAM agar (Difco) for enumeration of *L. monocytogenes* survivors, and tryptic soy agar (Difco) with 0.6% yeast extract (Acumedia, Neogen Corporation, Lansing, MI, USA) (TSAYE) for enumeration of total microbial populations. PALCAM agar plates were incubated at 30°C for 48 h and TSAYE plates were incubated at 25±2°C for 72 h. The detection limit for the analysis of frankfurter samples was -0.4 log CFU/cm$^2$.

The water in which frankfurters were heated was transferred to a bag (Whirl-Pak, 19×30 cm) and serial dilutions were prepared and surface-plated on PALCAM agar. For samples in which low numbers of survivors were expected (e.g., treatments at high power for 45, 60 and 75 s), the water was filtered through a Durapore® membrane filter (0.22 μm GV, Millipore Corporation, Billerica, MA, USA). The membrane was then placed in a Whirl-Pak bag (15×23 cm) containing 50 ml of MRD, shaken 30 times, and surface-plated on PALCAM agar. The detection limit for the analysis of water samples was -2.4 log CFU/ml.

Following microbiological analysis (and pH measurements of product samples, described below), frankfurter and water samples were kept at 4°C in the event that samples would have to be enriched if no *L. monocytogenes* survivors were obtained by direct plating. In such cases, samples were enriched using the USDA-FSIS method

(USDA-FSIS, 2008b) with some modifications. Specifically, 100 ml of University of Vermont broth (Difco) was added to each sample and incubated a 30°C for 24 ± 2 h. Then, 1 ml of this enrichment was transferred to 9 ml of Fraser Broth (Difco) and incubated at 35°C for up to 48 ± 2 h. After incubation for 24 and 48 h, tubes of Fraser broth showing signs of darkening were streak-plated onto PALCAM agar plates and incubated at 30°C for 48 ± 2 h. Following incubation, plates were checked for presence of typical *Listeria* colonies, which would have indicated that the sample was positive by enrichment. If no darkening of Fraser broth occurred, the sample was recorded as negative for *Listeria* by enrichment.

**Physicochemical analyses.** All frankfurter samples analyzed for microbial counts were homogenized (2 min; Masticator, IUL Instruments, Barcelona, Spain) after plating and pH measurements were obtained from an aliquot (5 ml) of the homogenate, using a Denver Instruments (Arvada, CO, USA) pH meter and glass electrode. Water activities ($a_w$) of both frankfurter formulations (i.e., with or without lactate/diacetate) were measured (AquaLab model series 3, Decagon Devices, Pullman, WA, USA) on day-0 of vacuum-packaged storage. Also, fat and moisture contents of both formulations were determined following the procedures of AOAC International official methods 960.39 and 950.46B, respectively (AOAC, 1998).

**Statistical analysis.** Two complete replications of the experiment were conducted, and each microwave oven treatment was applied to three different samples at each storage period. Data were analyzed using the Mixed Model procedure of SAS/STAT® (SAS Institute, 2007). Numbers of survivors (in log CFU/cm$^2$ or CFU/ml) was the dependent variable. The independent variables were formulation (with or without

lactate/diacetate), storage time in vacuum-sealed packages (18, 36 or 54 days), storage time in opened packages (0, 3 or 7 days), and microwave oven treatments (combinations of power/time: high/30 s, high/45 s, high/60 s, high/75 s, medium/60 s or medium/75s), as well as the interactions of these variables. Least-squares means were computed and separation was performed using Tukey's method ($\alpha$= 0.05).

## RESULTS AND DISCUSSION

**Physicochemical properties of frankfurters.** The moisture content of frankfurters formulated with and without lactate/diacetate ranged from 59.2±0.9 to 64.0±8.2%, and crude fat levels ranged from 13.3±3.5 to 17.0±1.1 %. Water activity values were 0.962±0.004 and 0.963±0.003 for frankfurters with and without lactate/diacetate, while pH values were 5.97±0.04 and 6.01±0.07, respectively. Fat and moisture content, pH and water activity for the two product formulations did not differ ($P \geq 0.05$). During storage, the pH of frankfurters with lactate/diacetate remained almost constant, regardless of storage condition (vacuum-sealed or aerobic) and storage temperature (Table 4.1). In the case of product without lactate/diacetate, the pH was not affected ($P \geq 0.05$ ) during storage in vacuum packages, but there was a trend of decreasing product pH during aerobic storage (7 d) after 36 and 54 days of storage in vacuum packages (Table 4.1). As expected, microwave oven treatments did not have an effect ($P \geq 0.05$) on the pH of frankfurters.

***Listeria monocytogenes* populations on untreated (no microwave heating) frankfurters.** Levels of *L. monocytogenes* on the day of inoculation (i.e., day-0 of vacuum-sealed storage) were 1.9±0.2 and 1.9±0.3 log CFU/cm$^2$ for frankfurters with and without lactate/diacetate, respectively. *L. monocytogenes* populations on frankfurters not

receiving a microwave treatment (control; Figures 4.1 to 4.6) were determined on samples that were immersed in water (21±2°C; 2 min), as indicated previously. In this way, the rinsing effect of the water in which samples were immersed for the microwave oven treatment was taken into consideration, thus more accurately determining the effect of the microwave oven treatments on inactivation of *L. monocytogenes*. The fate of the pathogen during vacuum-packaged storage at 4°C was reflected by the counts obtained for control (no microwave treatment) samples on day-0 of each cycle of aerobic storage (Figures 4.1 to 4.6). Pathogen counts on control (no microwave treatment) frankfurters without lactate/diacetate increased ($P < 0.05$) to 5.9±0.4 log CFU/cm$^2$ on vacuum-packaged samples stored at 4°C for 54 days, whereas counts remained unchanged ($P \geq 0.05$) during the same time period on control samples of product formulated with lactate/diacetate (0.7±0.0 log CFU/cm$^2$ on 54-day old product; Figures 4.1 to 4.6). Storage under aerobic conditions (up to 7 days at 7°C) following 18, 36 or 54 days of vacuum-packaged storage permitted additional increases in pathogen counts on control frankfurters (no microwave treatment) without lactate/diacetate, ranging from -0.4 to 3.5 log CFU/cm$^2$ within the 7-day period. No growth ($P \geq 0.05$) of *L. monocytogenes* was detected on control frankfurters with lactate/diacetate under any aerobic storage conditions. Thus, overall, compared to frankfurters with lactate/diacetate, those formulated without these antimicrobials allowed for prolific growth of the pathogen during both storage conditions, resulting in consistently higher counts of the pathogen before microwave treatment. Similar results have been reported by others (Bedie *et al.*, 2001; Barmpalia *et al.*, 2004; Stopforth *et al.*, 2006). In most cases, pathogen counts on PALCAM agar were similar to total microbial counts on TSAYE, irrespective of storage

condition (Figures 4.7 to 4.12). However, in one particular case, it was noteworthy that growth of *L. monocytogenes* was inhibited in the presence of high levels (6.1±1.1 log CFU/cm$^2$) of natural flora on frankfurters without lactate/diacetate stored for 36 days in vacuum-packages, followed by 7 days under aerobic conditions (Figures 4.5).

**Microwave oven inactivation of *L. monocytogenes* on frankfurters.** Frankfurters containing lactate/diacetate did not allow growth of *L. monocytogenes* during storage, and counts on control samples (no microwave treatment) were consistently < 1.0 log CFU/cm$^2$ (Figures 4.1 to 4.3). Survivors counts after microwave treatments of this type of product at high power for 60 or 75 s, differed ($P < 0.05$) from the control, and caused reductions from 1.0 to > 1.4 log CFU/cm$^2$ (DL: < -0.4 log CFU/cm$^2$), regardless of storage time or packaging condition of the product. Only treatment for 75 s consistently reduced counts to below the detection limit, irrespective of storage time. However, at least one out of the six samples that received this treatment after each storage period was positive for the pathogen by enrichment. Treatments at medium power (60 or 75 s) and high power for 30 or 45 s allowed for survivors in the range of 0.0 to 0.9 log CFU/cm$^2$. A similar trend was observed for total microbial populations (Figures 4.7 to 4.9).

On frankfurters without lactate/diacetate, where pathogen numbers steadily increased (1.5 to 7.2 log CFU/cm$^2$; $P<0.05$) on non-heated product as storage under vacuum and aerobic conditions progressed, treatments of medium power (60 or 75 s) and high power for 30 or 45 s were consistently ineffective, and numbers of survivors on these samples were not different ($P \geq 0.05$) than pathogen levels on control (no microwave treatment) samples (Figures 4.4 to 4.6). In most cases, survivor counts after

treatment at high power for 60 and/or 75 s differed ($P < 0.05$) from those on the control (1.5 to 7.2 log CFU/cm$^2$), with numbers of survivors following treatment depending on the initial level, as determined by length of storage and packaging condition of the frankfurters. Initial counts of up to 3.7 log CFU/cm$^2$, which were achieved after 18 days of vacuum storage followed by 7 days of aerobic storage, were reduced to below the detection limit (but detectable by enrichment on some samples) by microwave heating at high power for 75 s. This same treatment allowed for survival of the pathogen in numbers ranging from -0.1 (36 days vacuum followed by 7 days aerobic storage) to 2.3 log CFU/cm$^2$ (54 days vacuum followed by 7 days aerobic storage) as storage progressed and counts on control frankfurters increased above 3.7 log CFU/cm$^2$ and up to 7.2 log CFU/cm$^2$. Reductions in pathogen counts achieved by high power for 75 s on frankfurters without lactate/diacetate were as high as 5.9 log CFU/cm$^2$, stressing the importance of storage time, and therefore before heating initial counts, on the effectiveness of microwave heating. This treatment also was the most effective in reducing total microbial counts (Figure 4.10 to 4.12) on frankfurters without lactate/diacetate, and no other treatment differed ($P \geq 0.05$) from the non-heated control, regardless of storage time. Huang (2005) reported reductions of up to 7-log of *L. monocytogenes* on frankfurters can be achieved by microwave oven heating at 600 W. However, considerably longer times (12-15 min) than those used in this study were needed to reach such a level of inactivation.

It was observed in some cases that cell counts of *L. monocytogenes* on product without lactate/diacetate were numerically higher but not statistically different ($P \geq 0.05$) after some microwave oven treatments when compared to the control (Figures 4.4 to 4.6).

This phenomenon also was observed with total microbial counts on frankfurters without lactate/diacetate (Figures 4.10 and 4.11), and was probably due to sample variation. Overall, for frankfurters that allowed growth of *L. monocytogenes* during storage, the treatments need to be adjusted to higher power level and/or longer times in order to achieve higher levels of pathogen inactivation, compared to product formulated with lactate/diacetate, where numbers of the pathogen remained low during storage. Thus, reheating directions on package labels should be designed to account for the worst case scenario. The levels of *L. monocytogenes* survivors after some microwave oven heating treatments reported here may potentially cause disease not only in highly susceptible individuals, but it may also cause febrile gastroenteritis in healthy, immunocompetent people (Maijala *et al.*, 2001; Ooi and Lorber, 2005), stressing the importance of appropriate reheating directions.

**Microbial contamination in heating water.** Among the few manufacturers' guidelines found on frankfurter labels, some recommend the use of water for reheating of such products, which may help in decreasing the prevalence of hot and cold spots. Depending on the storage time, unheated water for control (no microwave heating) treatments of frankfurters with and without lactate/diacetate contained -1.7 to -0.1 and -0.7 to 5.3 log CFU/ml of *L. monocytogenes*, respectively (Figures 4.13 to 4.18), that were transferred from the surface of frankfurters. Initial counts < -1.7 log CFU/ml in the water used for heating of frankfurters with lactate/diacetate (Figure 4.13 to 4.15) were reduced to below the detection limit (DL: < -2.4 log CFU/ml) by heating at high power for 60 s. Initial counts between -1.6 and -0.9 log CFU/ml required high power treatment for 75 s to achieve reductions below the detection limit (Figures 4.13 to 4.15). For the water used in

the heating of product without lactate/diacetate (Figure 4.16 to 4.18), high power for 75 s reduced initial counts of ≤ 1.4 log CFU/ml to below the detection limit, but when the level increased between 1.5 and 5.3 log CFU/ml, due to storage time of the frankfurters, all the treatments applied allowed *L. monocytogenes* survival in the heating water (-0.1 to 5.1 log CFU/ml). The water used for reheating of frankfurters contaminated with *L. monocytogenes* may become a vehicle for cross-contamination of the kitchen environment, since it would be discarded through the sink, contaminating it, and potentially creating a reservoir for the pathogen that may later be transferred to other surfaces and even foods, by either splashing and/or aerosols (Bloomfield and Scott, 1997). The kitchen sink has already been implicated in a scenario of household cross-contamination with *L. monocytogenes*. Wagner *et al.* (2007) recovered the same isolate from the kitchen sink, three different food samples and stools from household members. Thus, reheating instructions for frankfurters in the microwave oven must be developed to address both the contamination potentially present on the frankfurters and that transferred to the heating water.

**Temperature of frankfurters and water following microwave oven treatments.** Data in Table 4.2 show the values of temperature that were recorded after each treatment for frankfurters and water used for heating. The wide range of temperatures observed is a consequence of the non-uniform heating that is commonly observed during microwave heating. Other authors also have reported a high variability in the temperatures recorded not only between, but also within, replicates (Göksoy *et al.*, 2000). This high variability also may be a consequence of the shape and positioning of the frankfurters inside the oven, because these two factors may promote an irreproducible

temperature distribution (Nott and Hall, 2005). Except for high power for 75 s, no other treatment produced an increase in frankfurter temperature to or above 74°C, which is the minimum recommended for reheating of food in a microwave oven (USDHHS-FDA, 2005). In addition, even when temperatures above 74°C were recorded in some cases, the presence of hot and cold spots on the surface of single frankfurters may lead to the survival of the pathogen on those colder places.

Standing time after treatment may help in obtaining a more uniform distribution of the heat, by conduction after the microwave power has been turned off, and can improve microbial destruction (Hollywood *et al.*, 1991; Ramaswamy and Pillet-Will, 1992). In this study, 2 min of standing time followed each treatment, to allow for temperature increase in colder spots. The average increase in temperature after this standing time was between 0 and 6°C. This value is comparable to that reported by Sawyer (1985), who obtained a post-processing temperature increase of between 2 and 14°C after heating one chicken frankfurter for 30 s at 100% power (663 W) and allowing 45 s of standing time. However, the increase in temperature obtained in the present study after 2 min of standing time does not allow for the coldest spot to reach 74°C, as recommended in the Food Code (USDHHS-FDA, 2005) and in some manufacturer's reheating instructions.

## CONCLUSIONS

Frankfurters should be reheated for 75 s at high power (1100 W) to reduce counts of *L. monocytogenes* by 3.7 log CFU/cm$^2$ on frankfurters and by 1.4 log CFU/ml in the reheating water, respectively. Longer times are needed when the product has supported growth of the pathogen to levels > 3.7 log CFU/cm$^2$ due to prolonged storage time and/or lack of lactate/diacetate in the formulation. Directions for reheating of frankfurters in the

microwave oven that do not specify power level along with a recommended reheating time may not be adequate when the product is formulated without lactate/diacetate, due to the potential for this type of product to support growth of the pathogen to high numbers. Thus, reheating instructions must be designed specifically for each type of product, and considering variations in microwave appliance maximum output power, amount of food to be reheated, age of the product and presence of antimicrobial compounds in the formulation.

Table 4.1. The pH values (means averaged over storage time ± SD) of frankfurters stored in vacuum-sealed (4°C, 54 days) and opened (aerobic; 7°C, 7 days) packages.

| Storage time (days) | | Frankfurters formulation | |
|---|---|---|---|
| Vacuum-packaged (4°C) | Aerobic (7°C) | With lactate/diacetate | Without lactate/diacetate |
| 18 | 0 | $6.01\pm0.04^{c}$ | $6.10\pm0.05^{a}$ |
| | 3 | $6.07\pm0.05^{a}$ | $6.14\pm0.04^{a}$ |
| | 7 | $6.03\pm0.06^{ab}$ | $6.09\pm0.09^{a}$ |
| 36 | 0 | $6.04\pm0.08^{ab}$ | $6.12\pm0.10^{a}$ |
| | 3 | $6.05\pm0.06^{ab}$ | $6.13\pm0.07^{a}$ |
| | 7 | $6.02\pm0.07^{b}$ | $6.01\pm0.15^{b}$ |
| 54 | 0 | $6.06\pm0.07^{ab}$ | $6.08\pm0.1^{a}$ |
| | 3 | $6.08\pm0.06^{a}$ | $6.10\pm0.06^{a}$ |
| | 7 | $6.05\pm0.08^{ab}$ | $5.87\pm0.17^{c}$ |

$^{abc}$ Means with same superscript letter within a column are not significantly different ( $P\geq$ 0.05).

Table 4.2. Mean ± 2°C (and range) of maximum surface temperatures of frankfurters formulated with (LD) and without (NoLD) lactate/diacetate, and of heating water after treatment in a household microwave oven.

| Microwave Heating | | Frankfurters | | | | Water used for reheating of frankfurters | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | After treatment | | After 2 min standing time | | After treatment | | After 2 min standing time | |
| Watts (Power level) | Time (s) | LD | NoLD | LD | NoLD | LD | NoLD | LD | NoLD |
| 550 (Medium) | 60 | 41 (34-65) | 38 (28-46) | 38 (27-54) | 35 (27-41) | 41 (38-63) | 38 (37-45) | 38 (35-60) | 36 (35-40) |
| | 75 | 48 (41-54) | 50 (43-58) | 43 (38-61) | 45 (39-49) | 47 (46-53) | 49 (42-54) | 33 (43-60) | 44 (39-48) |
| 1100 (High) | 30 | 41 (30-53) | 42 (29-48) | 37 (30-53) | 35 (19-42) | 41 (47-35) | 42 (39-46) | 38 (42-34) | 35 (20-42) |
| | 45 | 51 (22-60) | 52 (40-62) | 45 (37-50) | 53 (39-52) | 51 (47-59) | 52 (47-58) | 45 (43-49) | 45 (43-53) |
| | 60 | 62 (55-69) | 62 (58-72) | 54 (38-63) | 46 (48-64) | 60 (54-67) | 56 (56-69) | 54 (50-60) | 52 (51-63) |
| | 75 | 62 (32-87) | 65 (60-78) | 52 (54-76) | 55 (55-68) | 66 (61-78) | 63 (59-71) | 57 (56-66) | 53 (53-62) |

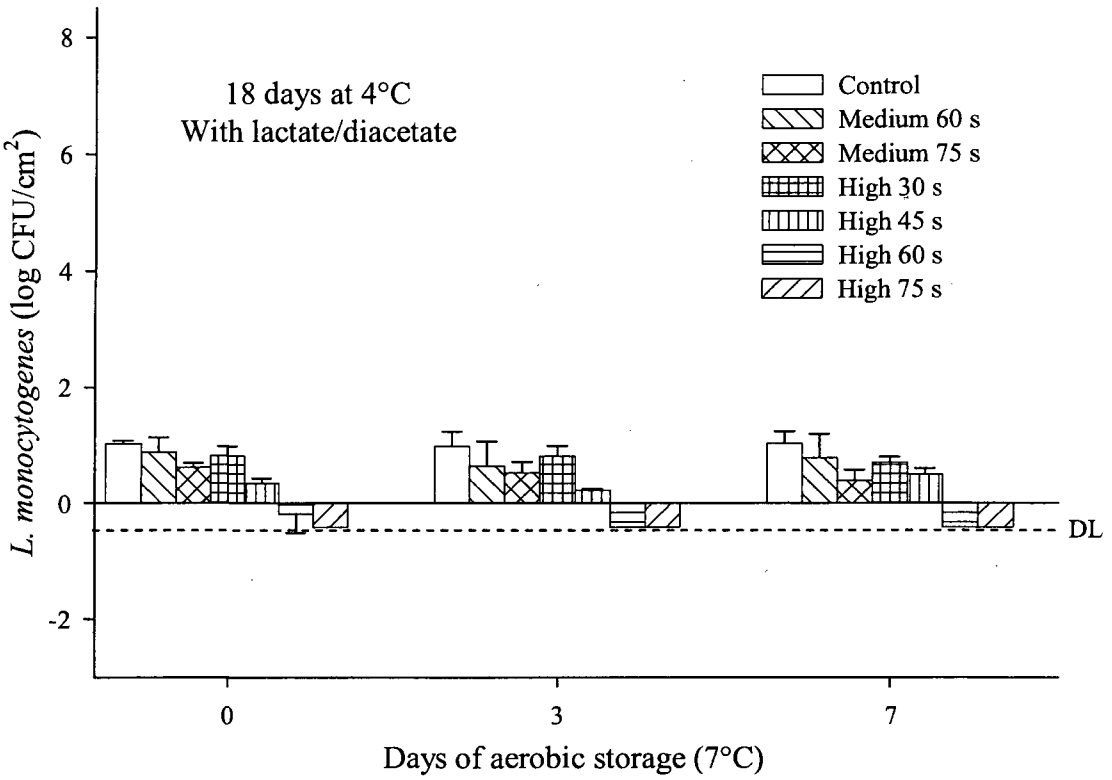


Figure 4.1 (Appendix Table 1). *Listeria monocytogenes* counts on frankfurters formulated with lactate/diacetate, after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

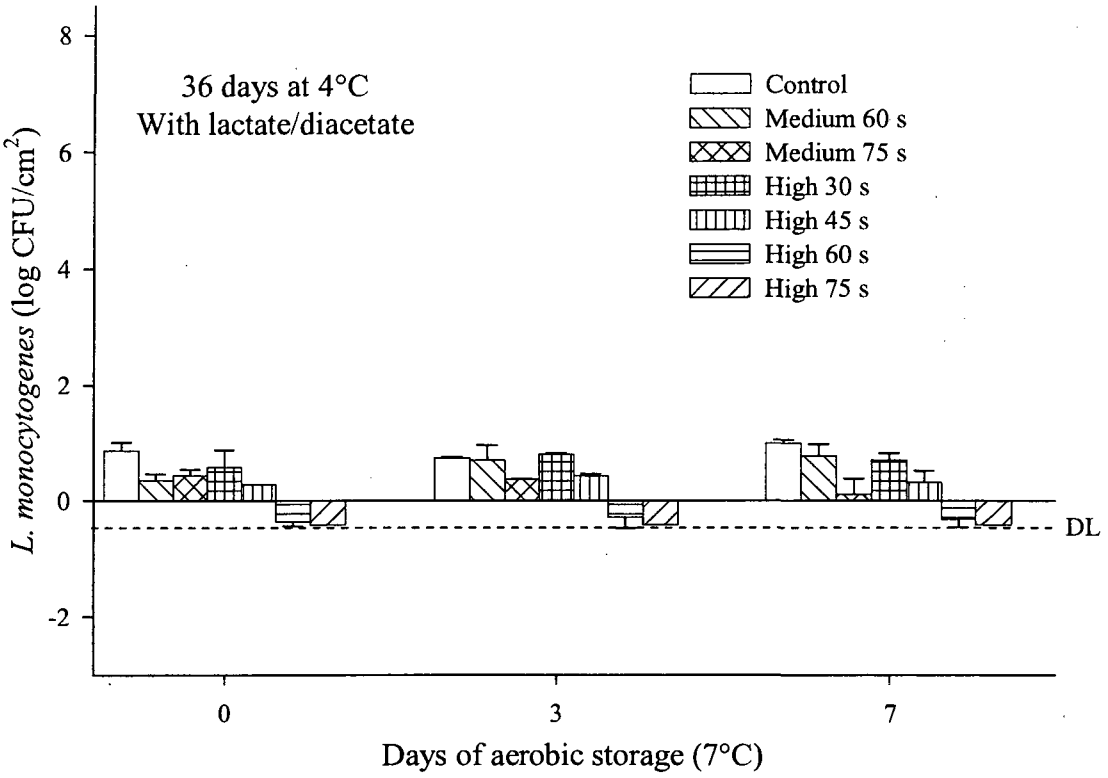


Figure 4.2 (Appendix Table 2). *Listeria monocytogenes* counts on frankfurters formulated with lactate/diacetate, after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

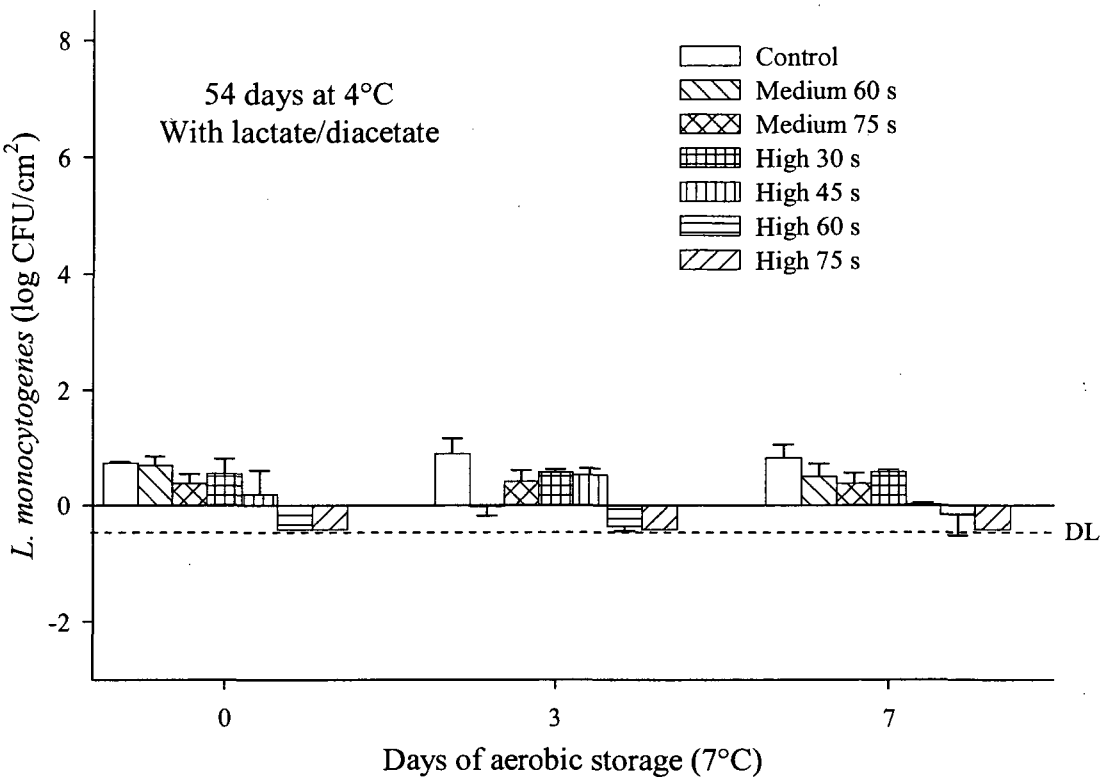


Figure 4.3 (Appendix Table 3). *Listeria monocytogenes* counts on frankfurters formulated with lactate/diacetate, after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

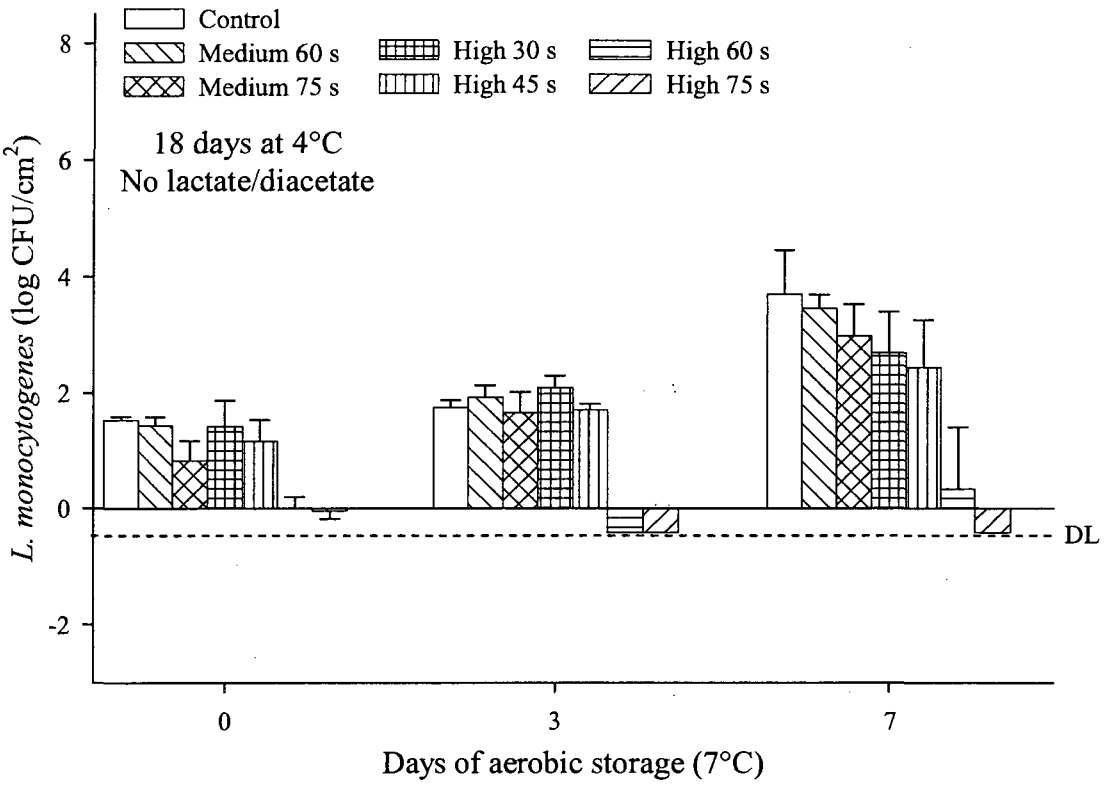


Figure 4.4 (Appendix Table 4). *Listeria monocytogenes* counts on frankfurters formulated without lactate/diacetate, after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

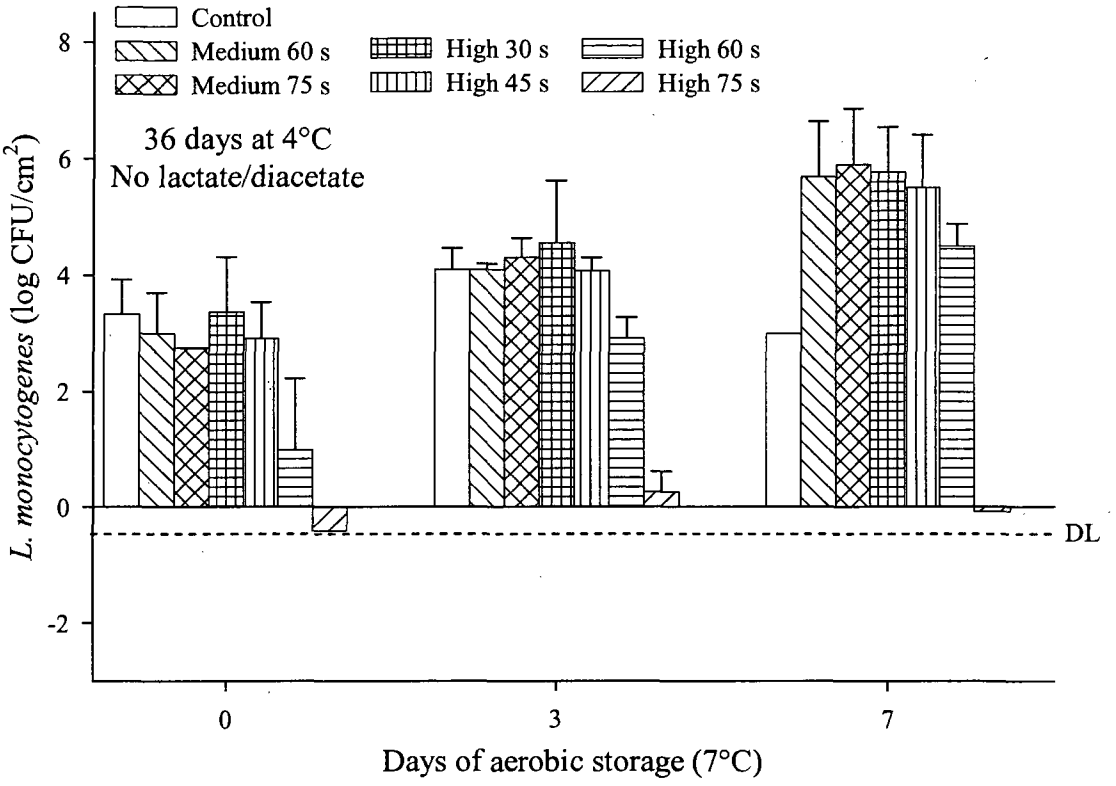


Figure 4.5 (Appendix Table 5). *Listeria monocytogenes* counts on frankfurters formulated without lactate/diacetate, after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

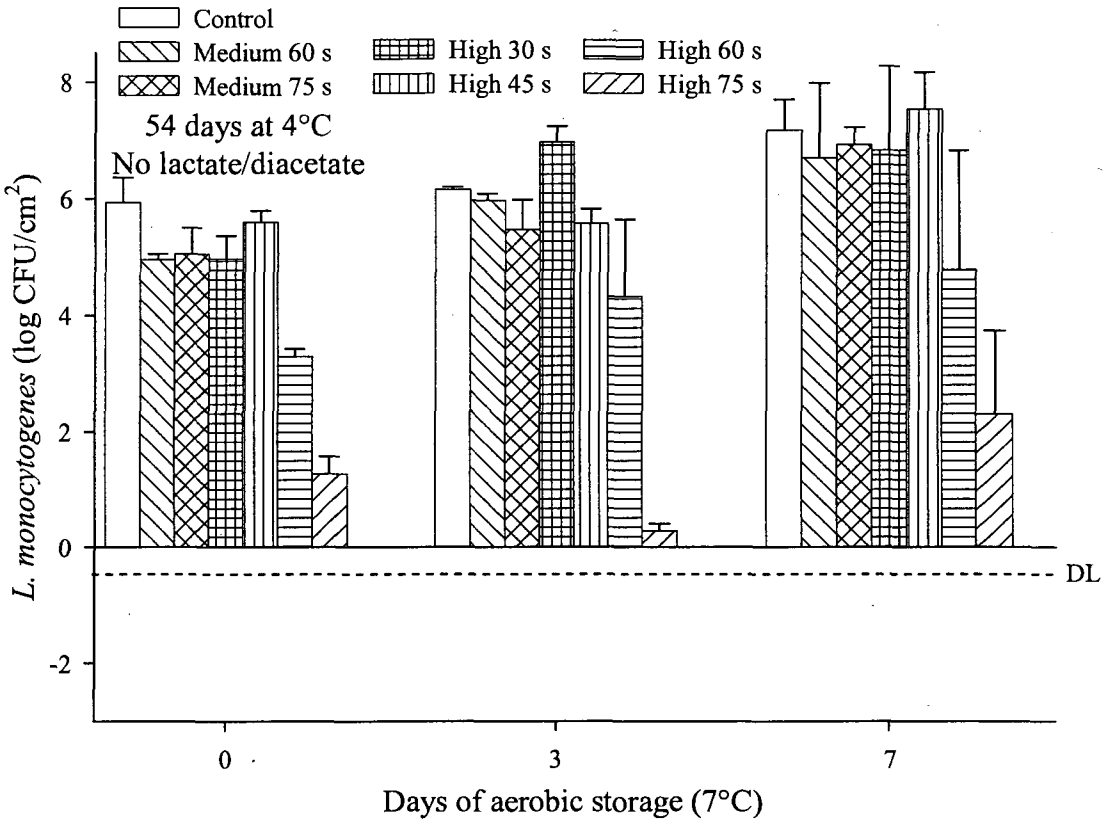


Figure 4.6 (Appendix Table 6). *Listeria monocytogenes* counts on frankfurters formulated without lactate/diacetate, after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

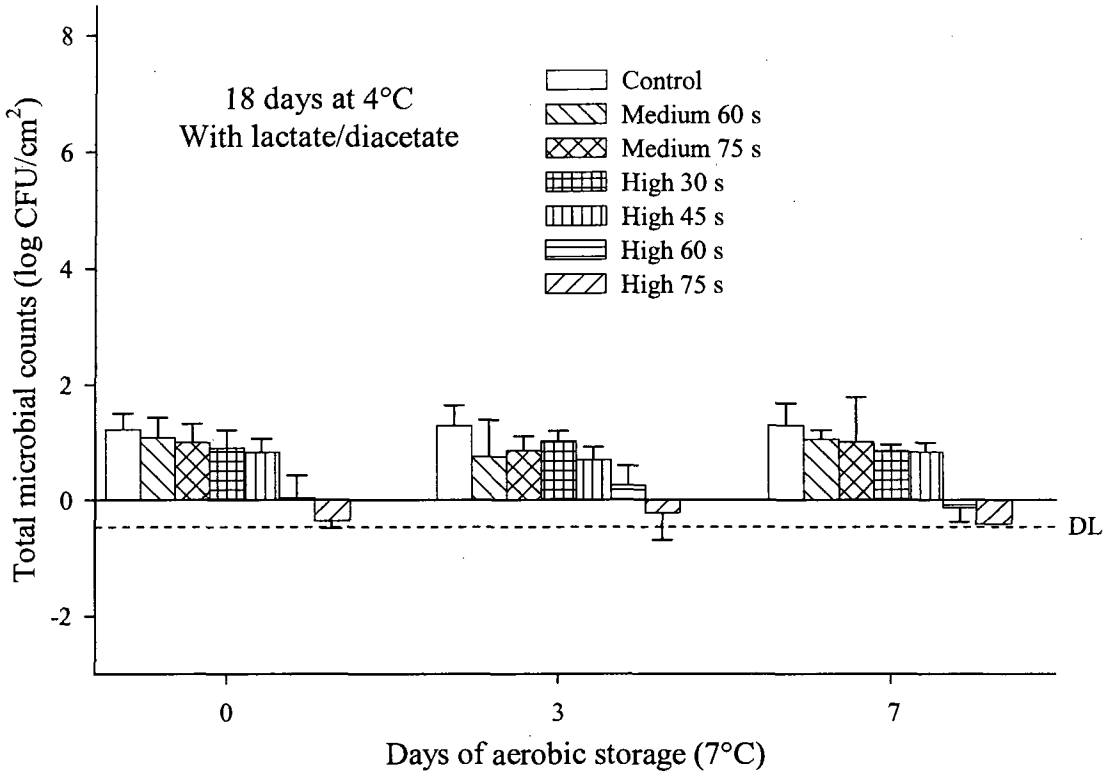


Figure 4.7 (Appendix Table 7). Total microbial counts on frankfurters formulated with lactate/diacetate, after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

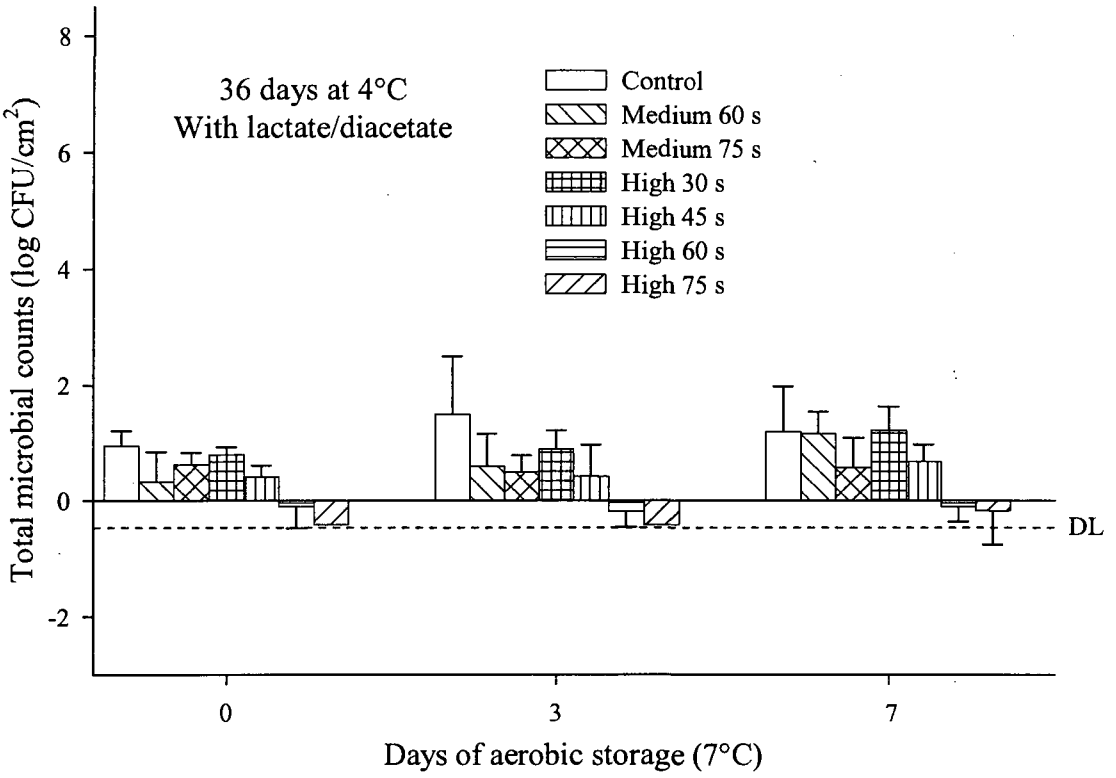


Figure 4.8 (Appendix Table 8). Total microbial counts on frankfurters formulated with lactate/diacetate, after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

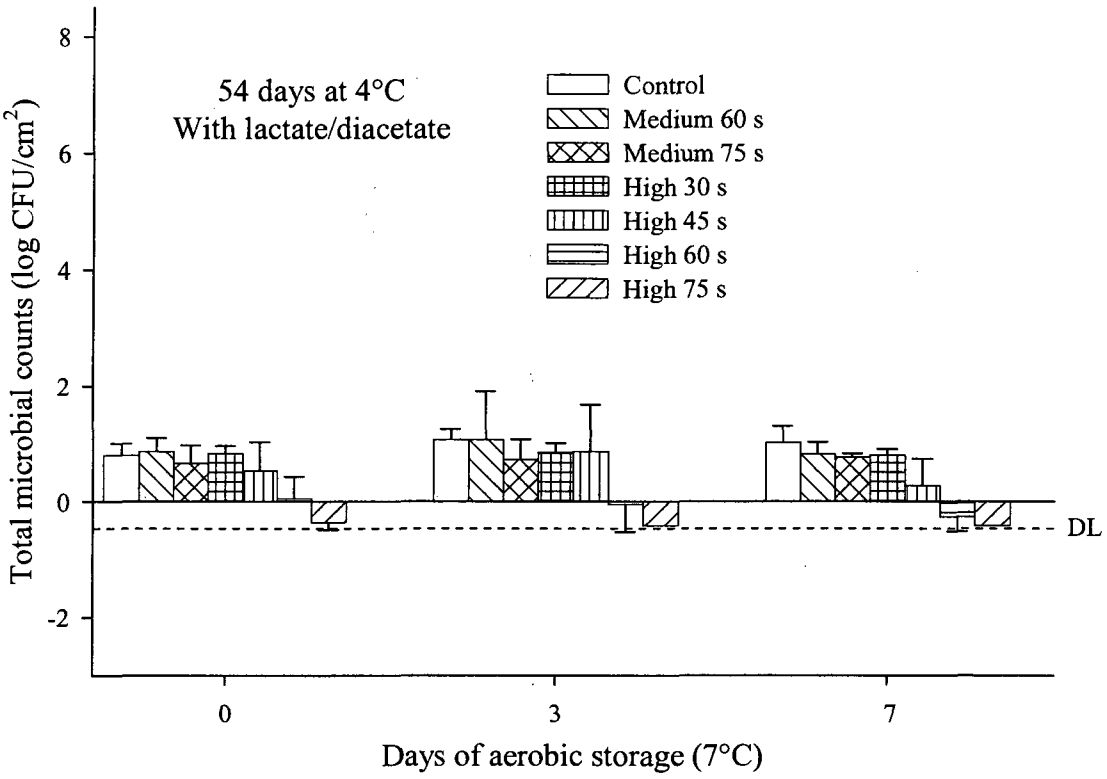


Figure 4.9 (Appendix Table 9). Total microbial counts on frankfurters formulated with lactate/diacetate, after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

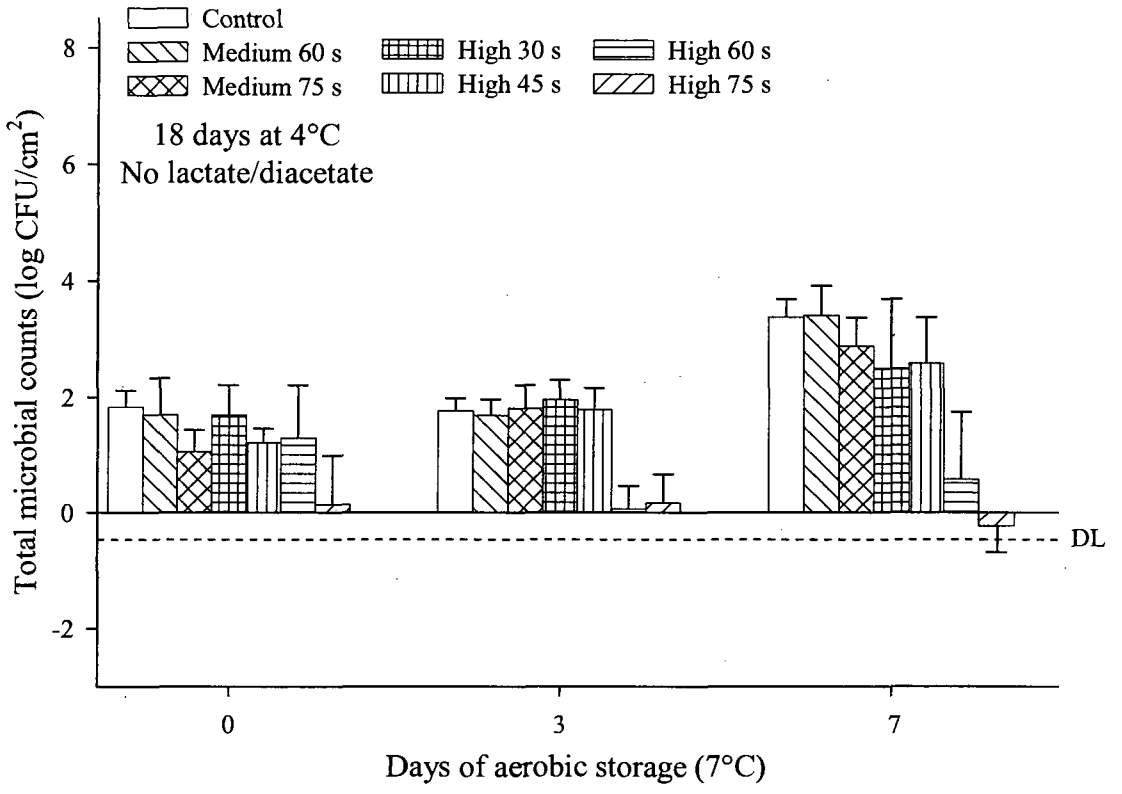


Figure 4.10 (Appendix Table 10). Total microbial counts on frankfurters formulated without lactate/diacetate, after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

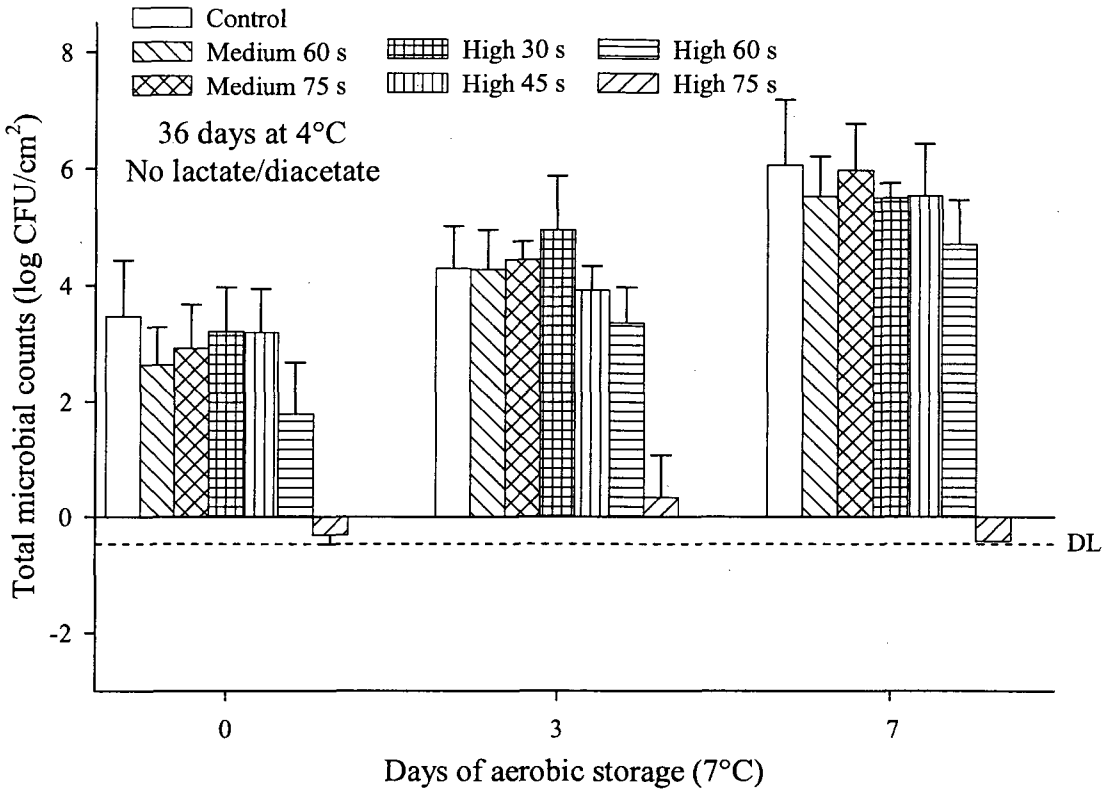


Figure 4.11 (Appendix Table 11). Total microbial counts on frankfurters formulated without lactate/diacetate, after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

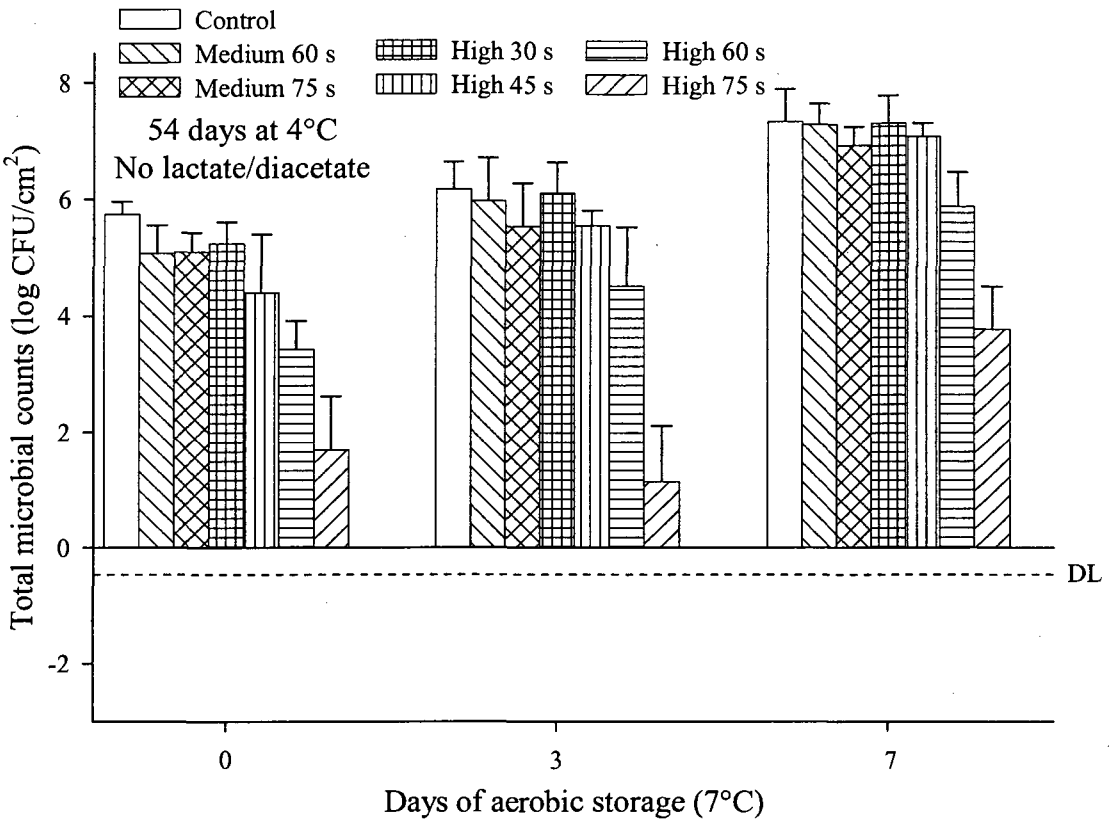


Figure 4.12 (Appendix Table 12). Total microbial counts on frankfurters formulated without lactate/diacetate, after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

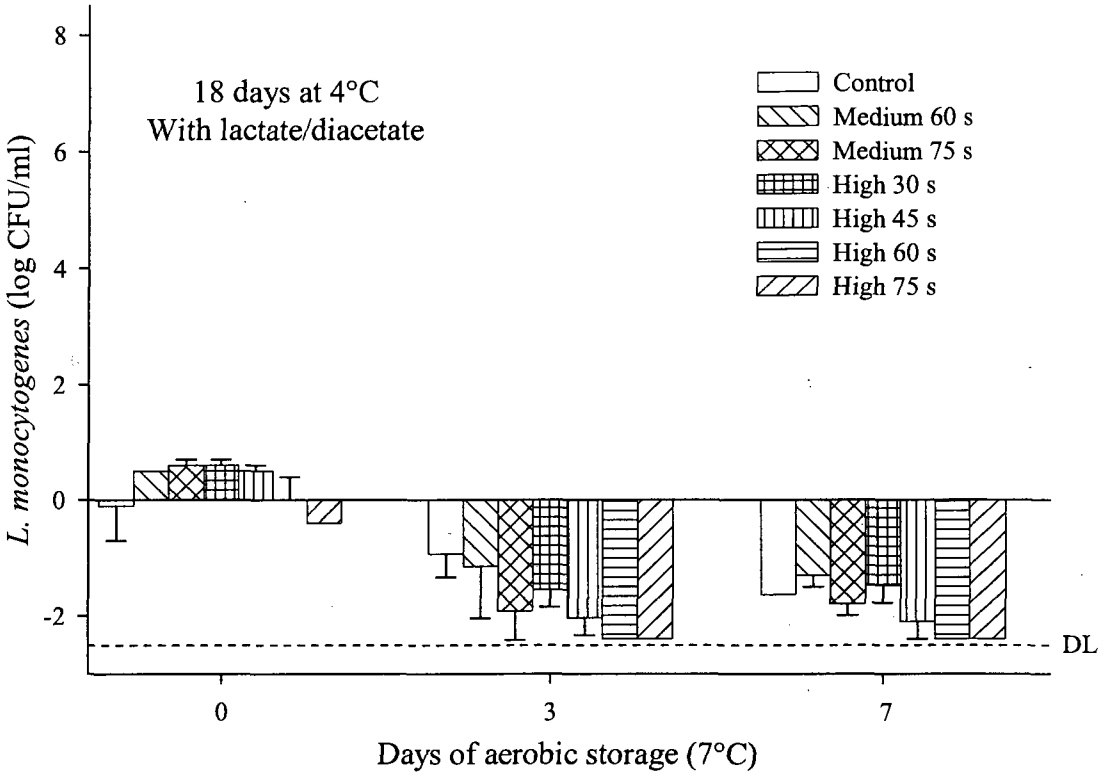


Figure 4.13 (Appendix Table 13). *Listeria monocytogenes* counts in water used to reheat frankfurters formulated with lactate/diacetate, after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

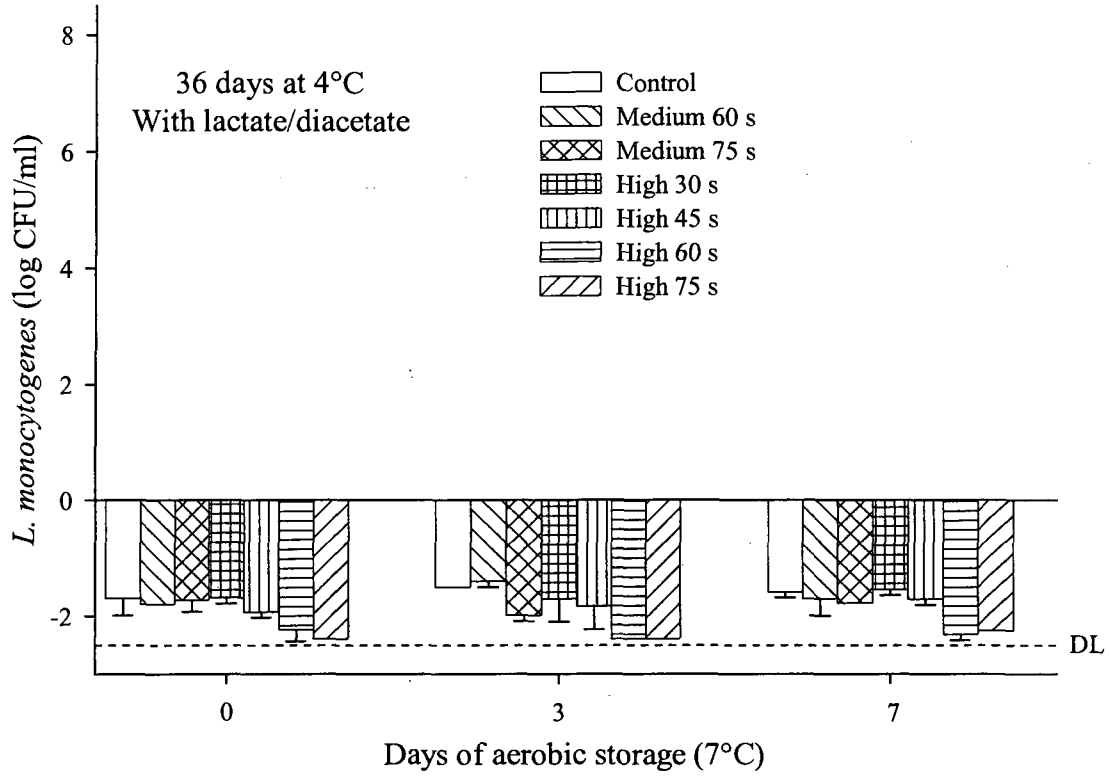


Figure 4.14 (Appendix Table 14). *Listeria monocytogenes* counts in water used to reheat frankfurters formulated with lactate/diacetate, after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

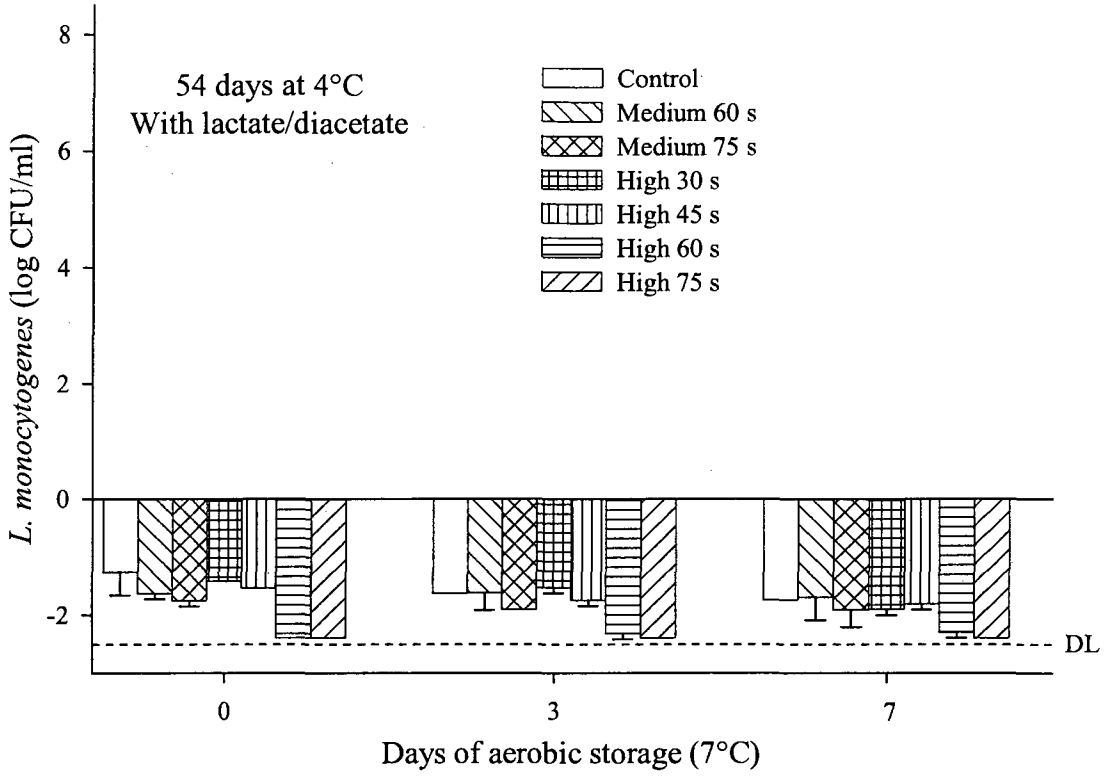


Figure 4.15 (Appendix Table 15). *Listeria monocytogenes* counts in water used to reheat frankfurters formulated with lactate/diacetate, after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

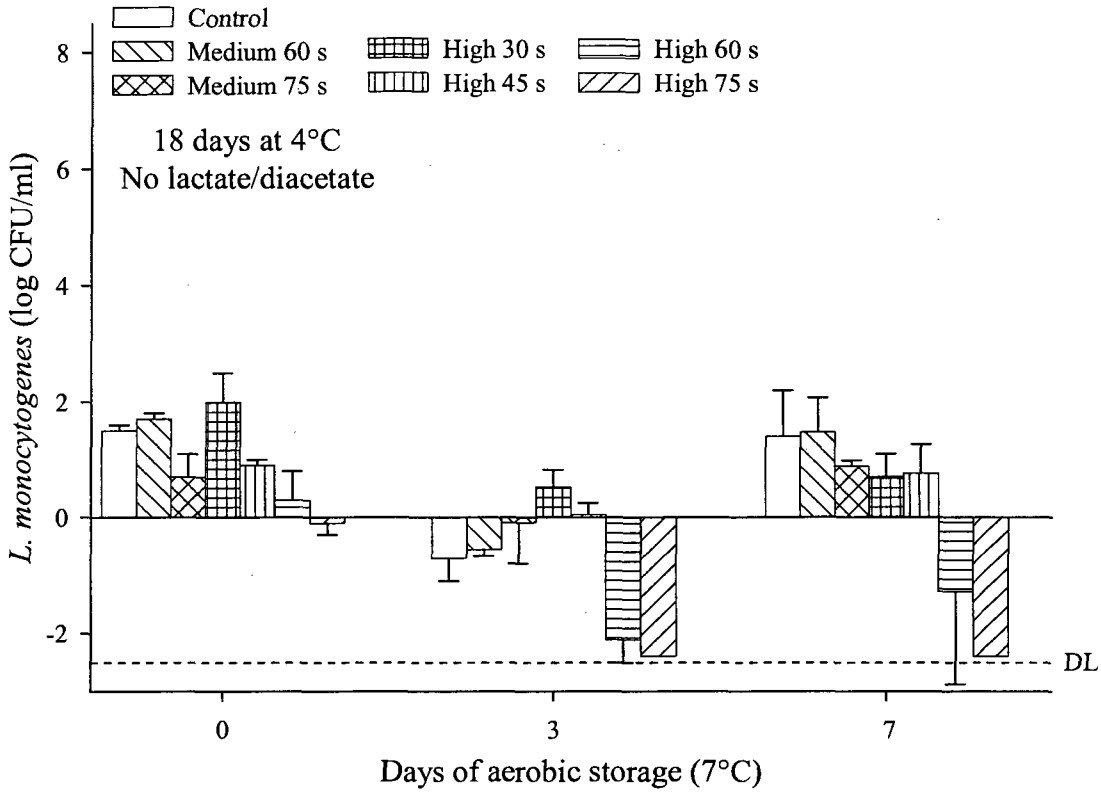


Figure 4.16 (Appendix Table 16). *Listeria monocytogenes* counts in water used to reheat frankfurters formulated without lactate/diacetate, after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

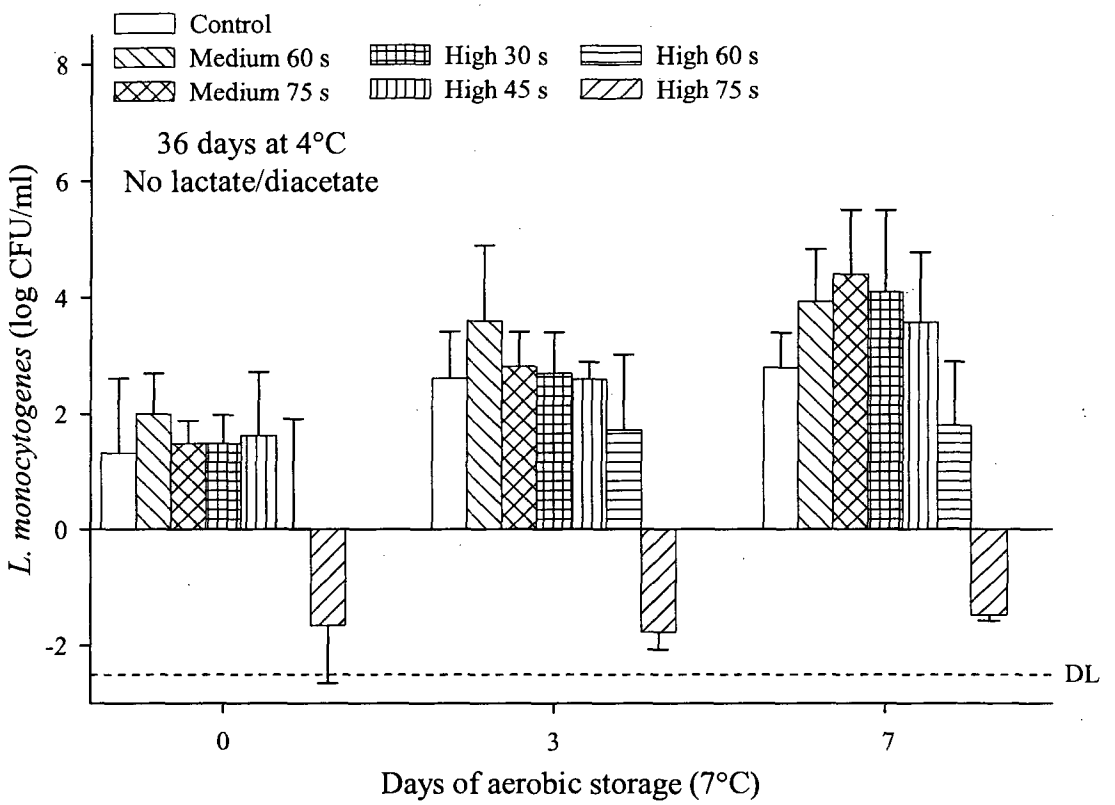


Figure 4.17 (Appendix Table 17). *Listeria monocytogenes* counts in water used to reheat frankfurters formulated without lactate/diacetate, after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

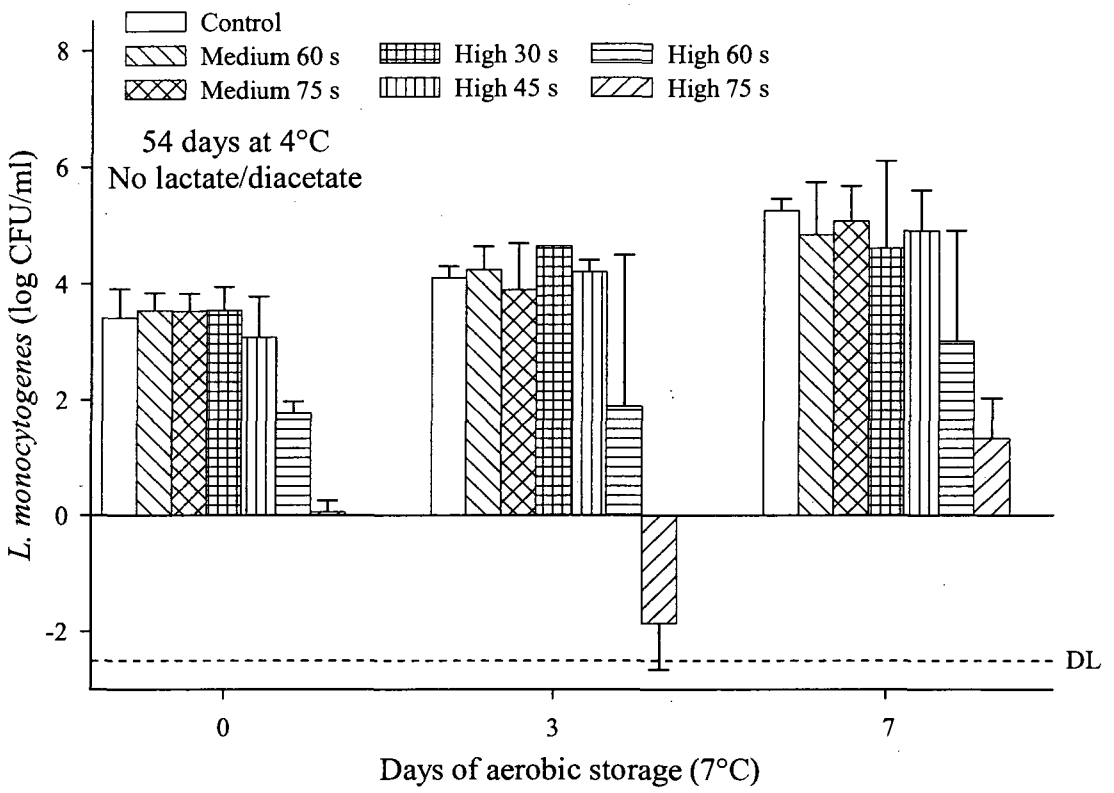


Figure 4.18 (Appendix Table 18). *Listeria monocytogenes* counts in water used to reheat frankfurters formulated without lactate/diacetate, after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

# CHAPTER V

## Use of hot water for inactivation of *Listeria monocytogenes* on frankfurters during storage


## ABSTRACT

Hot water may be used to kill potential contamination of *Listeria monocytogenes* on frankfurters immediately before consumption by sensitive individuals. However, studies are needed to provide data on the extent of heating necessary for product safety. This study evaluated the effectiveness of different time and water-temperature combinations to destroy *L. monocytogenes* contamination on frankfurters stored for different periods of time. A 10-strain composite of *L. monocytogenes* was inoculated (1.7 log CFU/cm$^2$) onto frankfurters formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD), and then vacuum-packaged and stored at 4°C to simulate manufacturer/retail storage conditions. On days 18, 40 and 60, packages were opened, reclosed and stored at 7°C, simulating aerobic, household conditions. At 0, 7 and 14 days of aerobic storage, frankfurters were exposed to hot water (80 or 94°C) that was either maintained at constant temperature (for 0, 30 60, 120 or 300 s) or removed from the heat (for 180, 300 or 420 s). Frankfurter samples, and the heating water in which they were immersed, were analyzed for total microbial counts and *L. monocytogenes* populations.

The 80°C (60, 120 s) and 94°C (30, 60 s) treatments reduced pathogen counts on frankfurters with PL/SD to at/below the detection limit (< -0.4 log CFU/cm$^2$) from initial levels of 0.6-0.9 log CFU/cm$^2$ on control samples (frankfurters immersed in 25°C water for 300 s). For frankfurters without PL/SD, where pathogen numbers on the control reached 5.3 log CFU/cm$^2$ on 60-day old vacuum-packaged product stored aerobically for 7 days, hot water treatments reduced counts by 0.3 (30 s/80°C) to > 5.7 (300 s/94°C) log CFU/cm$^2$. No survivors were detected in the heated water after any treatment (detection limit < -2.5 log CFU/ml). Frankfurters formulated with antimicrobials that limit the level of contamination to < 0.9 log CFU/cm$^2$ should be reheated in hot water at 80 or 94°C for at least 120 or 30 s, respectively, to reduce the pathogen counts to below the detection level. When frankfurters have allowed growth of *L. monocytogenes* to > 2.4 log CFU/cm$^2$, due to prolonged storage and/or absence of antimicrobials in the formulation, reheating in hot water should be performed at 94°C for 30 to 300 s.

## INTRODUCTION

*Listeria monocytogenes* is the causative agent of listeriosis, a disease that produces an estimated 2,500 cases in the U.S. every year (99% of them foodborne), with a hospitalization rate of 92% and a case-fatality rate of 20% (Mead *et al.,* 1999). It mostly affects susceptible individuals such as pregnant women and their fetuses, the elderly and the immunocompromised (Swaminathan and Gerner-Smidt, 2007). *L. monocytogenes* is a ubiquitous organism that can be found in different foods such as salads, cheeses and ready-to eat (RTE) meat and poultry products (Gombas *et al.,* 2003; Wallace *et al.,* 2003; Swaminathan and Gerner-Smidt, 2007). In the case of RTE meat and poultry products, cross-contamination and/or recontamination with *L. monocytogenes* usually occurs after

the product has undergone the lethality (i.e., cooking) treatment (Tompkin, 2002; Reij and Aantrekker, 2004), for example, during slicing of deli meats or peeling of frankfurters (Wenger *et al.,* 1990; Wang and Muriana, 1994; Wallace *et al.,* 2003). Frankfurters, among other RTE meat products, can support growth of the pathogen to high numbers and, according to the 2003 *L. monocytogenes* risk assessment (USDHHS-FDA-CFSAN/USDA-FSIS, 2003), non-reheated frankfurters are considered high risk, both on a per serving and per annum basis. Therefore, without further treatment before consumption, frankfurters contaminated with this pathogen represent a risk for consumers, especially to those with a compromised immune system.

The consumer's role in food safety is important since they are responsible for the last treatments (i.e., cooking and/or reheating) of food products immediately before consumption (Smith *et al.,* 2008). A survey by Porto *et al.* (2004) reported that 72% of people reheat frankfurters before eating, and 33% preferred boiling over other methods (such as grilling, microwaving and frying). There are not uniform reheating instructions on frankfurters labels. Only a few brands provide consumers with reheating directions, but no information is available on the effectiveness of such recommendations relative to the inactivation of *L. monocytogenes*. Appropriate label reheating instructions for this type of product are especially important for the population groups which are at higher risk for foodborne listeriosis infection, such as the elderly, pregnant women and other immunocompromised individuals (Painter and Slutsker, 2007). This study evaluated the efficacy of combinations of time and water-temperature for destruction of *L. monocytogenes* contamination on frankfurters formulated with or without potassium

lactate and sodium diacetate, during storage under simulated manufacturer/retail and household conditions.

## MATERIALS AND METHODS

**Preparation of frankfurters.** Frankfurter emulsions were formulated with or without 1.5% potassium lactate (PL, Purac Purasal® HiPure P, Lincolnshire, IL, USA) and 0.1% sodium diacetate (SD, Niacet Corporation, Niagara Falls, NY, USA) as antimicrobials. The meat mixture consisted of 40% beef (beef chuck, 76-78% lean) and 60% pork (pork shoulder, 70-72% lean). Spices and salts (sodium chloride, dextrose, dry mustard, corn syrup solids, polyphosphate, sodium nitrate, sodium erythorbate, paprika, onion powder, garlic powder, coriander and white pepper) were added according to the formulation of Samelis *et al.* (2002). After emulsification in a vacuum bowl chopper (RMF, Kansas City, MO, USA), the batter was stuffed into cellulose casings, linked at approximately 9 cm lengths, cooked and cooled (4°C) overnight, as described by Byelashov *et al.* (2008). Frankfurters were then manually peeled and moved to the microbiology laboratory for inoculation, packaging, storage, treatment and testing.

**Preparation of inoculum and inoculation of frankfurters.** The inoculum consisted of a mixture of 10 *L. monocytogenes* strains, including 558 (serotype 1/2, pork meat isolate), NA-1 (serotype 3b, pork sausage isolate), N-7150 (serotype 3a, meat isolate), N1-225 and N1-227 (serotype 4b, clinical and food isolates, respectively, associated with the same outbreak), R2-500 and R2-501 (serotype 4b, food and clinical isolates, respectively, associated with the same outbreak), R2-763, R2-764, and R2-765 (serotype 4b, clinical, food and environmental isolates, respectively, associated with the same outbreak). Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764, and R2-765

(Fugett *et al.,* 2006) were kindly provided by Dr. M. Wiedmann (Cornell University, Ithaca, NY, USA). Each strain was individually grown overnight at 37°C in tryptic soy broth (Difco, Becton Dickinson, Sparks, MD, USA) supplemented with 0.6% yeast extract (Acumedia, Lansing, MI, USA). Cells were harvested and habituated in autoclave-sterilized frankfurter extract (10%, w/v) for 72 h at 7°C as described by Lianou *et al.* (2007). After habituation, the 10 strains were mixed, serially diluted in frankfurter extract, and 0.2 ml of the diluted mixture was used to inoculate the surface of each frankfurter, using a sterile glass spreader (Byelashov *et al.* 2008). Inoculated frankfurters were then placed at 4°C for 15 min to allow for cell attachment. Six frankfurters were placed in zip-top vacuum bags (Zip Vak 15.2x20.3 cm, nylon/EVA copolymer, Winpak, Winnipeg, MB, Canada), and were vacuum-packaged (LVII Super, Hollymatic Corp., Countryside, IL, USA) and stored at 4°C for up to 60 days (simulating manufacturing and/or retail storage conditions). On days 18, 40 and 60, the zip-lock of bags was opened to release the vacuum-seal and then reclosed and stored at 7°C for up to 7 days (simulating aerobic, home storage conditions).

**Hot water treatments.** For selection of the treatments (Figure 5.1), recommendations found on some commercial packages of frankfurters from certain manufacturers were considered. Such recommendations included "Boil in water for 5 min", "Place in boiling water, cover and remove from heat, let stand 5-7 min", and "Heat 2/3 cup of water in skillet, add franks, cover and simmer 7-9 min". Treatments in this study were applied by placing two frankfurters (approx. 28 g each) in a stainless steel bowl (22.5 cm diameter, 10 cm deep, 2.84 L capacity) containing sterile distilled water (350 ml) preheated to 80°C or 94°C on a hot plate (Corning Hot Plate Model PC-101,

Corning Incorporated, New York, NY, USA) (Figure 1). For the 80°C-treatments, the bowl containing the frankfurters and water was left on the heating source for 0, 30, 60, or 120 s. For the 94°C-treatments, the bowl containing the frankfurters and water was either left on the heating source (0, 30, 60, 120, or 300 s) or removed and left standing for 180, 300 or 420 s. An untreated control (dry control, no water treatment) and two ambient-temperature controls (two frankfurters submerged in 25°C water for 300 or 420 s) were also included (Figure 5.1).

**Microbiological analyses.** Immediately after each treatment, frankfurters were transferred to a Whirl-Pak® bag (15×23 cm, Nasco, Modesto, CA, USA) containing 50 ml of maximum recovery diluent (MRD; 0.85% NaCl and 0.1% peptone) and vertically shaken 30 times to release cells from the surface of the samples (Barmpalia *et al.,* 2004). The rinsate was serially diluted with buffered peptone water (0.1%, Difco) and plated onto PALCAM agar (Difco) and tryptic soy agar (Difco) supplemented with 0.6 % yeast extract (TSAYE) for enumeration of *L. monocytogenes* survivors and total microbial populations, respectively. PALCAM agar plates were incubated at 30°C for 48 h and TSAYE plates were incubated at 25±2°C for 72 h. The detection limit for the microbiological analysis of frankfurters was -0.4 log CFU/cm$^2$. Serial dilutions of the water used for heating were were prepared and plated on PALCAM agar for enumeration of possible *L. monocytogenes* survivors. The detection limit for the analysis of water samples was -2.4 log CFU/ml.

Frankfurters and water samples were maintained at 4°C after microbiological analysis (and pH measurements of product samples, described below) for possible enrichment in the event that no *L. monocytogenes* survivors would be recovered by direct

plating. In such cases, the U.S. Department of Agriculture-Food Safety and Inspection Service method (USDA-FSIS, 2008b) was followed with some modifications. In summary, 100 ml of University of Vermont broth (UVM, Difco) was added to each sample and incubated for 24±2 h at 30°C. After incubation, 1 ml of the UVM enrichment was transferred to 9 ml of Fraser broth (Difco) for secondary enrichment at 35°C. Fraser broth tubes were checked for darkening after 24 and 48 h of incubation. If no darkening appeared, the sample was recorded as negative for *L. monocytogenes* by enrichment. If darkening of the medium occurred, a loopful was streaked onto PALCAM agar plates and incubated at 30°C for 48±2 h. Samples with PALCAM agar plates having typical *Listeria* colonies were recorded as positive for the pathogen by enrichment.

**Physicochemical analyses.** After plating, frankfurter samples were homogenized (2 min; Masticator, IUL Instruments, Barcelona, Spain) and pH measurements were obtained from a 5 ml aliquot of the homogenate, using a Denver Instruments (Arvada, CO, USA) pH meter and glass electrode. Water activities ($a_w$) of the two frankfurter formulations (i.e., with or without PL/SD) were measured (AquaLab model series 3, Decagon Devices, Pullman, WA, USA) on day-0 of vacuum-packaged storage. Fat and moisture content analyses were conducted following the AOAC International methods 960.39 and 950.46B, respectively (AOAC, 1998).

**Statistical analysis.** Two complete replications were conducted, in a randomized block design. For each replication, three samples received the same treatment on each sampling day. Data were analyzed with vacuum storage time, aerobic storage time, hot water treatments, and the interactions of vacuum storage time × hot water treatments and aerobic storage time × hot water treatments as independent variables, using the Glimmix

Procedure of SAS/STAT® (SAS Institute, 2007). Least-squares means were calculated, and mean separation was performed with Tukey's method, using a level of significance of 0.05.

## RESULTS AND DISCUSSION

**Physicochemical properties of frankfurters.** Values of $a_w$, fat and moisture content were similar between frankfurters with and without PL/SD. Fat content was 15.37±0.97% and 15.43±0.5% for product with and without PL/SD, respectively. As expected, $a_w$ and moisture content were slightly lower in the product formulated with PL/SD (0.964±0.005 and 59.22±0.59, respectively) as compared to product without PL/SD (0.970±0.008 and 61.09±0.51, respectively). The pH values of frankfurters with and without PL/SD on the day of inoculation were 5.92±0.07and 5.93±0.10, respectively. As expected, there was no effect ($P \geq 0.05$) of hot water treatments on pH values of the product (Table 5.1). For frankfurters with PL/SD, pH remained constant ($P \geq 0.05$) through storage. However, there was an effect of storage time (both in vacuum and aerobic packages) on the pH of frankfurters without PL/SD, most likely due to growth of *L. monocytogenes* and other background flora to high levels in these products (Figures 5.2 to 5.7). For this formulation, 60-day old vacuum-packaged samples had a lower ($P < 0.05$) pH than corresponding samples stored for 18 days. In general, during each aerobic storage cycle, the pH of 0- and 7-day samples were not different ($P \geq 0.05$), but decreased ($P < 0.05$) on samples stored for 14 days.

**Effect of storage time on *L. monocytogenes* counts on frankfurters.** A dry control was used to evaluate changes in pathogen counts during storage. On day-0 (inoculation day), counts on frankfurters with and without PL/SD in the formulation were

1.8±0.0 and 1.7±0.1 log CFU/cm$^2$, respectively. During vacuum storage (4°C), these initial numbers remained unchanged ($P \geq 0.05$) for up to 18 days on frankfurters without PL/SD in the formulation, and then increased to 2.7±1.5 and 4.5±2.1 log CFU/cm$^2$ after 40 and 60 days, respectively (Figure 5.2 to 5.4). Once the packages were opened and stored at 7°C, *L. monocytogenes* counts increased by 0.6 to 1.6 log CFU/cm$^2$ for every 7 days of storage, (Figures 5.2 to 5.4). Total microbial counts also increased during storage, and were comparable to those of *L. monocytogenes* (Figures 5.5 to 5.7). Growth of *L. monocytogenes* was inhibited during vacuum-packaged storage of frankfurters containing PL/SD (Figures 5.8 to 5.10). Inhibition of growth also was observed during aerobic storage, with final numbers of 1.2±0.2 log CFU/cm$^2$ after 14 days aerobic storage following 60 days of vacuum storage (Figures 5.8 to 5.10); total microbial counts also were inhibited (Figures 5.11 to 5.13). These results highlight the importance of including antimicrobials in the formulation of frankfurters that inhibit growth of *L. monocytogenes* during refrigerated storage (Bedie *et al.,* 2001; Samelis *et al.,* 2002; Barmpalia *et al.,* 2004; Geornaras *et al.*, 2006) since it has been reported that consumers may store this type of product for periods of time exceeding recommendations (Cates *et al.,* 2006), a practice that may allow for growth of *L. monocytogenes* to high numbers in the absence of inhibitors.

**Effect of hot water treatments on *L. monocytogenes* counts on frankfurters.** In order to more accurately determine the effect of hot water treatments on *L. monocytogenes,* the rinsing effect of the water in which samples were immersed was taken into consideration by the inclusion of two ambient controls: two frankfurters immersed in water at 25°C for 300 or 420 s. There was no difference ($P \geq 0.05$) between

the counts found on frankfurters after these two control treatments; therefore, the results and discussion presented in the following sections are based on the ambient control immersed in water for 300 s, and referred to as control, which is common for both formulations (with and without PL/SD).

The effectiveness of hot water treatments at constant temperature (80 or 94°C) on frankfurters formulated without PL/SD, as expected, was influenced by initial counts on frankfurters, which depended on storage conditions (vacuum vs. aerobic; 4°C vs. 7°C) and age of the product. Longer storage times allowed for an increase in *L. monocytogenes* counts up to 5.3±2.7 log CFU/cm$^2$ on the control (ambient control 300 s, Figures 5.2 to 5.4). Naturally, these high numbers required longer times and/or higher temperatures to be reduced to below the detection limit (< -0.4 log CFU/cm$^2$). Initial counts on the control of less than 3 log CFU/cm$^2$ were reduced to below the detection limit when treated for 120 s at 80°C or ≥ 60 s at 94°C. As counts on the control increased to 3-4 log CFU/cm$^2$, no treatment at 80°C was effective in reducing counts to below the detection limit, and the most effective treatments were ≥ 120 s at 94°C with reductions of ≥ 4.2 log CFU/cm$^2$. The only treatment at constant temperature that reduced initial counts of > 4 log CFU/cm$^2$ to below the detection limit was 300 s at 94°C, but the pathogen still was detected by enrichment of some samples. Treatments that involved removal of frankfurters from the heating source (180, 300 and 420 s) consistently rendered the product with counts below the detection limit, regardless of initial levels, and accounted for reductions of up to ≥ 5.7 log CFU/cm$^2$. However, some samples were positive by enrichment.

At the water temperature of 94°C, reductions achieved after 300 s of treatment were similar when the temperature was kept constant and when bowls were removed from the heating source. Treatments associated with manufacturers' recommendations (boiling for 5 min, and placing frankfurters in boiling water, removed from heat and let stand for 5-7 min) (Figures 5.2 to 5.4) were effective in reducing *L. monocytogenes* initial counts to below the detection limit, with reductions of up to 5.7 log CFU/cm$^2$ on frankfurters without PL/SD. However, the pathogen was detected in some frankfurter samples by enrichment, indicating that these directions for reheating may potentially allow for survival of small numbers of the pathogen on product formulated without PL/SD that is stored under conditions that permit growth of the pathogen to high levels (> 5.3 log CFU/cm$^2$). Treatments of ≥ 60 s at 80°C and ≥ 30 s at 94°C applied to frankfurters formulated with PL/SD consistently reduced initial counts of the pathogen (0.6±0.7 to 0.9±0.7 log CFU/cm$^2$) to at/below the detection limit (but sometimes detectable by enrichment), regardless of storage time.

***L. monocytogenes* survivors in water.** *L. monocytogenes* was detected (-0.7±1.7 to 5.2±1.4 log CFU/ml) in the water used for ambient (25°C) water control treatments (Figures 5.14 to 5.16), indicating that cells were transferred from the frankfurters into the water. However, no survivors were found remaining, by direct plating or enrichment, in any of the heated water samples, regardless of frankfurter formulation. Therefore, it is important to devise treatments that destroy *L. monocytogenes* not only on frankfurters, but also in the water used for reheating, to avoid cross-contamination of the environment and other foods via the water (Wagner *et al.*, 2007).

## CONCLUSIONS

Under the conditions of this study, results showed that *L. monocytogenes* contamination levels of $\leq 2$ log CFU/cm$^2$ on frankfurters can be reduced to below detection ($< -0.4$ log CFU/cm$^2$) with short exposure to hot water (at least 60 s at 94°C). However, when pathogen numbers on frankfurters increased to above 4 log CFU/cm$^2$ due to storage conditions, longer times (at least 300 s) were needed. Treatments based on manufacturers' recommendations tested in this study (boiling for 5 min, and placing frankfurters in boiling water, remove from heat and let stand for 5-7 min) allowed for survival of *L. monocytogenes* detectable only by enrichment, even with initial numbers up to 5.3 log CFU/cm$^2$. Boiling renders water used for frankfurter reheating (at either 80 or 94°C) safe for discarding without risk for cross-contamination of other kitchen surfaces with *L. monocytogenes*.

It has been suggested that food labels are an important tool for providing consumers with critical information (Brandt, *et al.*, 2003), such as reheating instructions and safe handling of the product. However, in order to provide consumers with reliable directions, cooking and reheating instructions on labels should be validated and based on scientific data. The data provided here may be useful to the industry in the development of science-based recommendations for reheating of frankfurters by consumers in their homes.

Table 5.1. Mean±standard deviation pH values of frankfurters stored in vacuum-sealed (4°C, up to 60 days) and opened (aerobic; 7°C, up to 14 days) packages.

| Storage time | | Frankfurter formulation | |
|---|---|---|---|
| Vacuum (4°C) | Aerobic (7°C) | With PL/SD | Without PL/SD |
| 18 | 0 | 5.94±0.13[a] | 6.09±0.16[a] |
| | 7 | 5.97±0.11[a] | 6.01±0.08[ab] |
| | 14 | 5.96±0.11[a] | 5.98±0.19[bc] |
| 40 | 0 | 5.97±0.10[a] | 6.02±0.20[ab] |
| | 7 | 5.97±0.12[a] | 5.99±0.23[bc] |
| | 14 | 5.94±0.20[a] | 5.88±0.33[d] |
| 60 | 0 | 6.00±0.12[a] | 5.96±0.38[bc] |
| | 7 | 5.97±0.13[a] | 5.91±0.36[dc] |
| | 14 | 5.94±0.22[a] | 5.70±0.39[e] |

[abc] Means with the same superscript within a column are not significantly different, $P \geq 0.05$

PL/SD: Potassium Lactate and Sodium Diacetate

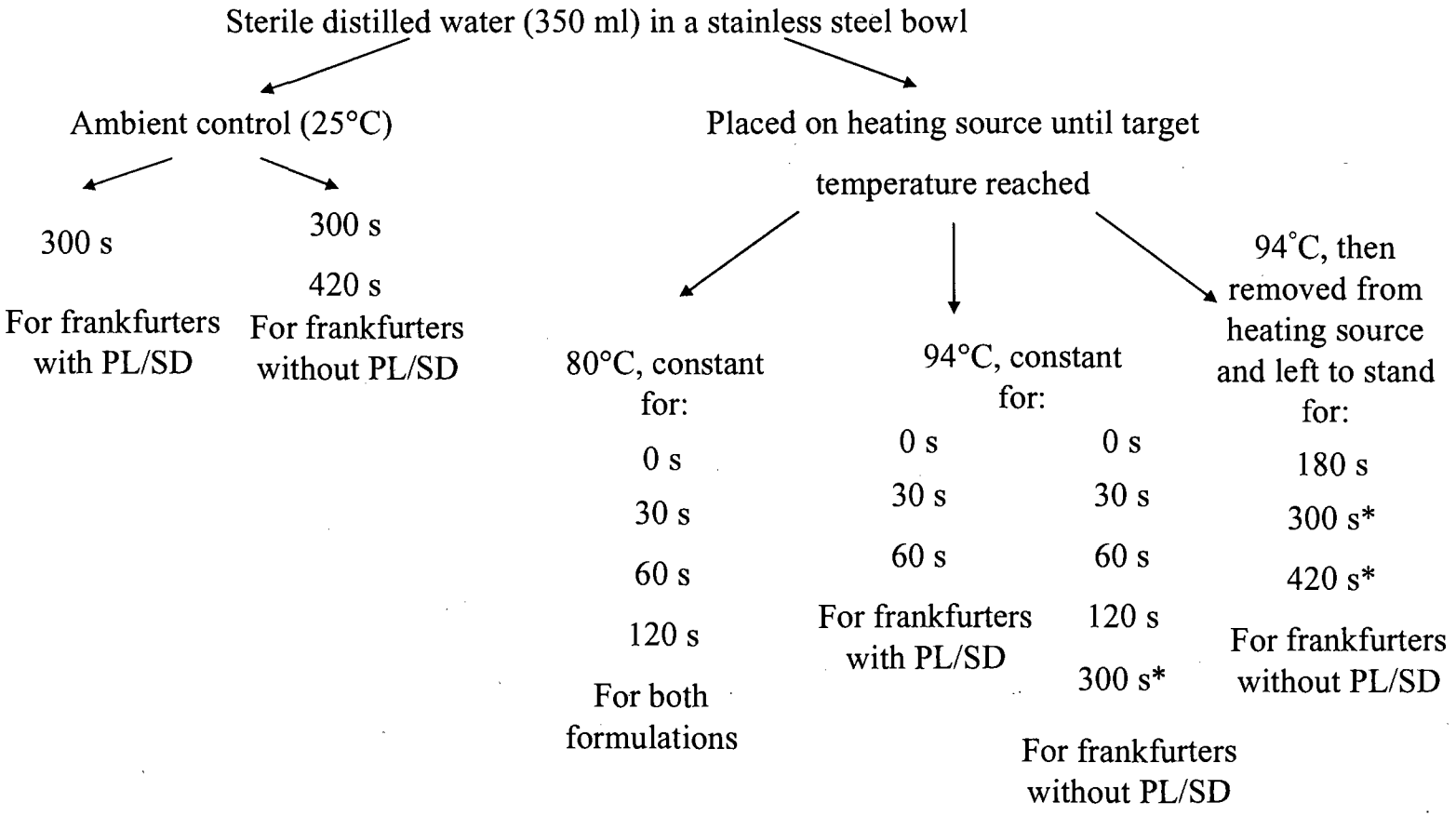


Figure 5.1. Hot water treatments applied to frankfurters formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD) for inactivation of *Listeria monocytogenes* before consumption.
* Treatments according to actual recommendations found on commercial packages of frankfurters from certain manufacturers.
PL/SD: Potassium Lactate and Sodium Diacetate.

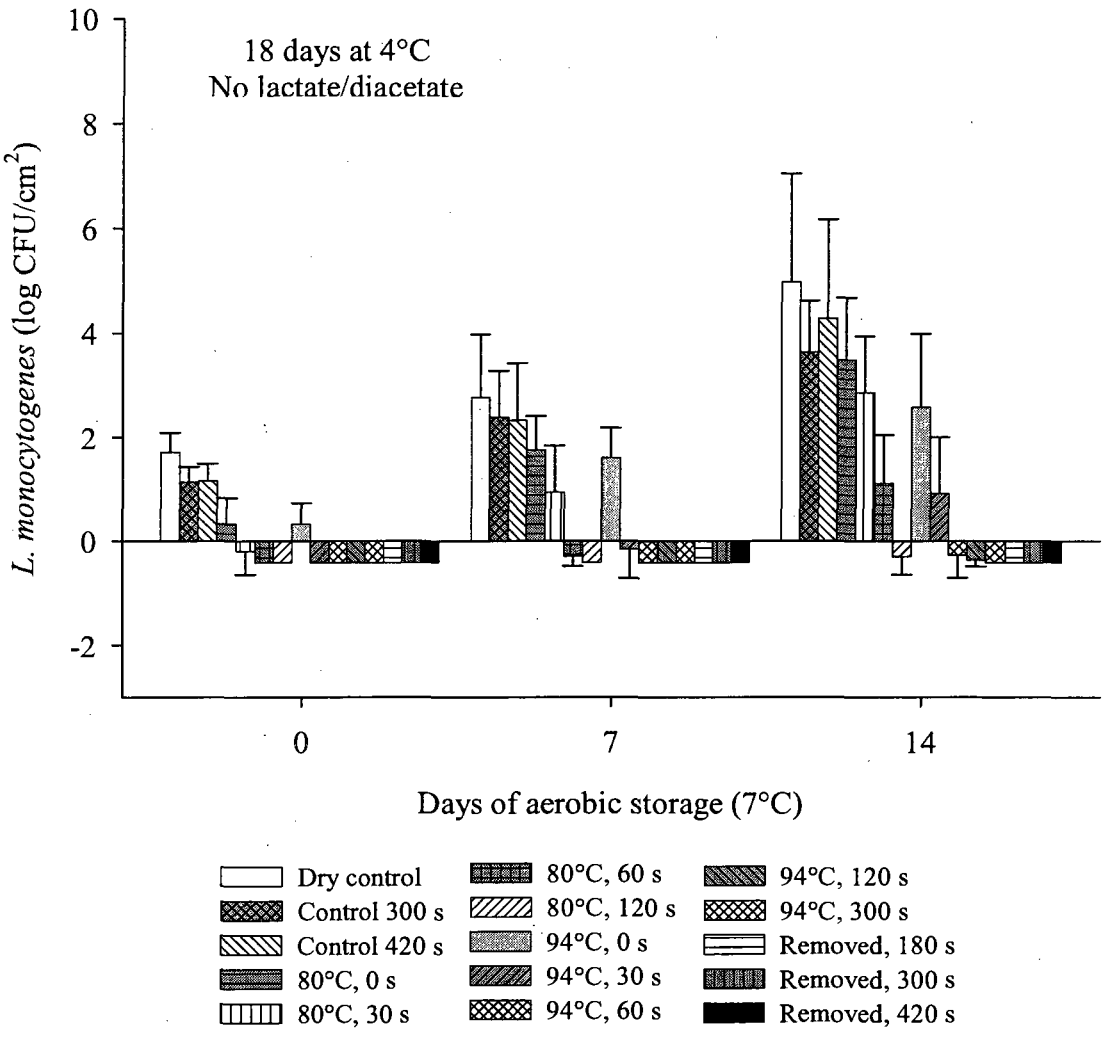


Figure 5.2 (Appendix Table 19). *Listeria monocytogenes* counts on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 18 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

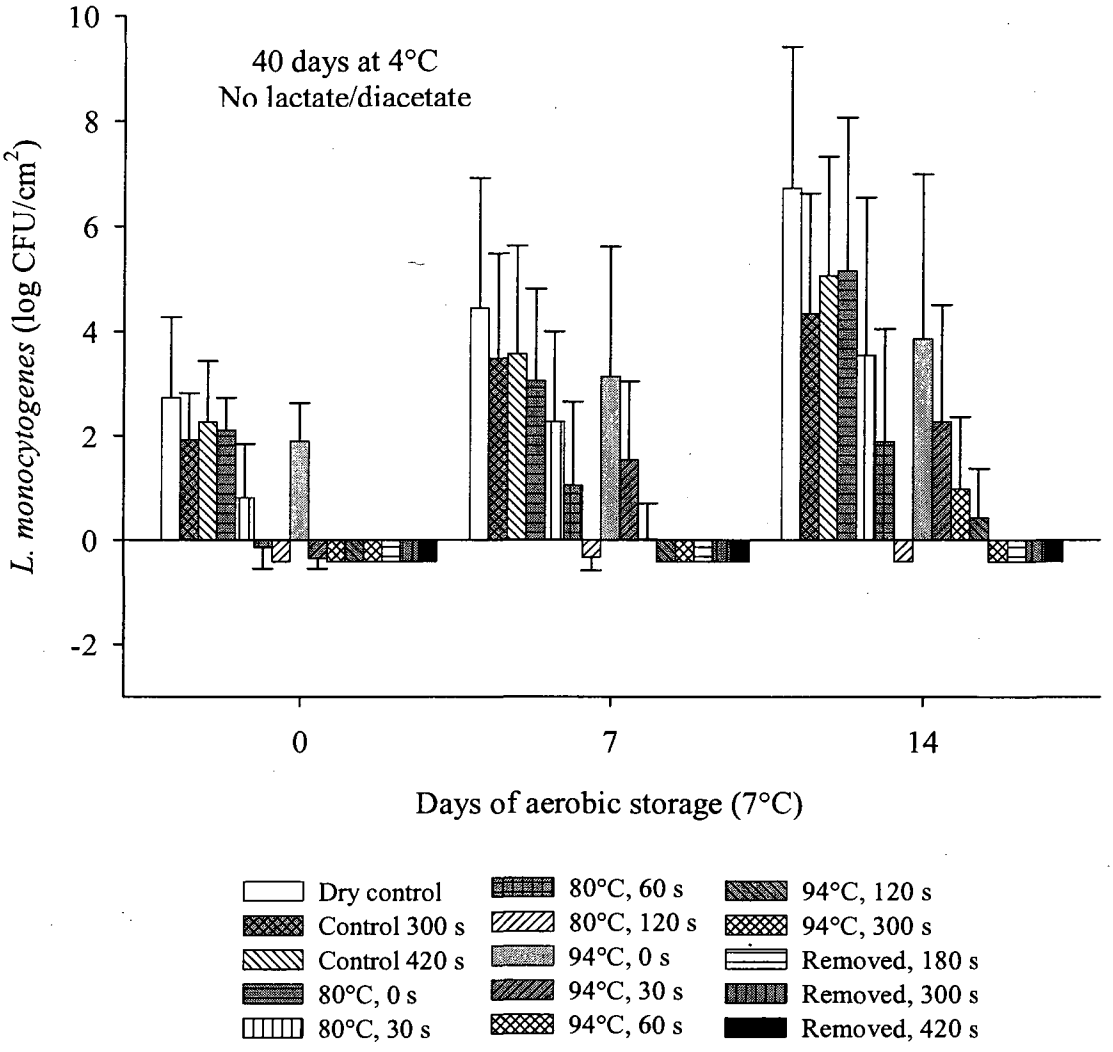


Figure 5.3 (Appendix Table 20). *Listeria monocytogenes* counts on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 40 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

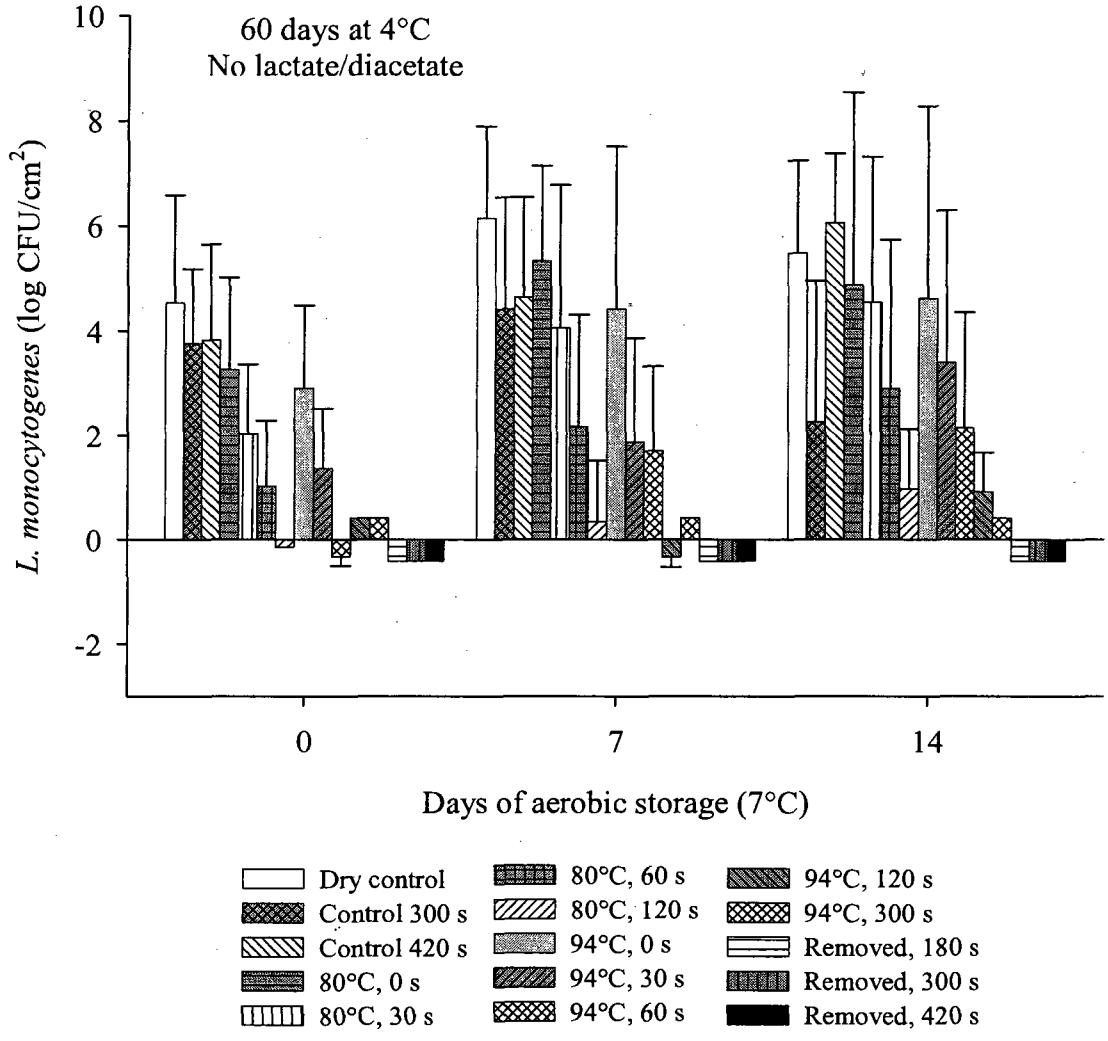


Figure 5.4 (Appendix Table 21). *Listeria monocytogenes* counts on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 60 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

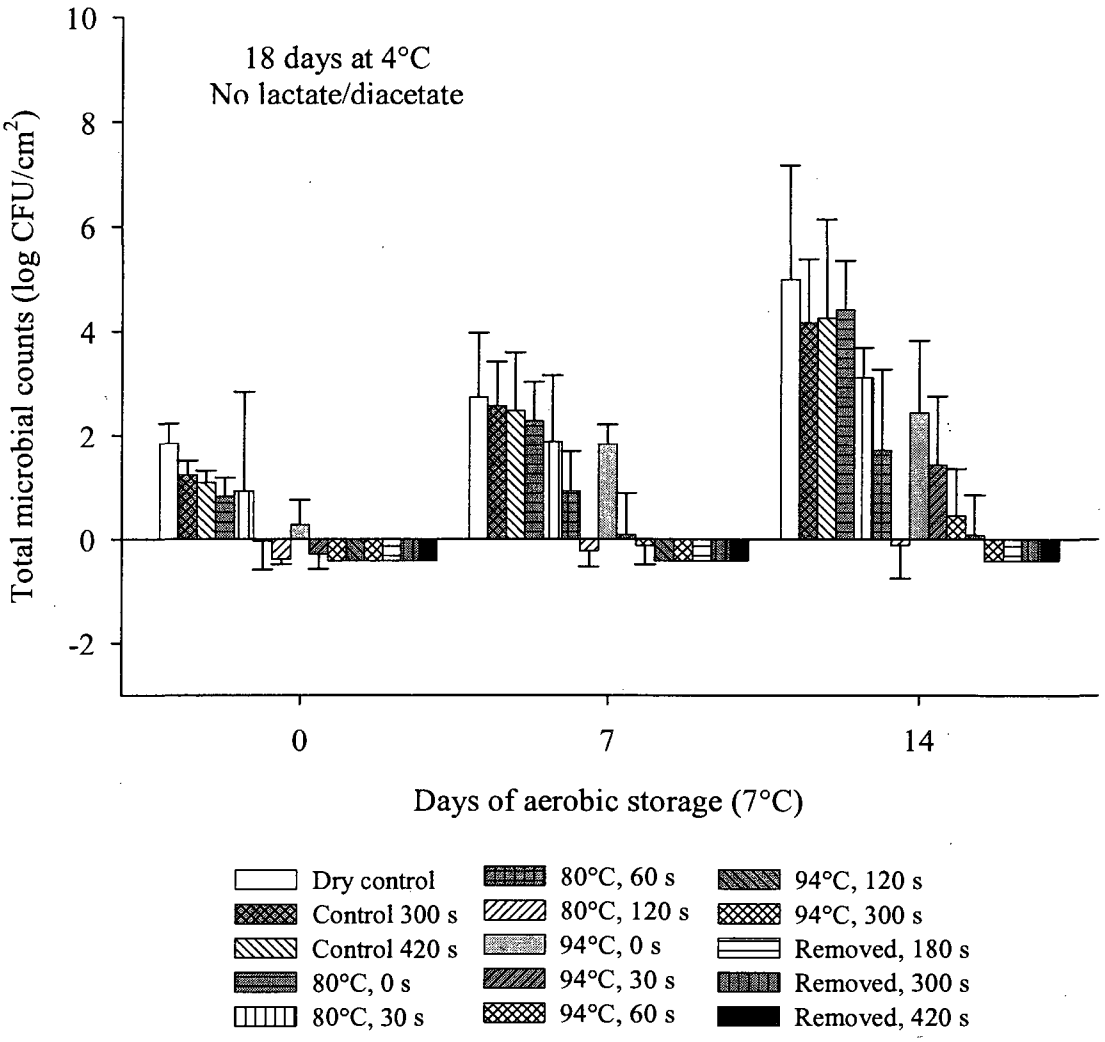


Figure 5.5 (Appendix Table 22). Total microbial counts on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 18 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

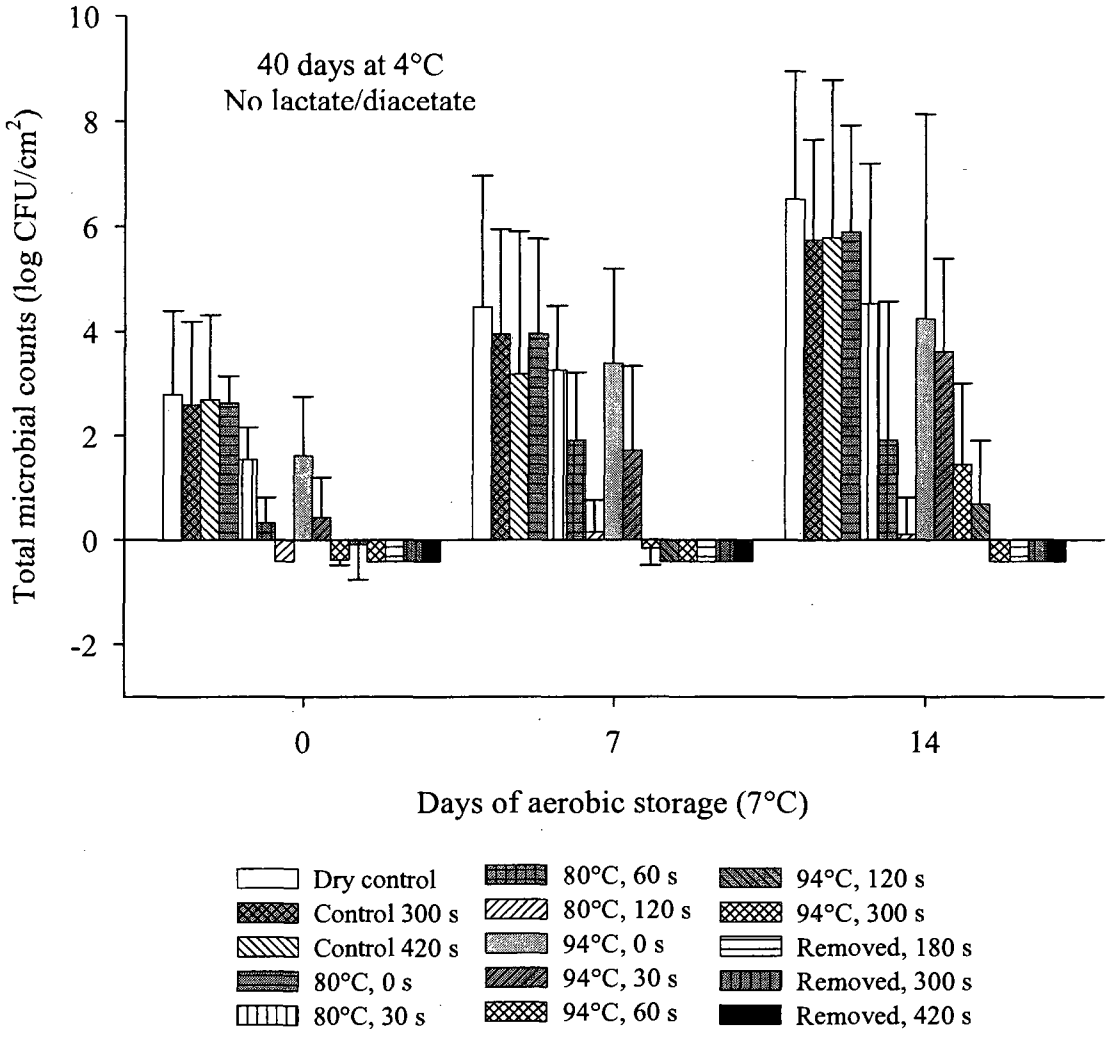


Figure 5.6 (Appendix Table 23). Total microbial counts on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 40 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

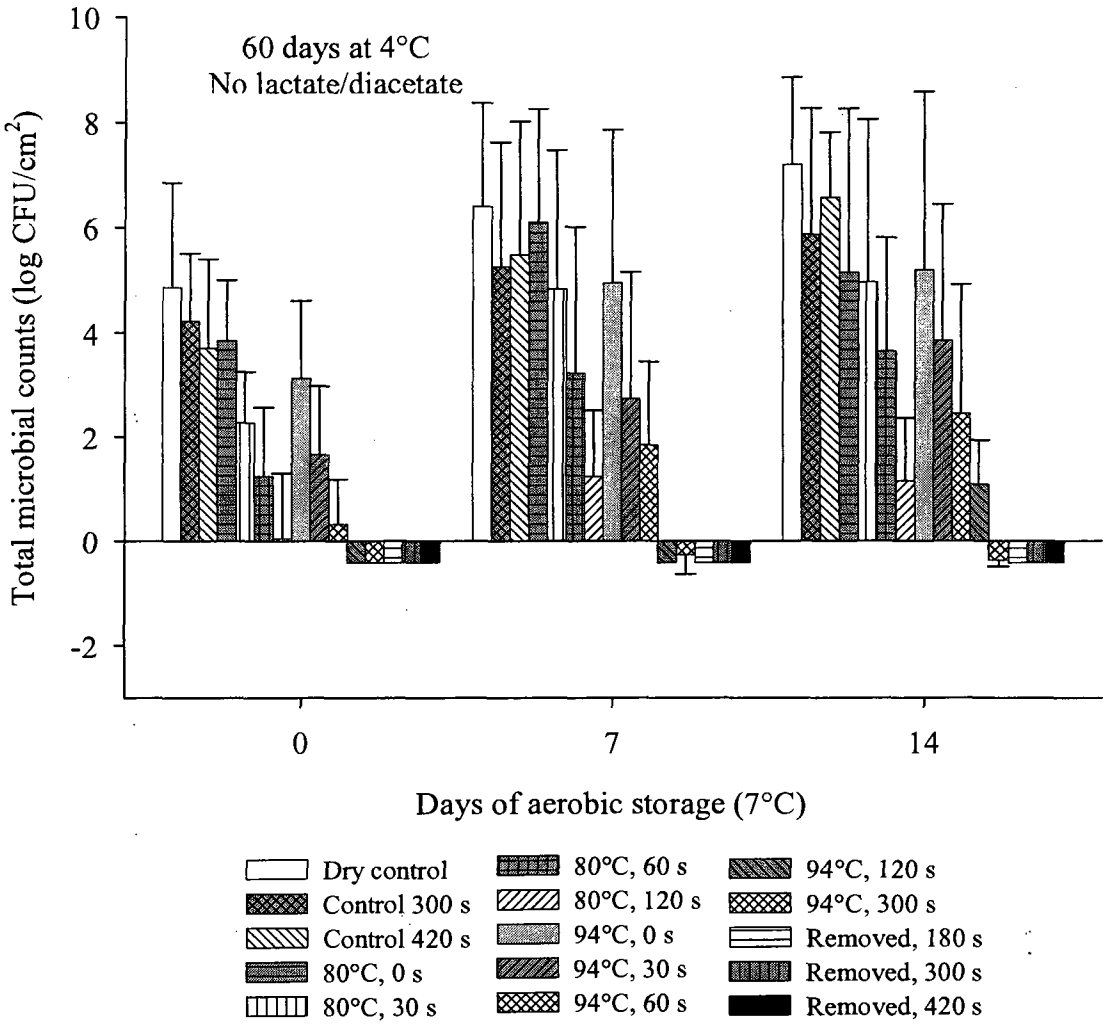


Figure 5.7 (Appendix Table 24). Total microbial counts on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 60 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

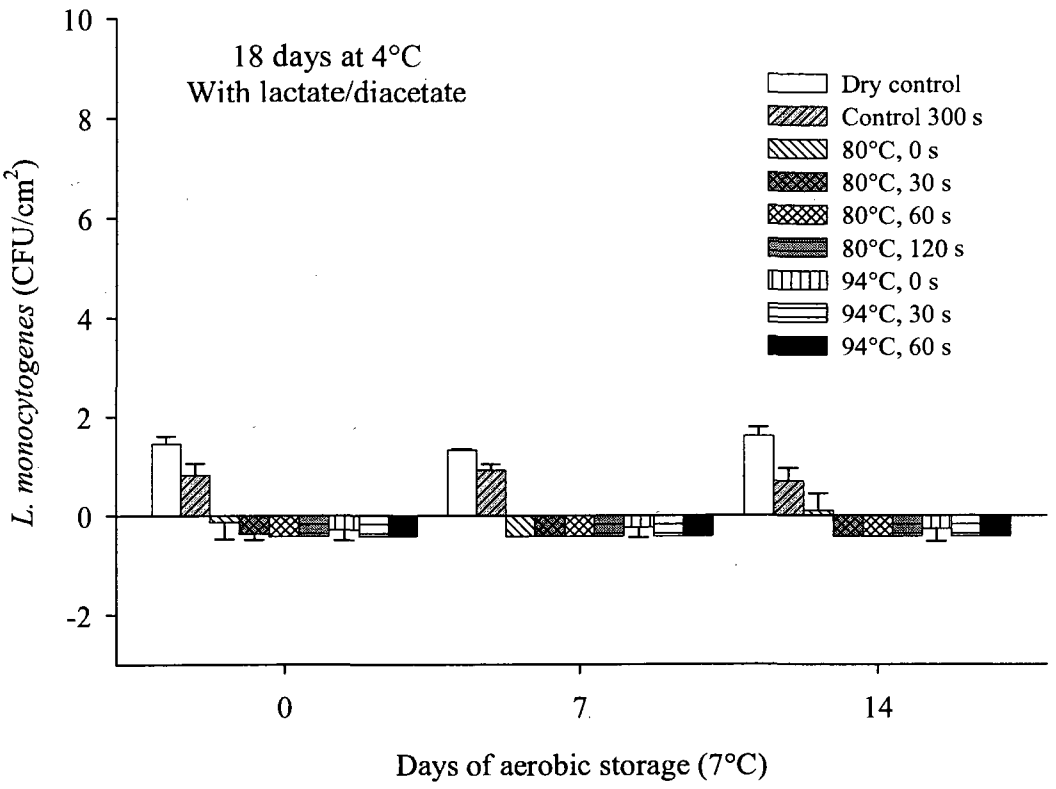


Figure 5.8 (Appendix Table 25). *Listeria monocytogenes* counts on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 18 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

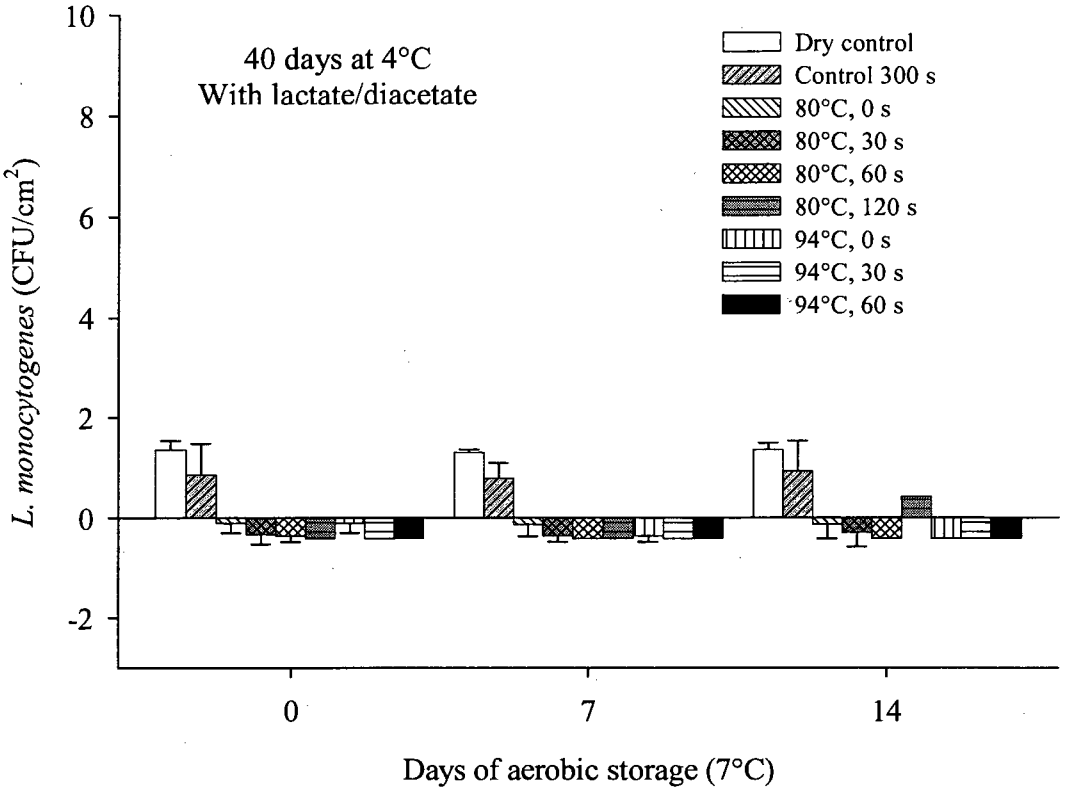


Figure 5.9 (Appendix Table 26). *Listeria monocytogenes* counts on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 40 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

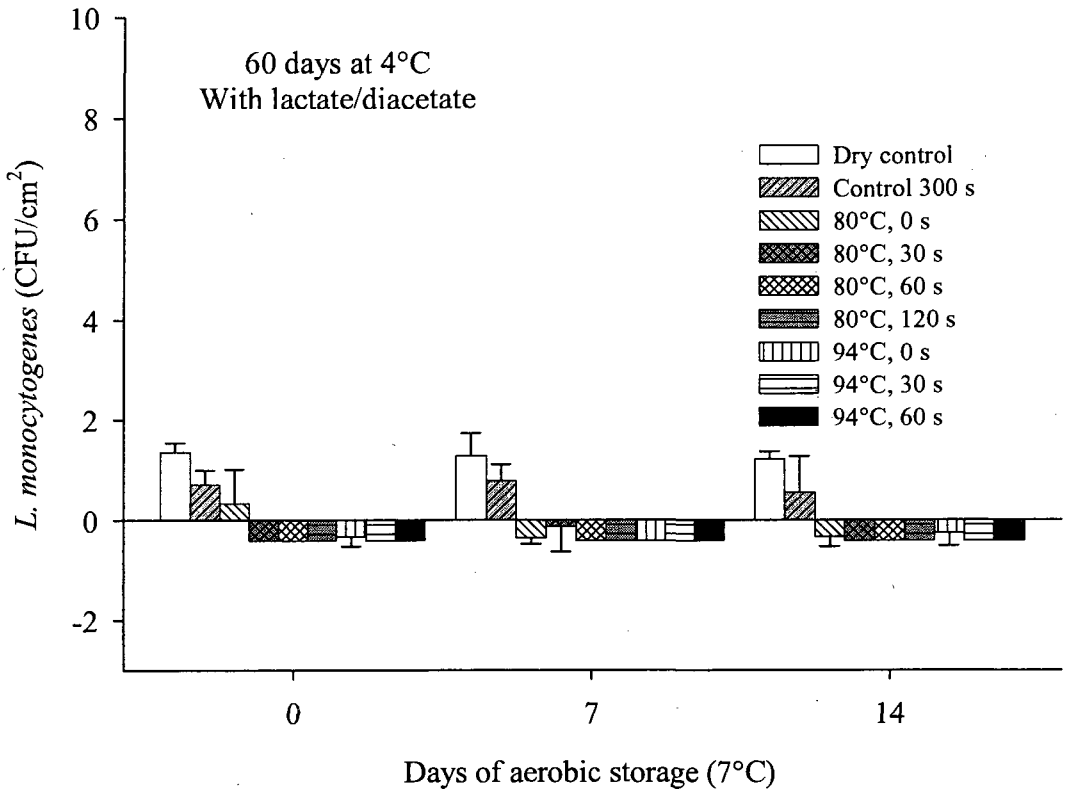


Figure 5.10 (Appendix Table 27). *Listeria monocytogenes* counts on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 60 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

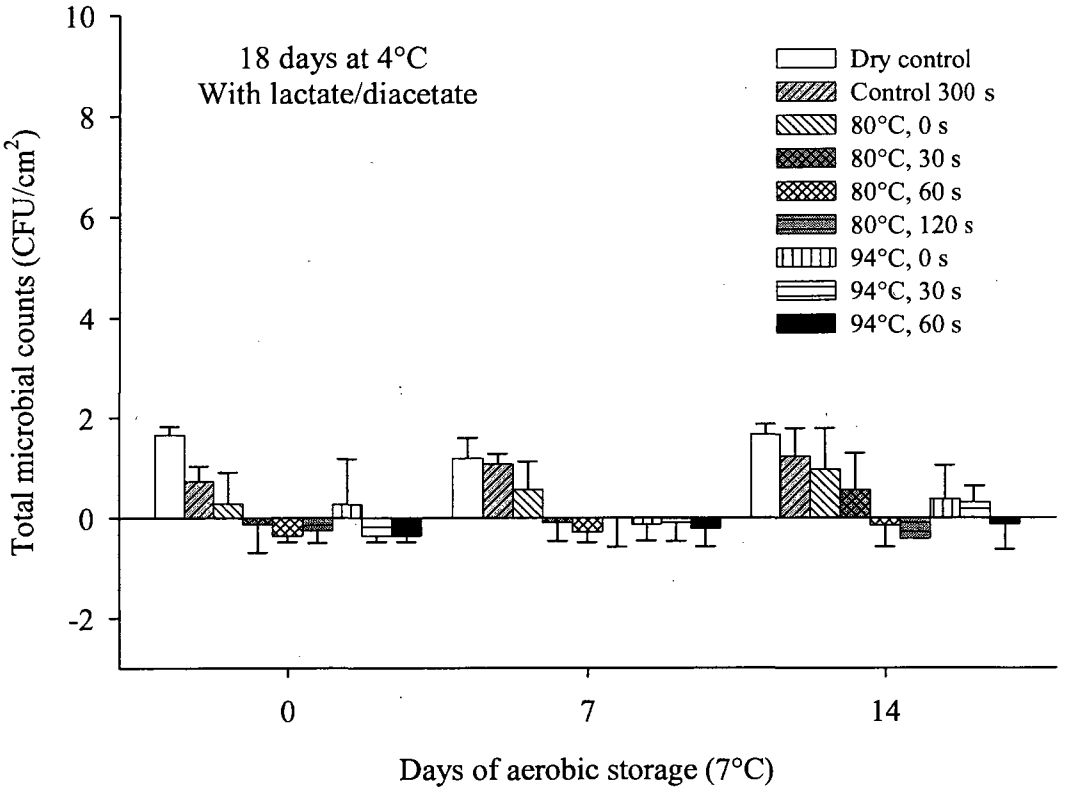


Figure 5.11 (Appendix Table 28). Total microbial counts on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 18 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

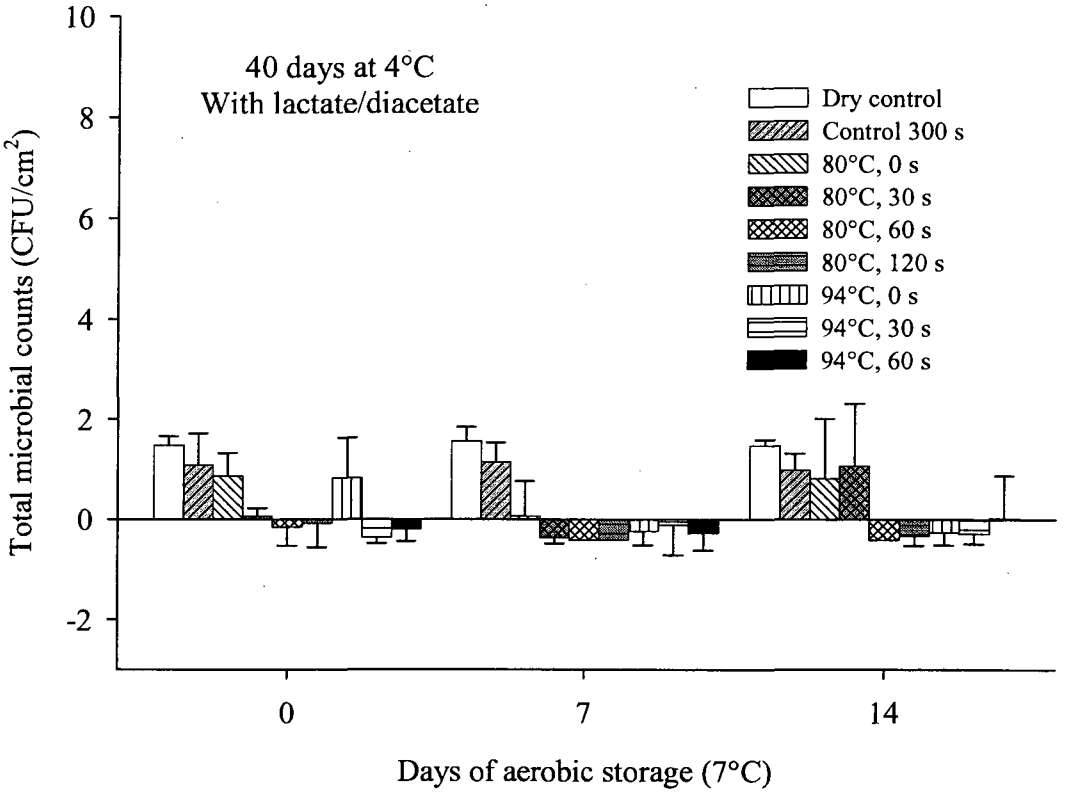


Figure 5.12 (Appendix Table 29). Total microbial counts on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 40 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

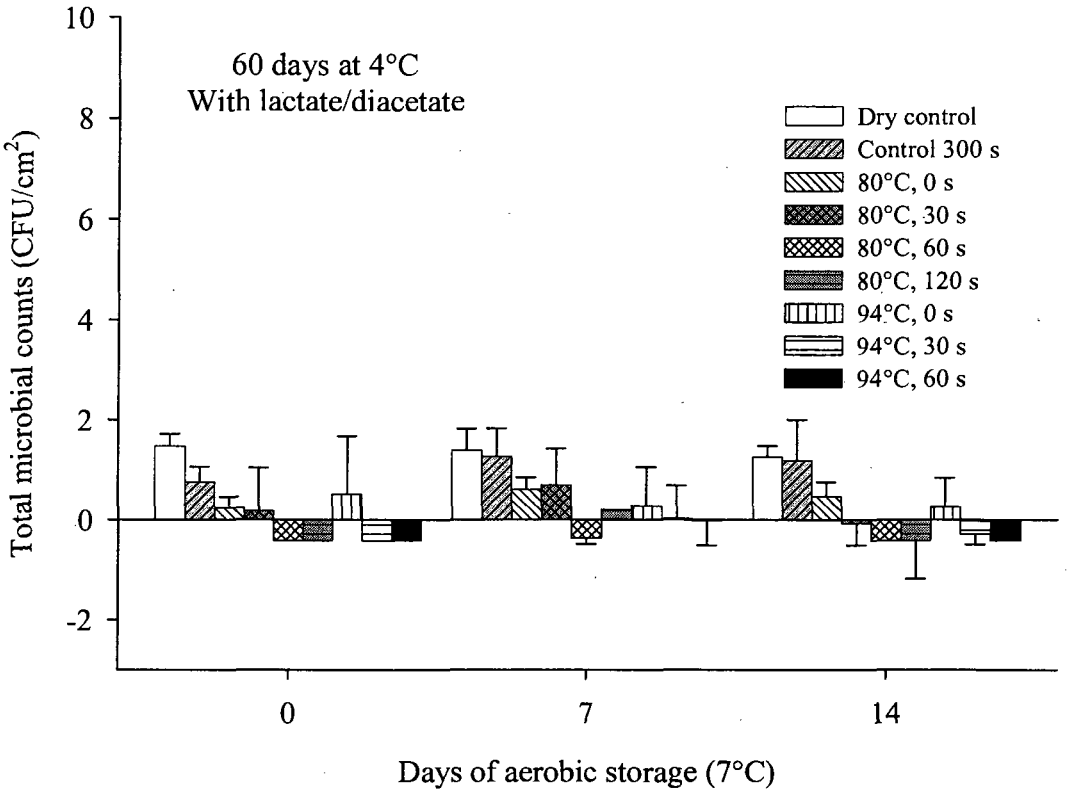


Figure 5.13 (Appendix Table 30). Total microbial counts on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 60 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

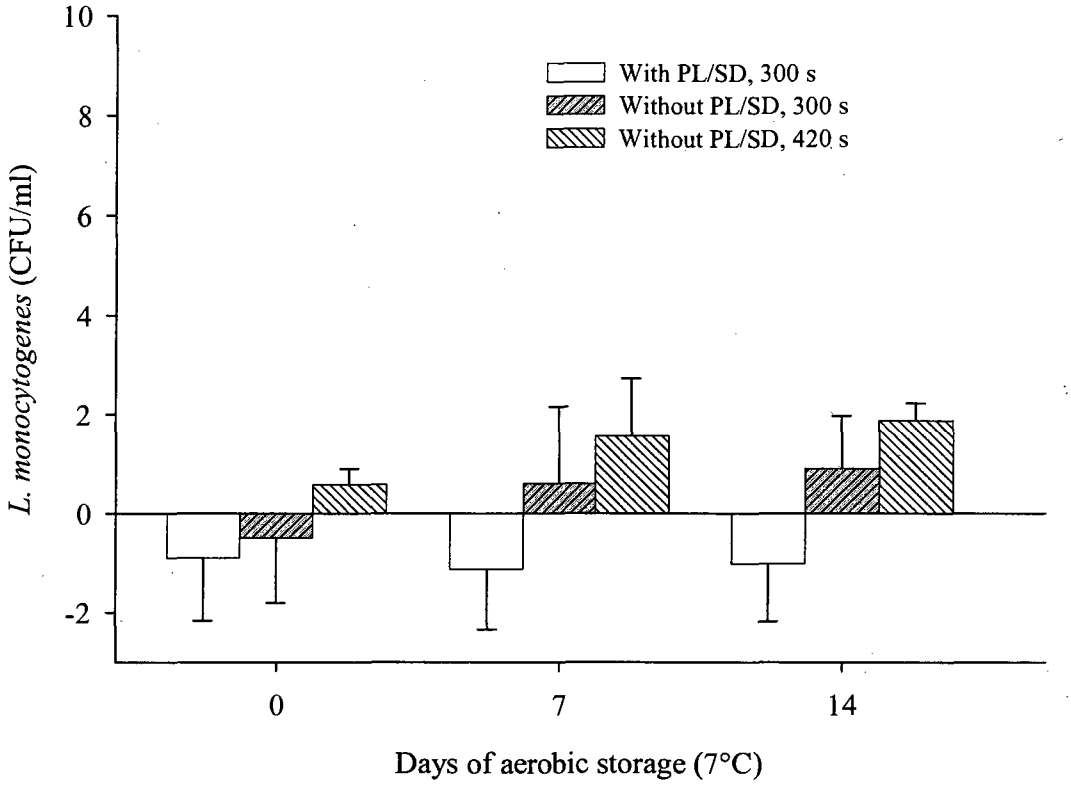


Figure 5.14 (Appendix Table 31). *Listeria monocytogenes* counts in water used for ambient control treatments of frankfurters formulated with and without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD), at 18 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

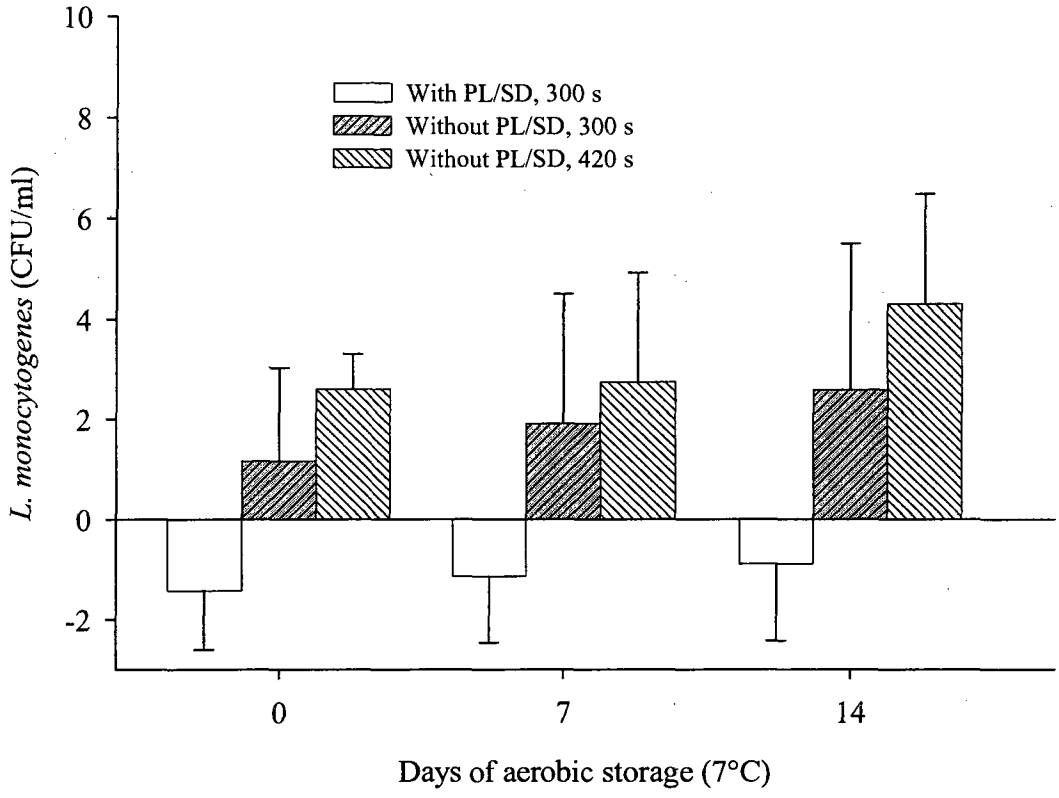


Figure 5.15 (Appendix Table 32). *Listeria monocytogenes* counts in water used for ambient control treatments of frankfurters formulated with and without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD), at 40 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

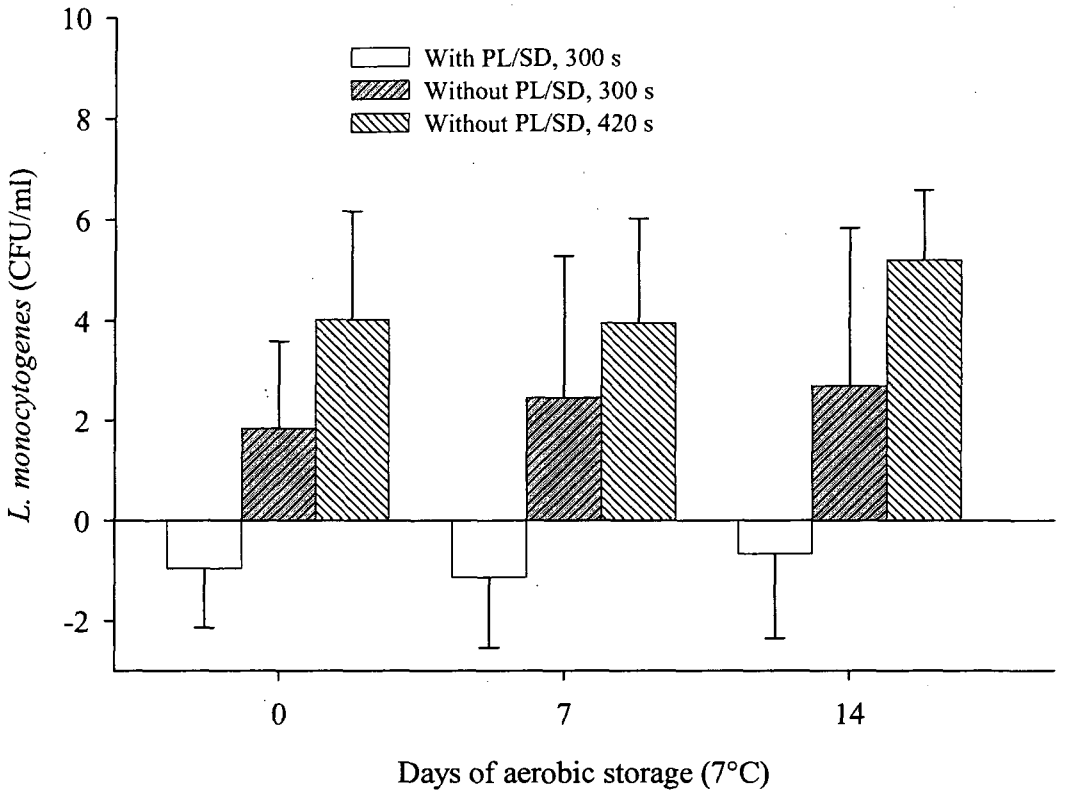


Figure 5.16 (Appendix Table 33). *Listeria monocytogenes* counts in water used for ambient control treatments of frankfurters formulated with and without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD), at 60 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

## CHAPTER VI

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# APPENDIX I

## Letter of approval by the Office of Regulatory Compliance at Colorado State University

Colorado State University

Office of Regulatory Compliance
Office of Vice President for Research
and Information Technology
Fort Collins, CO 80523-2011
(970) 491-1553
FAX: (970) 491-2293

MEMORANDUM

TO: Pat Kendall, FSHN, 1571

JUL 21 2005

FROM: Janell A. Meldrem, Administrator
Human Research Committee

SUBJECT: **PROJECT APPROVAL**
Title: Incidence, Significance and Control of Listeria Monocytogenes in the Home Environment
Protocol No.: 05-200H
Funding Agency: USDA-NIFSI

DATE: July 19, 2005

The above-referenced project was approved by the Human Research Committee on July 15, 2005 for the period July 15, 2005 to July 11, 2006 with the condition that the attached consent form is signed by the subjects and each subject is given a copy of the form. It is the investigator's responsibility to obtain this consent form from all subjects. *NO changes may be made to this document without first obtaining the approval of the Committee.* The approved cover letter must also be used.

**Approval is for 70 participants from Colorado with the condition that The Ohio State University IRB approval is submitted once obtained.**

A status report of this project will be required within a 12-month period from the date of approval. Renewal is the Principal Investigator's responsibility, but as a courtesy, you will be sent a reminder approximately two months before the protocol expires. The Principal Investigator will report on the numbers of subjects who have participated this year and project-to-date, about problems encountered, and provide a verifying copy of the consent form or cover letter used. The necessary form (H-101) is available from the Regulatory Compliance web page (see below). Should the protocol not be renewed before expiration, all activities must cease until the protocol has been re-reviewed.

It is the responsibility of the investigator to immediately inform the Committee of any serious complications, unexpected risks, or injuries resulting from this research. It is also the investigator's responsibility to notify the Committee of any changes in experimental design, participant population, or consent procedures or documents. This can be done with a memo which completely describes the changes and their consequences (new consent form or cover letter, or altered survey instrument, for example). Students serving as Co-Principal Investigators may not alter projects without first obtaining PI approval. The PI is ultimately responsible for the conduct of the project. Upon completion of the project, an H-101 should be submitted as a close-out report.

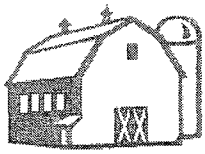
This approval is issued under Colorado State University's OHRP Federal Wide Assurance 00000647. If approval did not accompany a proposal when it was submitted to a sponsor, it is the researcher's responsibility to provide the sponsor with the approval notice.

Please direct any questions about the Committee's action on this project to me for routing to the Committee.

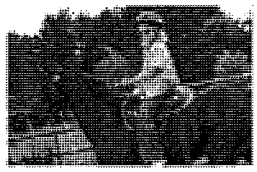
Attachment

## APPENDIX II

### Flier used for recruiting of households



# Rural Families
## needed for a
# CSU Research Study
## Spring 2006



Researchers in the Department of Food Science & Human Nutrition and the Department of Animal Sciences at Colorado State University are conducting a study to learn more about *Listeria*, a microorganism that causes illness in humans.

**We are seeking rural families who live on premises with or without ruminant farm animals and are willing to meet in their homes with a research assistant for 4 visits to conduct the following:**

- Complete a household survey, food questionnaire, food interview, and kitchen audit (1 visit up to 2 hrs)
- Microbiological samplings to test for *Listeria* in the home/farm environment (1st visit + 3 brief visits ~ 4 weeks apart)

- **Purpose**
  To learn more about how *Listeria* contamination may occur and to develop risk control measures and education materials for farmers/ranchers and consumers to better help protect themselves.
- **Time Involved**
  Up to 2 hours for the 1st visit and up to 30 minutes for each of the next 3 visits.
- **When/Where**
  ***February to May 2006*** (at dates/times mutually agreed upon)

**Compensation: $65 for your participation**

We would like to explain the various activities of this study by phone so that we may answer any questions you may have. If you decide to participate, we can schedule the 1st visit at that time. We look forward to your call!

**For more information contact Ruth Inglis-Widrick:**
**Phone: (970) 491-3747 Email:** Ruth.Inglis-Widrick@colostate.edu

***Project Directors:***
Pat Kendall (970) 491-1945 Email: kendall@cahs.colostate.edu
John Sofos (970) 491-7703 Email: john.sofos@colostate.edu

## APPENDIX III

**Non-Farm Household Survey. To be completed by the person with primary responsibility for the care and management of the household.**

**General Household Questions**

1) **How old is your house?**

**(4)** Less than 5 years

**(3)** 5-14 years

**(2)** 15-24 years

**(1)** Greater than 25 years

**(0)** Not sure

2) **What type of cooling system do you use in warm weather?** Check all that apply.

**(3)** Always **(2)** Usually **(1)** Sometimes

**(0)** Never

| | | | | |
|---|---|---|---|---|
| **(a)** Central A/C | ? | ? | ? | ? |
| **(b)** Window A/C | ? | ? | ? | ? |
| **(c)** Evap. cooler | ? | ? | ? | ? |
| **(d)** Fresh air | ? | ? | ? | ? |
| **(e)** Fans | ? | ? | ? | ? |

3) **Which of the following is the source of water for the house?**

Well water → please indicate type of well:

**(1)** dug **(2)** drilled **(0)**unsure

**(3)** Municipal **(4)** Spring

4) **If your water supply is not municipal, how frequently do you have it tested for bacterial contamination, i.e., coliforms?**

**(5)** Annually

**(4)** Every couple of years

**(3)** Only if the water tastes funny

**(2)** Never

**(1)** Don't recall

**(0)** I'm on municipal water

5) **What do household members use primarily for drinking water in the home?**

**(1)** Chlorinated municipal tap water

**(2)** Tap water from well or spring

**(3)** Filtered tap water (municipal, well or spring)

**(4)** Bottled water

**(5)** Other: _____

6) **Where do you usually do the household laundry?**

**(2)** Home **(1)** Laundromat → **Skip to Question #10**

**(0)** Other → **Skip to Question #10**

7) **If you do laundry at home, please indicate how you do the following.** Check all that apply.

| **(a) Washing** | **(b) Drying** |
|---|---|
| **(2)** Automatic washer | **(2)** Automatic dryer |
| **(1)** Non-automatic washer | **(1)** Clothesline |

**(3) BOTH** **(3) BOTH**

*Part of question 7 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Drying

Automatic dryer = 5

Clothesline = 0

BOTH = 2.5

**8) Do you use a separate washer (automatic or non-automatic washer) and dryer for farm clothing?**

**(a) Separate Washer** **(b) Separate Dryer**

**(3)** Yes **(3)** Yes

**(2)** No **(2)** No

**(1)** Occasionally **(1)** □Occasionally

**(0)** Don't use a dryer

**9) Do you clean the drum and lid area of the washing machine on a regular basis?**

**(2)** Yes **(1)** No

*Question 9 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Yes = 5; No = 0

**10) What water temperature setting do you usually use for the wash cycle of your laundry?**

**(1)** Cold **(2)** Warm **(3)** Hot **(0)** Not sure

**(a) Colors**

**(b) Whites**

**(4) cold and warm (1&2)**

**(5) warm and hot (2&3)**

**(6) cold and hot (1&3)**

**11) Do you wash kitchen towels separately from other laundry?**

**(2)** Yes **(1)** No

**12) How often do you sweep or vacuum the kitchen floor?**

**(4)** Daily

**(3)** 2-3 times/week

**(2)** Weekly

**(1)** Every 2 weeks or so

**(0)** Don't recall

*Question 12 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Daily = 5

2-3 times/week = 3

Weekly = 2

Every 2 weeks or so = 0

Don't recall = 0

**13) How often do you change the hand towels in the bathrooms?**

**(5)** Daily

**(4)** 2-3 times/week

**(3)** Weekly

**(2)** Every 2 weeks or so

**(1)** When visibly soiled

**(6)** Use paper towels

**(7) daily and use PT (5&6)**

**(8) 2-3 times and PT (4&6)**

**(9) weekly and PT (3&6)**

**(10) 2 weekly and PT (2&6)**

**(11) soiled and PT (1&6)**

*Part of question 13 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Daily = 2.5

2-3 times/week = 2.5

Weekly = 2

Every 2 weeks or so = 2

When visibly soiled = 0

Use paper towels = 5

**14) How often do you change the hand towels in the kitchen?**

**(5)** Daily

**(4)** 2-3 times/week

**(3)** Weekly

**(2)** Every 2 weeks or so

**(1)** When visibly soiled

**(6)** Use paper towels

**(7) daily and use PT (5&6)**

**(8) 2-3 times and PT (4&6)**

**(9) weekly and PT (3&6)**

**(10) 2 weekly and PT (2&6)**

**(11) soiled and PT (1&6)**

Comments:

---

*Part of question 14 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Daily = 2.5

2-3 times/week = 2.5

Weekly = 2

Every 2 weeks or so = 2

When visibly soiled = 0

Use paper towels = 5

**15) How long do you use the following items before they are replaced or sanitized?**

| **(a) Sponge** | **(b) Dish Cloth** |
|---|---|
| **(6)** One day | One day |
| **(5)** 2-3 days | 2-3 days |
| **(4)** One week | One week |
| **(3)** 2-3 weeks | 2-3 weeks |
| **(2)** Until visibly soiled | Until visibly soiled |
| **(0)** Don't recall | Don't recall |
| **(1)** Don't use | Don't use |

*Question 15 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

One day= 4

2-3 days = 4

One week = 3

2-3 weeks = 2

Until visibly soiled = 1

Don't recall = 0

Don't use = 5

**16) What is your primary means for washing dishes?**

**(2)** Dishwashing machine

**(1)** Hand-wash in the sink

**(3) BOTH**

*Question 16 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Dishwashing machine = 5

Hand-wash in the sink = 5

BOTH = 3.5

**17) If you hand-wash kitchen items, how do usually dry them?** Check all that apply.

**(a)** Cloth towel **1=no, 2=yes**

**(b)** Air dry

**(c)** Disposable paper towels

Comments:_____

*Part of question 17 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Cloth towel: Yes = 0, No = 5

Air dry: Yes = 5, No = 0

Disposable paper towels: Yes = 5, No = 0

**18) When cleaning the kitchen sink and counters, how frequently do you use the following?** Check one category per item.

**(3) Always** **(2) Usually**

**(1) Sometimes** **(0) Never**

**(a)** Sponge

**(b)** Dish cloth

**(c)** Paper towels

**(d)** Soap & water

**(e)** Antibacterial wipes/spray

**(f)** Other, please specify:

_____

**19) How often do you wash hands with soap and warm running water directly after handling the following uncooked products?**

| **(a)Meats** | **(b)Eggs** | **(c)Fruits & Veggies** |
|---|---|---|
| **(3)** Always | Always | Always |
| **(2)** Usually | Usually | Usually |
| **(1)** Sometimes | Sometimes | Sometimes |
| **(0)** Never\ | Never | Never |

*Question 19 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Always = 5; Usually = 3; Sometimes = 1; Never = 0

**20) How would you judge if the following meats are adequately cooked prior to consumption?** Check all that apply for each food item.

**(20.1)Meat Thermometer (20.2)Time (20.3)Visual**

**(a)** Whole chicken

**(b)** Ground beef

**(c)** Steak

**(d)** Pork roast

**1 = no, 2 = yes**

□

*Question 20 was included in the Perishable Food Handling and Cooking Index (PFHCI) and was coded as follows:*

| | Meat Thermometer | Time | Visual |
|---|---|---|---|
| **(a)** Whole chicken | Yes = 5, No = 0 | Yes = 0, No = 5 | Yes = 0, No = 5 |
| **(b)** Ground beef | Yes = 5, No = 0 | Yes = 0, No = 5 | Yes = 0, No = 5 |
| **(c)** Steak | Yes = 5, No = 0 | Yes = 0, No = 5 | Yes = 0, No = 5 |
| **(d)** Pork roast | Yes = 5, No = 0 | Yes = 0, No = 5 | Yes = 0, No = 5 |

**21) During a normal work day, how often do you typically wash your hands?**

**(1)** □ Less than 5 times

**(2)** □ 5-10 times a day

**(3)** □ Greater than 10 times a day

*Question 21 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Less than 5 times = 0

5-10 times a day = 2.5

Greater than 10 times a day = 5

**22) Do you wash your hands with soap and water prior to eating?**

**(3)** Always

**(2)** Usually

**(1)** Sometimes

**(0)** Never

Comments:

_____

*Question 22 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Always = 5

Usually = 4

Sometimes = 2

Never = 0

**Animal/Pet Questions**

Note: Because you are participating in this study as a **"non-farm"** household, the researchers assume that you have **not** had contact with ruminant animals **(including dairy cows, beef cattle, deer, elk, bison, sheep, goats)** in the past 3 months nor expect to in the next 3 months. If this changes for any reason, please notify the researcher immediately so that this information can be documented.

**23) What <u>outdoor</u> animals (and how many) do you raise or have contact with on a regular basis?** Please check all that apply. **1=no(yes, have animals), 2=yes**

**(a)** Poultry ( # _____ ) **(enter #)**

**(b)** Ostrich/Emus ( # _____ )

**(c)** Horses ( # _____ )

**(d)** Pigs ( # _____ )

**(e)** Alpacas/Llamas ( # _____ )

**(f)** Other: _____ ( # _____ )

**(g)** None → **If no outdoor animals are raised, please proceed to Q. #29.**

*Question 23 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Yes, have the animals = 0

No, does not have the animals = 5

**24) Do all family members have contact with the outdoor animals?**

**(1)** Yes

**(2)** No → Who does not touch the animals or handle manure? List age and gender below:

---

*Question 24 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Yes = 0

No = 5

**25) Which of the following procedures are practiced immediately after completing animal chores?** Please check any that apply.

**(a)** Hand washing **(1=no, 2=yes)**

**(b)** Boots are changed or disinfected

**(c)** Change of clothing

**(d)** None of the above

**(e)** Other

*Question 25 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Hand washing: Yes = 5, No =0

Boots are changed or disinfected: Yes = 5, No =0

Change of clothing: Yes = 5, No =0

None of the above: Yes = 0, No =5

**26) Do you routinely change clothing and footwear after animal contact or visiting an area where animals are housed, prior to entering the house?**

| **(a) Clothing** | **(b) Footwear** |
|---|---|
| **(2)** Yes | **(2)** Yes |
| **(1)** No | **(1)** No |

Comments:

---

*Question 26 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Clothing: Yes = 5, No = 0; Footwear: Yes = 5, No = 0

**27) After animal care, when do you usually wash your hands?** Check one.

**(4)** Before coming into the house

**(3)** Immediately upon entering the house

**(2)** Both of the above

**(1)** Don't wash

**(0)** Can't recall

Comments:

---

*Question 27 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Before coming into the house = 5

Immediately upon entering the house = 4

Both of the above = 5

Don't wash = 0

Can't recall = missing value (.)

**28) If you wash-up in the house, which sink do you usually use?**

**(2)** Kitchen

**(3)** Bathroom

**(4)** Laundry/utility room

**(0)** Not applicable

**(1)** Other:

*Question 28 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Kitchen = 0

Bathroom = 3

Laundry/utility room = 5

Not applicable = missing value (.)

**29) What pets (and how many) do you own or have contact with on a regular basis?** Please check all that apply.

**(a)** Reptiles (snake, turtle, iguana,etc.)

(# ___ )

**(b)** Pet Birds (# _____ )

**(c)** Rabbits (# _____ ) **(enter #)**

**(d)** Dogs (# _____ )

**(e)** Cats (# _____ )

**(f)** Other:

_____

**(g)** None → **If no pets, please proceed to Q. #37.**

**(1=no(yes, have animals), 2=yes)**

*Question 29 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Yes, have the animals = 0

No, does not have the animals = 5

**30) How often are hands washed immediately after completing pet chores?**

**(3)** Always

**(2)** Usually

**(1)** Sometimes

**(0)** Never/rarely

Comments:_____

*Question 30 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Always = 5

Usually = 4

Sometimes = 2

Never/rarely = 1

**31) Do house cats (family pets) go outside?**

**(1)** Always

**(2)** Usually

**(3)** Sometimes

**(4)** Never

**(0)** We don't have house cats.

*Question 31 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Always = 0

Usually = 1

Sometimes = 3

Never = 5

We don't have house cats = 5

**32) Are "barn" cats allowed inside the house?**

**(1)** Always

**(2)** Usually

**(3)** Sometimes

**(4)** Never

**(0)** We don't have any "barn" cats

*Question 32 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Always = 0

Usually = 1

Sometimes = 3

Never = 5

We don't have "barn" cats = 5

**33) Do the house cats and dogs have current rabies vaccinations?**

**(2)** Yes **(1)** No

**(0)** We don't have house cats or dogs

**34) Are dogs allowed inside the house?**

**(1)** Yes

**(2)** No

**(0)** We don't have dogs

*Question 34 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Yes = 0; No = 5

**35) Please indicate the location for the following activities.** Check all that apply.

| **(35.1)Feeding dogs** | **(35.2)Feeding <u>all</u> cats** | **(35.3)Cat litter box** |
|---|---|---|
| **(a)** Kitchen | Kitchen | Kitchen |
| **(b)** Laundry | Laundry | Laundry |
| **(c)** Basement | Basement | Basement |
| **(d)** Garage | Garage | Garage |
| **(e)** Barn | Barn | Goes out |
| **(f)** Outside | Outside | No house cats |
| **(g)** No dogs | No cats | |

**(1=no, 2=yes)**

*Part of question 35 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Is any animal feed in the kitchen? Yes = 0, No = 5

**36) Diet for dogs and cats**

| **(a.1)Raw milk** House cats | **(a.2)Raw meat** |
|---|---|
| **(1)** Yes | Yes |
| **(2)** No | No |
| **(0)** No cats | No cats |

| **(b.1) Raw milk** Barn cats | **(b.2)Raw meat** |
|---|---|
| **(1)** Yes | Yes |
| **(2)** No | No |

(0) No cats No cats

**(c.1) Raw milk (c.2)Raw meat**

Dogs

(1) Yes Yes

(2) No No

(3) No dogs No dogs

*Part of question 35 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Is any animal feed raw milk? Yes = 0, No = 5

**General Health Questions**

Zoonoses are diseases caused by infectious agents (germs), which can spread between animals and humans. Examples include but are not limited to *E. coli*, *Listeria*, *Campylobacter*, *Salmonella*, *Toxoplasma* and *Cryptosporidium.* Individuals who are very young, elderly, pregnant and/or suffer from chronic and debilitating conditions (such as cancer, coronary heart disease, Crohn's disease and asthma) can be at greater risk for acquiring these infections.

**37) Has your physician/health care provider discussed strategies to help prevent zoonotic infections in a family member who may be suffering from a chronic or debilitating illness, is contemplating pregnancy, or is currently pregnant?**

(3) Yes If yes, describe:

---

(2) No (1) Not sure (0) Not applicable

**38) Do you feel your knowledge regarding infection, transmission, and prevention of disease is sufficient to protect you, your family, and your employees from infection with zoonotic diseases?**

(3) Yes

(2) No

(1) Not sure

(0) Would like more information

**(4) yes and would like more info (3&0)**

**(5) no and would like more info (2&0)**

**(6) not sure and would like more info (1&0)**

**39) What are your primary sources of information regarding zoonoses?** Check all that apply.

**(a)** Family physician/ healthcare provider

**(b)** Veterinarian

**(c)** Extension agent

**(d)** Web based health information

**(e)** Television/newspaper/magazines

**(f)** No source

**(g)** Other:

---

**(1=no, 2=yes)**

**40) Has your family physician/health care provider ever discussed potential occupational health hazards associated with infectious diseases of animal origin?**

(2) Yes (1) No (0) Don't recall

**41) If so, were you satisfied with the infectious disease prevention information provided?**

(2) Yes

(1) No

(0) Never discussed health hazards associated with infectious diseases of animal origin

**42) Have you or a family member sought medical treatment for bloody diarrhea in the last three months?**

(1) Yes

(2) No

(0) Don't recall

**Household Demographics**

**43) Please indicate the number of children in each age group that live on your farm.**

**(a)** _____ 0 - 12 months

**(b)** _____ 13 to 48 months (4 years)

**(c)** _____ age 4 to 18 years

**44) Number of adults over age 18.** Please list number of individuals by the appropriate age categories.

**(44.1) Males**
**(44.2)Females**

**(a)** 19 to 28_____19 to 28_____

**(b)** 29 to 38_____29 to 38_____

**(c)** 39 to 48_____39 to 48_____

**(d)** 49 to 58_____49 to 58_____

**(e)** 59 to 68_____59 to 68_____

**(f)** over 68_____over 68

_____**(Enter #)**

**45) Highest level of education completed by any adult household members?**

**(1)** Some high school

**(2)** High school graduate

**(3)** Some college or technical school

**(4)** 4 year college degree

**(5)** Post-graduate studies

**(6)** Post-graduate degree

**46) Please check any of the following conditions that apply (either currently or within the past year) to someone who resides in your household:**

**(a)** Pregnant

**(b)** Diabetes

**(c)** Liver or kidney disease

**(d)** Cancer

**(e)** Organ transplant

**(f)** Heart disease

**(g)** Other conditions affecting immune system

Please specify: _____

Comments: _____

**47) Do any household members have other work-related exposure to animals not mentioned in this survey?**

**(2)** No

**(1)** Yes → **If so, identify the household member(s), age, gender and occupation**

Member #1

_____

Member #2

_____

Member #3

_____

Member #4

_____

**(enter member information in textbox)**

***Thank you for your time and effort! Please return this completed survey to the research assistant at the first visit.***

## APPENDIX IV

**Farm Household Survey. To be completed by the person with primary responsibility for the care and management of the household.**

**General Household Questions**

1) **How old is your house?**

**(4)** Less than 5 years

**(3)** 5-14 years

**(2)** 15-24 years

**(1)** Greater than 25 years

**(0)** Not sure

2) **What type of cooling system do you use in warm weather?** Check all that apply.

**(3)** Always **(2)** Usually **(1)** Sometimes

**(0)** Never

| | | | | |
|---|---|---|---|---|
| **(a)** Central A/C? | ? | ? | ? | ? |
| **(b)** Window A/C | ? | ? | ? | ? |
| **(c)** Evap. cooler | ? | ? | ? | ? |
| **(d)** Fresh air | ? | ? | ? | ? |
| **(e)** Fans | ? | ? | ? | ? |

3) **Which of the following is the source of water for the house?**

Well water → please indicate type of well:

**(1)** dug **(2)** drilled **(0)**unsure

**(3)** Municipal **(4)** Spring

4) **If your water supply is not municipal, how frequently do you have it tested for bacterial contamination, i.e., coliforms?**

**(5)** Annually

**(4)** Every couple of years

**(3)** Only if the water tastes funny

**(2)** Never

**(1)** Don't recall

**(0)** I'm on municipal water

5) **What do household members use primarily for drinking water in the home?**

**(1)** Chlorinated municipal tap water

**(2)** Tap water from well or spring

**(3)** Filtered tap water (municipal, well or spring)

**(4)** Bottled water

**(5)** Other: _____

6) **Where do you usually do the household laundry?**

**(2)** Home **(1)** Laundromat → **Skip to Question #10**

**(0)** Other → **Skip to Question #10**

7) **If you do laundry at home, please indicate how you do the following.** Check all that apply.

| **(a) Washing** | **(b) Drying** |
|---|---|
| **(2)** Automatic washer | **(2)** Automatic dryer |
| **(1)** Non-automatic washer | **(1)** Clothesline |
| **(3) BOTH** | **(3) BOTH** |

*Part of question 7 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Drying

Automatic dryer = 5

Clothesline = 0

BOTH = 2.5

**8) Do you use a separate washer (automatic or non-automatic washer) and dryer for farm clothing?**

**(a) Separate Washer** **(b) Separate Dryer**

**(3)** Yes **(3)** Yes

**(2)** No **(2)** No

**(1)** Occasionally **(1)** □Occasionally

**(0)** Don't use a dryer

**9) Do you clean the drum and lid area of the washing machine on a regular basis?**

**(2)** Yes **(1)** No

*Question 9 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Yes = 5; No = 0

**10) What water temperature setting do you usually use for the wash cycle of your laundry?**

**(1)** Cold **(2)** Warm **(3)** Hot **(0)** Not sure

**(a) Colors**

**(b) Whites**

**(4) cold and warm (1&2)**

**(5) warm and hot (2&3)**

**(6) cold and hot (1&3)**

**11) Do you wash kitchen towels separately from other laundry?**

**(2)** Yes **(1)** No

**12) How often do you sweep or vacuum the kitchen floor?**

**(4)** Daily

**(3)** 2-3 times/week

**(2)** Weekly

**(1)** Every 2 weeks or so

**(0)** Don't recall

*Question 12 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Daily = 5

2-3 times/week = 3

Weekly = 2

Every 2 weeks or so = 0

Don't recall = 0

**13) How often do you change the hand towels in the bathrooms?**

**(5)** Daily

**(4)** 2-3 times/week

**(3)** Weekly

**(2)** Every 2 weeks or so

**(1)** When visibly soiled

**(6)** Use paper towels

**(7) daily and use PT (5&6)**

**(8) 2-3 times and PT (4&6)**

**(9) weekly and PT (3&6)**

**(10) 2 weekly and PT (2&6)**

**(11) soiled and PT (1&6)**

*Part of question 13 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Daily = 2.5

2-3 times/week = 2.5

Weekly = 2

Every 2 weeks or so = 2

When visibly soiled = 0

Use paper towels = 5

**14) How often do you change the hand towels in the kitchen?**

**(5)** Daily

**(4)** 2-3 times/week

**(3)** Weekly

**(2)** Every 2 weeks or so

**(1)** When visibly soiled

**(6)** Use paper towels

**(7) daily and use PT (5&6)**

**(8) 2-3 times and PT (4&6)**

**(9) weekly and PT (3&6)**

**(10) 2 weekly and PT (2&6)**

**(11) soiled and PT (1&6)**

Comments:

_____

*Part of question 14 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Daily = 2.5

2-3 times/week = 2.5

Weekly = 2

Every 2 weeks or so = 2

When visibly soiled = 0

Use paper towels = 5

**15) How long do you use the following items before they are replaced or sanitized?**

| **(a)** <u>Sponge</u> | **(b)** <u>Dish Cloth</u> |
|---|---|
| **(6)** One day | One day |
| **(5)** 2-3 days | 2-3 days |
| **(4)** One week | One week |
| **(3)** 2-3 weeks | 2-3 weeks |
| **(2)** Until visibly soiled | Until visibly soiled |
| **(0)** Don't recall | Don't recall |
| **(1)** Don't use | Don't use |

*Question 15 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

One day= 4

2-3 days = 4

One week = 3

2-3 weeks = 2

Until visibly soiled = 1

Don't recall = 0

Don't use = 5

**16) What is your primary means for washing dishes?**

**(2)** Dishwashing machine

**(1)** Hand-wash in the sink

**(3) BOTH**

*Question 16 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Dishwashing machine = 5

Hand-wash in the sink = 5

BOTH = 3.5

**17) If you hand-wash kitchen items, how do usually dry them?** Check <u>all</u> that apply.

**(a)** Cloth towel **1=no, 2=yes**

**(b)** Air dry

**(c)** Disposable paper towels

Comments:_____

*Part of question 17 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Cloth towel: Yes = 0, No = 5

Air dry: Yes = 5, No = 0

Disposable paper towels: Yes = 5, No = 0

**18) When cleaning the kitchen sink and counters, how frequently do you use the following?** Check one category per item.

**(3) Always** **(2) Usually**

**(1) Sometimes** **(0) Never**

**(a)** Sponge

**(b)** Dish cloth

**(c)** Paper towels

**(d)** Soap & water

**(e)** Antibacterial wipes/spray

**(f)** Other, please specify:

_____

**19) How often do you wash hands with soap and warm running water directly after handling the following uncooked products?**

**(a)Meats** **(b)Eggs** **(c)Fruits & Veggies**

**(3)** Always Always Always

**(2)** Usually Usually Usually

**(1)** Sometimes Sometimes Sometimes

**(0)** Never\ Never Never

*Question 19 was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

Always = 5; Usually = 3; Sometimes = 1; Never = 0

**20) How would you judge if the following meats are adequately cooked prior to consumption?** Check all that apply for each food item.

**(20.1)Meat Thermometer (20.2)Time (20.3)Visual**

**(a)** Whole chicken

**(b)** Ground beef

**(c)** Steak

**(d)** Pork roast

**1 = no, 2 = yes**

*Question 20 was included in the Perishable Food Handling and Cooking Index (PFHCI) and was coded as follows:*

| | **Meat Thermometer** | **Time** | **Visual** |
|---|---|---|---|
| **(a)** Whole chicken | Yes = 5, No = 0 | Yes = 0, No = 5 | Yes = 0, No = 5 |
| **(b)** Ground beef | Yes = 5, No = 0 | Yes = 0, No = 5 | Yes = 0, No = 5 |
| **(c)** Steak | Yes = 5, No = 0 | Yes = 0, No = 5 | Yes = 0, No = 5 |
| **(d)** Pork roast | Yes = 5, No = 0 | Yes = 0, No = 5 | Yes = 0, No = 5 |

Yes = 0, No = 5

**21) During a normal work day, how often do you typically wash your hands?**

**(1)** Less than 5 times

**(2)** 5-10 times a day

**(3)** Greater than 10 times a day

**22) Do you wash your hands with soap and water prior to eating?**

**(3)** Always

**(2)** Usually

**(1)** Sometimes

**(0)** Never

Comments: _____

## Animal/Pet Questions

**23) What pets (and how many) do you own or have contact with on a regular basis?** Please check all that apply. **(Enter # of animals)**

**(a)** Reptiles(snake, turtle, iguana, etc.)( #__ )

**(b)** Pet birds (# _____ )

**(c)** Rabbits (# _____ )

**(d)** Dogs (# _____ )

**(e)** Cats (# _____ )

**(f)** Other: **textbox**_____

**(g)**☐ None → **If no pets, please proceed to Q. #31. (1= yes, 0= no → yes, have pets)**

*Question 23 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Yes, have the animals = 0

No, does not have the animals = 5

**24) How often are hands washed immediately after completing indoor pet chores?**

**(3)** Always

**(2)** Usually

**(1)** Sometimes

**(0)** Never/rarely

Comments:_____

*Question 24 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Always = 5

Usually = 4

Sometimes = 2

Never/rarely = 1

**25) Do house cats (family pets) go outside?**

**(1)** Always

**(2)** Usually

**(3)** Sometimes

**(4)** Never

**(0)** We don't have house cats

*Question 25 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Always = 0

Usually = 1

Sometimes = 3

Never = 5

We don't have house cats = 5

**26) Are "barn" cats allowed inside the house?**

**(1)** Always

**(2)** Usually

**(3)** Sometimes

**(4)** Never

**(0)** We don't have any "barn" cats

*Question 26 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Always = 0

Usually = 1

Sometimes = 3

Never = 5

We don't have "barn" cats = 5

**27) Do the house cats or dogs have current rabies vaccinations?**

**(2)** Yes **(1)** No

**(0)** We don't have any house cats or dogs

**28) Are dogs allowed inside the house?**

**(1)** Yes

**(2)** No

**(0)** We don't have dogs

*Question 28 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Yes = 0; No = 5

**29) Please indicate the location for the following activities.** Check all that apply.

| **(29.1)Feeding dogs** | **(29.2)Feeding all cats** | **(29.3)Cat litter box** |
|---|---|---|
| **(a)** Kitchen | Kitchen | Kitchen |
| **(b)** Laundry | Laundry | Laundry |
| **(c)** Basement | Basement | Basement |
| **(d)** Garage | Garage | Garage |
| **(e)** Barn | Barn | Goes out |
| **(f)** Outside | Outside | No house cats |
| **(g)** No dogs | No cats | |

**(1=no, 2=yes)**

*Part of question295 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Is any animal feed in the kitchen? Yes = 0, No = 5

**30) Diet for dogs and cats**

| **(a.1)Raw milk** House cats | **(a.2)Raw meat** |
|---|---|
| **(1)** Yes | Yes |
| **(2)** No | No |
| **(0)** No cats | No cats |
| **(b.1) Raw milk** Barn cats | **(b.2) Raw meat** |
| **(1)** Yes | Yes |
| **(2)** No | No |
| **(0)** No cats | No cats |
| **(c.1) Raw milk** Dogs | **(c.2) Raw meat** |
| **(1)** Yes | Yes |
| **(2)** No | No |
| **(3)** No dogs | No dogs |

*Part of question 30 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Is any animal feed raw milk? Yes= 0, No= 5

## General Health Questions

Zoonoses are diseases caused by infectious agents (germs), which can spread between animals and humans. Examples include but are not limited to *E. coli*, *Listeria*, *Campylobacter*, *Salmonella*, *Toxoplasma* and *Cryptosporidium*. Individuals who are very young, elderly, pregnant and/or suffer from chronic and

debilitating conditions (such as cancer, coronary heart disease, Crohn's disease and asthma) can be at greater risk for acquiring these infections.

**31) Has your physician/health care provider discussed strategies to help prevent zoonotic infections in a family member who may be suffering from a chronic or debilitating illness, is contemplating pregnancy or is currently pregnant?**

**(3)** Yes

**(2)** No

**(1)** Not sure

**(0)** Not applicable

If so, please describe:

_____

**32) Do you feel your knowledge regarding infection, transmission, and prevention of disease is sufficient to protect you, your family, and your employees from infection with zoonotic diseases?**

**(3)** Yes

**(2)** No

**(1)** Not sure

**(0)** Would like more information

**(4) yes and would like more info (3&0)**

**(5) no and would like more info (2&0)**

**(6) not sure and would like more info (1&0)**

**33) What are your primary sources of information regarding zoonoses?** Check all that apply.

**(a)** Family physician/ healthcare provider

**(b)** Veterinarian

**(c)** Extension agent

**(d)** Web-based health information

**(e)** Television/newspaper/magazines

**(f)** No source

**(g)** Other: _____

**1 = no, 2 = yes**

**34) Has your family physician/health care provider ever discussed potential occupational health hazards associated with infectious diseases of animal origin?**

**(2)** Yes

**(1)** No

**(0)** Don't recall

**35) If so, were you satisfied with the infectious disease prevention information provided?**

**(2)** Yes

**(1)** No

**(0)** Never discussed health hazards associated with infectious diseases of animal origin

**36) Have you or a family member sought medical treatment for bloody diarrhea in the last three months?**

**(1)** Yes

**(2)** No

**(0)** Don't recall

**37) Do you recall if anyone in the family has been diagnosed with a disease that was also diagnosed in a farm animal(s) at the same time?** ***example: Salmonellosis in a calf***

**(1)** Yes

**(2)** No

**(0)** Don't recall

## Household Demographics

**38) Please indicate the number of children in each age group that live on your farm.**

**(a)** _____0 - 12 months

**(b)** _____13 to 48 months (4 years)

**(c)** _____age 4 to 18 years

**Enter #**

**39) Number of adults over age 18.** Please list number of individuals by the appropriate age categories.

**(39.1)Males**
**(39.2)Females**

**(a)** 19 to 28_____19 to 28_____

**(b)** 29 to 38_____29 to 38_____

**(c)** 39 to 48_____39 to 48_____

**(d)** 49 to 58_____49 to 58_____

**(e)** 59 to 68_____59 to 68_____

**(f)** over 68_____over 68_____

**40) Highest level of education completed by any adult household members?**

**(1)** Some high school

**(2)** High school graduate

**(3)**Some college or technical school

**(4)** 4 year college degree

**(5)** Post-graduate studies

**(6)** Post-graduate degree

**41) Please check any of the following conditions that apply (either currently or within the past year) to someone who resides in your household:**

**(a)** Pregnant

**(b)** Diabetes

**(c)** Liver or kidney disease

**(d)** Cancer

**(e)** Organ transplant

**(f)** Heart disease

**(g)** Other conditions affecting immune system

Please specify: _____

Comments:_____

**0 = no, 1 = yes**

**42) Do any household members have other work-related exposure to animals not mentioned in this survey?**

**(2)** No

**(1)** Yes → Yes → **If so, identify the household member(s), age, gender and occupation**

Member#1_____

Member#2_____

Member#3_____

Member#4_____

**Enter member info in textbox**

***Thank you for your time and effort! Please return this completed survey to the research assistant at the first visit.***

## APPENDIX V
## Food Handling and Eating Preferences Questionnaire

1. **This is a survey about food preferences and ways you fix food. It is not a test, and there are no wrong answers. When answering questions, check the box that applies to the way you usually do things.**

| | Never | Rarely | Some of the time | Most of the time | Always | Does not apply to me |
|---|---|---|---|---|---|---|
| a. I wash my hands with soap and warm running water before preparing food. | 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 0☐ |
| b. After playing with a pet and before getting a snack, I wash my hands with soap and warm running water. | 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 0☐ |
| c. After cutting raw meat, chicken, or seafood, I wash all items that came in contact with the raw food (e.g. cutting board, knife, counter top) with hot, soapy water before I continue cooking. | 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 0☐ |
| d. I thoroughly rinse fresh vegetables under running water before eating them | 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 0☐ |
| e. I wash the plate used to hold raw meat, poultry, or seafood with hot, soapy water before returning cooked food to the plate OR I use a clean plate. | 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 0☐ |
| f. I wash my hands with soap and warm running water after working with raw meat, chicken, or seafood and before I continue cooking. | 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 0☐ |
| g. When I cook fish, I check that the flesh flakes easily with a fork before serving. | 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 0☐ |
| h. I store my butter at room temperature. | 5☐ | 4☐ | 3☐ | 2☐ | 1☐ | 0☐ |

*Part if this question (items a-f) was included in the Indoors Crosscontamination Index (ICCI) and was coded as follows:*

Never = 1 Rarely = 2 Some of the time = 3 Most of the time = 4 Always = 5

Does not apply to me = missing value (.)

2. **Do you refrigerate the following foods within 2 hours of preparing and serving?**

| | YES | NO | |
|---|---|---|---|
| a. Cooked rice | 5☐ | 0☐ | |
| b. Fried chicken | 5☐ | 0☐ | .☐ NA |

c. Refried or cooked beans 5☐ 0☐

*This question was included in the Perishable Food Handling and Cooking Index (PFHCI) and was coded as follows:*

Yes = 5, No = 0

**3. Food Preferences. Do you or anyone in your household eat the following foods?**

| | YES | NO |
|---|---|---|
| a. rare hamburger | 0☐ | 5☐ |
| b. eggs with runny yolks | 0☐ | 5☐ |
| c. raw oysters/oysters on the half shell | 0☐ | 5☐ |
| d. cold deli or luncheon meats | 0☐ | 5☐ |
| e. homemade cookie dough | 0☐ | 5☐ |
| f. alfalfa or other raw sprouts | 0☐ | 5☐ |
| g. ceviche (marinated raw fish) | 0☐ | 5☐ |
| h. sushi or sushimi (made with raw fish) | 0☐ | 5☐ |
| i. raw milk (unpasteurized) | 0☐ | 5☐ |
| j. cold hot dogs | 0☐ | 5☐ |
| k. soft cheese made from unpasteurized milk (like Brie, Camembert and queso fresco) | 0☐ | 5☐ |
| l. Smoked fish | 0☐ | 5☐ |

*Part if this question (items g, h, I, k and l) was included in the Risky Foods Procurements Index (RFPI) and was coded as follows:*
Yes = 0, No = 5

**4. For each question below, please check what you usually do.**

| | YES | NO |
|---|---|---|
| a. If you have diarrhea, do you prepare food for others? | 0☐ | 5☐ |
| b. Do you use a thermometer to check the temperature of your refrigerator? | 0☐ | 5☐ |
| c. Do you use a thermometer to determine if hamburger patties have been cooked enough? | 0☐ | 5☐ |
| d. Do you use a thermometer to determine if leftovers have been reheated enough? | 0☐ | 5☐ |

e. Do you use a thermometer to determine if chicken breasts have been cooked enough? 0☐ 5☐

f. Do you check the expiration date on packages of luncheon meat prior to eating? 0☐ 5☐

## APPENDIX VI

**Farmer/Rancher Survey: To be completed by the person primarily responsible for the handling and management of ruminant animals raised on the farm/ranch.**

1) **Please check if any of the following ruminant animals are raised on your farm/ranch and the approximate number you have of each:**

**a.1** Beef Cattle # __**a.2**

**e.1** Deer # __**e.2**

**b.1** Dairy Cows # __**b.2**

**f.1** Sheep # __**f.2**

**c.1** Elk # __**c.2**

**g.1** Goats # __**g.2**

**d.1** Bison #__**d.2**

**1=yes, 0=no, if yes, enter #**

2) **Do you maintain a closed herd for any of the above animals?**

**(2)** Yes Which ones? _____

**(1)** No **(0)** N/A

3) **What other outdoor animals do you raise or have contact with on a regular basis?** Please check all that apply.

**a.** Poultry **d.** Alpacas/Llamas

**b.** Ostrich/Emu **e.** Horses

**c.** Pigs **f.** None

**g.** Other:

_____

**1=yes, 0=no**

*Question 3 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Yes, have the animals = 0

No, does not have the animals = 5

**1=yes, 0=no**

**a.** Family member*

**b.** Employee

4) **Do all family members have contact with the outdoor animals?**

**(1)** Yes **(0)** No → Who does not touch the animals or handle manure? List age and gender below:

*Question 24 was included in the Outdoor Crosscontamination Index (OCCI) and was coded as follows:*

Yes = 0

No = 5

5) **What is the source of the water supply for the house?**

**(1) Well or spring**

**(2) Municipal water line**

**(3)** both

6) **How is the water supply to the animal area separated from the water supply to the house?**

**(4)** 2 separate sources

**(2)** 1 well but 2 different water lines

**(1)** Not separated

**(3)** Back-flow prevention valve installed on animal water link

**(0)** Not sure

**(5)** (4and2 – 2 separate sources and 1 well but two separate sources)

7) **Other than a veterinarian, who treats the sick animals?**

**c.** Neighbor

* for a family member, please list age and gender of individuals involved:

_____

8) **Please indicate if any of the following health problems have been diagnosed in your animals by a veterinarian over the last 12 months.** Please check any that apply.

**1=yes, 0=no**

**a.** Cryptosporidiosis

**b.** Johne's Disease

**c.** Leptospirosis

**d.** Listeriosis or circling disease

**e.** Q fever

**f.** Salmonellosis

**g.** Psittacosis or Chlamydiosis

**h.** Chronic Wasting Disease

**i.** Other:

---

9) **Which of the following procedures are practiced immediately after completing farming chores?** Please check any that apply:

**1=yes, 0=no**

**a.** Hand washing

**b.** Boots are changed or disinfected

**c.** Change of clothing

**d.** None of the above

**e.** Other

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*Question 9 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Hand washing: Yes = 5, No =0

Boots are changed or disinfected: Yes = 5, No =0

Change of clothing: Yes = 5, No =0

None of the above: Yes = 0, No =5

10) **Do you routinely change clothing and footwear after animal contact or visiting areas where animals are housed, prior to entering the house?**

| **a. Clothing** | **b. Footwear** |
|---|---|
| **(1)** Yes | **(1)** Yes |
| **(0)** No | **(0)** No |

Comments:

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*Question 10 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Clothing: Yes = 5, No = 0; Footwear: Yes = 5, No = 0

11) **Do you use the same equipment (tractors, etc.) for handling feed and for handling waste?**

Yes → How often do you clean equipment?

**(1)** Never **(2)** Sometimes **(3)** Always
**(0)** No

Comments:

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## Visitors. If you do not allow visitors on the farm, skip to #13.

12) **How often do you do the following when you have visitors at your farm?**

**a. Have visitors to the farm sign a log book**

**(1)** Never **(2)** Sometimes **(3)** Always

**(0)** N/A

**b. Provide protective clothing to visitors**

**(1)** Never **(2)** Sometimes **(3)** Always

**(0)** N/A

**c. Have an employee accompany visitors during the entire visit**

**(1)** Never **(2)** Sometimes **(3)** Always

**(0)** N/A

**d. Provide protective footwear**

**(1)** Never **(2)** Sometimes **(3)** Always

**(0)** N/A

**13) In your opinion, which is the greatest threat for spreading infectious diseases to your farm or between animals on your farm?** Please check one.

**1=yes, 0=no**

**a.** Wild animals (including rodents)

**b.** Insects

**c.** Birds

**d.** Vehicular traffic on the farm/Visitors

**e.** Replacement cattle

**f.** Fairs

**g.** Chronically infected animals that don't show signs of illness

**h.** Other_____

**14) Which of the following is your preferred way to learn about technical or animal health related issues?** Please check one.

**1=yes, 0=no**

**a.** Written (magazine/journal articles, fact sheets, pamphlets)

**b.** Web based tutorials (Internet)

**c.** Extension workshops

**d.** Friends/Family

**e.** Consultations with experts

**f.** Other, please describe _____

**15) After farming and/or animal care, when do you usually wash your hands?** Check one.

**(4)** Before coming into the house

**(3)** Immediately upon entering the house

**(5)** Both of the above

**(2)** Can't recall

**(1)** Don't wash

*Question 15 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Before coming into the house = 5

Immediately upon entering the house = 4

Both of the above = 5

Don't wash = 0

Can't recall = missing value (.)

**16) If you wash-up in the house, which sink do you usually use?**

**1=yes, 0=no**

**a.** Kitchen

**b.** Bathroom

**c.** Laundry room

**d.** Not applicable

**e.** Other: _____

*Question 16 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Kitchen = 0

Bathroom = 3

Laundry/utility room = 5

Not applicable = missing value (.)

**17) How often do you change your hand towels used after livestock handling?**

**(5)** Daily

**(4)** 2-3 times/week

**(3)** Weekly

**(2)** Every 2 weeks or so

**(1)** When visibly soiled

**(6)**Use paper towels

**(7)** (daily and use paper towel)

**(8)** (2-3 times and use paper towel)

**(9)** (weekly and use paper towel)

**(10)** (2 weeks and use paper towel)

**(11)** (soiled and use paper towel)

**18) During a normal work day, how frequently do you typically wash your hands?**

**(1)** Less than 5 times

**(2)** 5-10 times a day

**(3)** Greater than 10 times a day

*Question 21 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Less than 5 times = 0

5-10 times a day = 2.5

Greater than 10 times a day = 5

**19) Do you wash your hands with soap and water prior to eating?**

**(3)** Always **(2)** Usually **(1)** □Sometimes

Comments:

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*Question 22 was included in the Personal Cleanliness Index (PCI) and was coded as follows:*

Always = 5

Usually = 4

Sometimes = 2

Never = 0

***Thank you! Please return this completed survey to the research assistant at the first visit.***

## APPENDIX VII
## Questions for Interview with Primary Household Food Preparer

Thanks for taking the time to let me interview you on your cooking practices and food preferences. There are no right or wrong answers-we just want to gather information about what consumers generally do. So that I won't have to take notes during this interview, I'd like to be able to audio-tape the interview. No one except myself and one other researcher will listen to this tape and we will destroy the tape when the study is completed. Is this OK with you? Do you have any questions before we begin?

**Introduction/ Icebreaker**

1) Who does most of the food shopping for your household?
**Q 1a. = self**
**Q 1b. = spouse/partner**
**Q 1c. = shared by both**
**Q 1d-1. = another household member**
**Q 1d-2. = who:** _____

2) Who is responsible for most of the meal preparation in your home? (list all who apply)
**Q 2a. = self**
**Q 2b. = spouse/partner**
**Q 2c. = shared by both**
**Q 2d-1. = another household member**
**Q 2d-2. = who:** _____

3) Do you primarily prepare homemade or convenience foods?
**Q 3a. = homemade**
**Q 3b. = convenience**
**Q 3c. = take out (restaurant delivery)**
**Q3d-1. = Other**
**Q3d-2. = What:** _____
**Q3e. = Comments:**

4) (*If applicable) Are you currently pregnant or have you been within the past 2 years? If so, what changes (if any) did you make in your food consumption habits?
**Q4a. No=(0) Yes =(1)**
**Changes**
**Q 4b. = avoided certain foods**
**Q 4c. = did not avoid certain foods**
**Q 4d. = changed eating habits**
**Q 4e. = no change in eating habits**

**Awareness/Knowledge of Foodborne Pathogens**
**I'd like to talk for a minute about hazards in food.**

5) Have you or anyone you know ever had a foodborne illness?
**Q 5. No = (0) Yes =(1)**

6) What microorganisms are you familiar with that can cause foodborne illness?
**Q 6a. = not familiar with any microorganisms**
**Q 6b. = Salmonella**
**Q 6c. = Ecoli**
**Q 6d. = Listeria**
**Q 6e-1. = other**
**Q 6e-2. = List other:_____**

*Part of this question was included in the Pathogen Awareness Index (PAI) and coded as follows:*
Salmonella: Yes=5, No=0
E. coli: Yes=5, No=0
Listeria: Yes=5, No=0

7) How can you tell if a food is contaminated? (What is it you're looking for?)
**Q 7a. = visual, smell, taste.**
**Q 7b-1. = other**
**Q 7b-2. = list other**
**Q 7c. = can't tell.**

*Part of this question was included in the Pathogen Awareness Index (PAI) and coded as follows:*
visual, smell, taste: Yes=5, No=0
can't tell: Yes=5, No=0

8) What microorganisms are you familiar with that can be transmitted by animals?
**Q 8a. = not sure**
**Q 8b. = Salmonella**
**Q 8c. = Ecoli**
**Q 8d. = Listeria**
**Q 8e-1. = Other**
**Q 8e-2. = List other:_____**

**Now I'd like to talk about some specific bacteria.**

9) <u>Salmonella</u>
**a)** For instance, have you ever heard of Salmonella?
**Q 9a. No = (0) Yes = (1)**

**b)** What foods are you familiar with that can be contaminated by Salmonella bacteria?
**Q9b-1. = Eggs**

**Q9b-2. = Poultry**
**Q9b-3. = Meat**
**Q 9b-4. = Animal foods**
**Q 9b-5. = Vegetables**
**Q 9b-6a. = Other**
**Q 9b-6b. = List other: _____**
**Q 9b-7. = Not sure**

c) What do you consider as most important in preventing contamination with Salmonella bacteria?

**Q 9c-1. =cook adequately**
**Q 9c-2. = hand washing**
**Q 9c-3. = wash meat before cooking**
**Q 9c-4a. = Other**
**Q 9c-4b = List other:_____**
**Q 9c-5. = Not sure**

**10) <u>E.Coli</u>**

**a)** Have you ever heard of E. coli?

**Q 10a. No =(0) Yes =(1)**

**b)** What foods have you heard of that can be contaminated with E. Coli bacteria?

**Q 10b-1. = Eggs**
**Q 10b-2. = Poultry**
**Q 10b-3. = Meat**
**Q 10b-4. = Vegetables**
**Q 10b-5a. = Other**
**Q 10b-5b. = List other:_____**
**Q 10b-6. = Not sure**

c) What do you consider as most important in preventing contamination with E. coli bacteria?

**Q 10c-1. = cook adequately**
**Q 10c-2. = hand washing/ preventing cross contamination**
**Q 10c-3. = wash meat before cooking**
**Q 10c-4. = proper refrigeration**
**Q 10c-5a. = Other: _____**
**Q 10c-5b. = List other:_____**
**Q 10c-6. = Not sure**

**11) <u>Campylobacter</u>**

**a)** Have you ever heard of Campylobacter?

**Q 11a. No= (0) Yes= (1)**

*This question was included in the Pathogen Awareness Index (PAI) and coded as follows:*

No= 0, Yes= 5

**b)** What foods have you heard of that can be contaminated by Campylobacter bacteria?

**Q 11b-1. = Eggs**
**Q 11b-2. = Poultry**
**Q 11b-3. = Meat**
**Q 11b-4. = Vegetables**
**Q 11b-5a. = Other**
**Q 11b-5b. = List other:_____**
**Q 11b-6. = Not sure**

**c)** What do you consider as most important in preventing contamination with Campylobacter bacteria?

**Q 11c-1. =cook adequately**
**Q 11c-2. = hand washing**
**Q 11c-3. = wash meat before cooking**
**Q 11c-4a. = Other**
**Q 11c-4b. = List other:_____**
**Q 11c-5. = Not sure**

**12) <u>Listeria</u>**

**a)** Have you ever heard of Listeria? **Q 12a. No= (0) Yes= (1)**

*This question was included in the Pathogen Awareness Index (PAI) and coded as follows:*
No= 0, Yes= 5

**b)** Can you tell me where you have heard about this bacteria?

**Q 12b-1. = doctor**
**Q 12b-2. = word of mouth**
**Q 12b-3. = newsletter/brochure**
**Q 12b-4. = not sure**

**c)** What foods have you heard of that can be contaminated by Listeria bacteria?

**Q 12c-1. = Eggs**
**Q 12c-2. = Poultry**
**Q 12c-3. = Meat**
**Q 12c-4. = Vegetables**
**Q 12c-5a. = Other**
**Q 12c-5b. = List other:_____**
**Q 12c-6. = Not sure**

**d)** What do you consider as most important in preventing contamination with Listeria bacteria?

**Q 12d-1. =cook adequately**
**Q 12d-2. = hand washing**

**Q 12d-3. = wash meat before cooking**
**Q 12d-4. =avoiding consumption of high risk foods**
**Q 12d-5a. = Other**
**Q 12d-5b. = List other:_____**
**Q 12d-6. = Not sure**

**13) Others:**

**a)** Are there any others you have heard of or want to mention?

**Q 13a. No= (0) Yes= (1)**
**Q 13b:** List:_____

**14)** Have you ever heard of antibiotic resistance?

**Q 14a-1. No= (0) Yes= (1)**

**a)** Are you concerned about it?

**Q 14a-2. No= (0) Yes= (1)**

**b)** What can you tell me about it?

**Q 14b.-1** Comments: _____

**Food Shopping/Procurement**

**Next I'd like to talk about the various sources of where you get you food from. As I go through each category of foods, please tell me if you have gotten any of these foods from the following sources in the past 12 months.**

**Let's start with eggs. Where do you get your eggs? (Go through a-h.)**
**What about Milk? Meat? Fruits? Veggies? (Go through a-h.)**

Over the past 12 months, did you obtain the following per category *(circle)*:

| | 15) EGGS | 16) MILK | 17) MEAT | 18) FRUITS | 19) VEGS |
|---|---|---|---|---|---|
| home grown: | a | a | a | a | |
| farm-direct or CSA* | b | b | b | b | |
| rancher-direct: | c | c | c | c | |
| local cooperative: | d | d | d | d | |
| farmers markets: | e | e | e | e | |
| grocery store: | f | f | f | f | |
| neighbor: | g | g | g | g | |
| other: | h | h | h | h | |

**CSA=Community Supported Agriculture (cow sharing)*

**20) DAIRY**

**a)** Is the milk you drink pasteurized?

**Q20-a. No= (0) Yes= (1)**

**b)** Does anyone in your household drink fresh, raw milk or eat cheese/yogurt made from raw milk?

**Q20b-1. No (N/A)= (0) Yes= (1)**
**Who?**

**Q20b-2. = Self or Spouse**
**Q20b-3. = Other household adult**
**Q20b-4. = Whole family (kids too)**
**Q20b-5a. = Other**
**Q20b-5b = List other: _____**
**What items?**
**Q20b-6. = raw milk (cow and/or goat)**
**Q20b-7. = raw-milk cheese**

c) **If YES,** is there anyone in your household (or visitors to your household) that you would NOT serve unpasteurized milk or milk products to?

**Q20c-1. No = (0) Yes (1)**
**Q20c-2 N/A = 1**
**Who?**
**Q20c-3. Pregnant woman**
**Q20c-4. Elderly person**
**Q20c-5. Young children**
**Q20c-6a. Other**
**Q20c-6b. List other: _____**

d) Do you pasteurize your own milk? How?

**Q20d. No= (0) Yes= (1)**

e) Have you ever made your own cheese, butter or dairy products?

**Q20e. No= (0) Yes= (1)**

f) If so, do you use pasteurized milk to make these products?

**Q20f. No= (0) Yes= (1)**

g) Does anyone in your household eat soft cheeses such as Brie, Camembert, feta or queso fresco?

**Q20g-1. No= (0) Yes= (1)**
Who?
**Q 20g-2. = N/A**
**Q20g-3. = Self and/or Spouse**
**Q20g-4. = Whole family (including kids)**
**Q20g-5a. = Other household member**
**Q20g-5b.= List other:_____**

h) Do you know if the soft cheese you eat in your home is made with pasteurized milk?

**Q20h-1. No=(0) Yes= (1)**
**Q20h-2. = Assume it is**

**21) EGGS**

a) What types of eggs does your family use? (Note all that apply)

**Q 21a-1. = shell eggs**
**Q 21a-2. = liquid eggs**

**Q 21a-3. = pasteurized shell eggs**
**Q 21a-4. = don't eat eggs**
**Q 21a-5a. = other**
**Q 21a-5b = List other: _____**

**b)** Where do you store fresh eggs?
**Q 21b-1. = carton (refrig shelf)**
**Q 21b-2. = egg holder (refrig door)**
**Q 21b-3. = room temp.**
**Q 21b-4a. = other**
**Q 21b-4b. = List other: _____**

**c)** How long do you generally keep your eggs?
**Q 21c-1. = up to 2 weeks**
**Q 21c-2 = up to 4 weeks**
**Q 21c-3. = don't know**
**Q 21c-4a. = other**
**Q 21c-4b. = List other: _____**

**d)** If farm-fresh eggs, are the outside shells cleaned or sanitized before storing?
**Q 21d-1. No= (0) Yes= (1)**
How?
**Q 21d-2. =rinse/wipe off outer shell**
**Q 21d-3. = wash outer shell w/ soap**
**Q 21d-4a. = other**
**Q 21d-4b. = List other: _____**

**e)** If farm-fresh eggs, are the outside shells cleaned just prior to eating?
**Q 21e-1. No= (0) Yes= (1)**
How?
**Q 21e-2. = rinse/wipe off outer shell**
**Q 21e-3. = wash outer shell w/ soap**
**Q 21e-4a. = other**
**Q 21e-4b. = List other: _____**

**RTE FOODS**

**Now I'd like to ask some questions about ready-to-eat products. These include foods such as hotdogs, luncheon and deli meats...foods that do not require any additional cooking prior to eating.**

22) **Hotdogs**

**a)** How often do you purchase hotdogs?
**Q 22a-1. = Never**
**Q 22a-2. = (1-2x/yr)**
**Q 22a-3. = (every 2-3 mo)**
**Q 22a-4. = monthly**

**Q 22a-5. = weekly**
**Q 22a-6. = Comments:**

**b)** How are these generally eaten?
**Q 22b-1. = hot**
**Q 22b-2. = cold right out of the package**
**Q 22b-3. = N/A**
**Q 22b-4. = Comments:**

*Part of this question was included in the Risky Foods Procurement Index (RFPI) and coded as follows:*
hot = 5
cold right out of the package = 0
N/A = missing value (.)

**c)** How do you store packages of hotdogs that you do not plan to use right away?
**Q 22c-1. = refrigerate**
**Q 22c-2. = freeze until use**
**Q 22c-3. = other**
**Q22c-4. = List: _____**

**d)** If you freeze them, how do you thaw these before eating?
**Q 22d-1. = refrigerator**
**Q 22d-2. = counter**
**Q 22d-3. = microwave**
**Q 22d-4a. = other**
**Q 22d-4b. = List other: _____**

**23) <u>Deli Meats</u>**
**a)** How often do you purchase pre-packaged luncheon or deli meats?
**Q23a-1. = weekly**
**Q23a-2. = monthly**
**Q23a-3. = 2 to 6 times/year**
**Q23a-4. = don't purchase**
**Q23a-5. = Comments: _____**

*This question was included in the Risky Foods Procurement Index (RFPI) and coded as follows:*
weekly = 1
monthly = 3
2 to 6 times/year = 4
don't purchase = 5

b) How are these generally eaten?

**Q23b-1. = hot**
**Q23b-2. = cold**
**Q23b-3. = N/A**
**Q23b-4. = Comments: _____**

*This question was included in the Risky Foods Procurement Index (RFPI) and coded as follows:*
hot = 5
cold right out of the package = 0
N/A = missing value (.)

c) How do you store packages of luncheon or deli meats that you do not plan to use right away?

**Q23c-1. = refrigerate**
**Q23c-2. = freeze until use**
**Q23c-3. = N/A**
**Q23c-4a. = other**
**Q23c-4b. = List other: _____**

d) How often do you purchase other processed or cured meats, like **Salami** or trail bologna*?
(*trail bologna is a cured product made from wild game)

**Q23d-1. = don't purchase**
**Q23d-2. = (1-2x/yr)**
**Q23d-3. = (every 2-3 mo)**
**Q23d-4. = monthly**
**Q23d-5. = weekly**
**Q23d-6. = Comments: _____**

e) How are these generally eaten?

**Q23e-1. = hot**
**Q23e-2. = cold**
**Q23e-3. = N/A**
**Q23e-4. = Comments: _____**

f) Where does this come from?

**Q23f-1. = homemade (yours/others)**
**Q23f-2. = store-bought**
**Q23f-3. = roadside/friend**
**Q23f-4. = Comments: _____**

g) If you freeze them, how do you thaw these before eating?

**Q23g-1. = refrigerator**
**Q23g-2. = counter**
**Q23g-3. = microwave**

**Q23g-4a. = other**
**Q23g-4b. = List other:_____**
**Q23g-5. = Comments: _____**

**h)** What is the longest period of time you would store an <u>unopened</u> package of deli meats or hotdogs before discarding?

**Q23h-1. = used immediately**
**Q23h-2. = 2 -3 days**
**Q23h-3. = 1 week**
**Q23h-4. = 10 days**
**Q23h-5. = 2 weeks or longer**
**Q23h-6. = freeze until ready to use**
**Q23h-7. = Comments: _____**

**i)** How long would you store a package of deli meat or hotdogs once it has been <u>opened</u>?

**Q23i-1. = used immediately**
**Q23i-2. = ~2 -3 days**
**Q23i-3. = ~1 week**
**Q23i-4. = 10 days**
**Q23i-5. = 2 weeks or longer**
**Q23i-6. = thaw amount needed each use**
**Q23i-7. = Comments:_____**

*This question was included in the Risky Foods Procurement Index (RFPI) and coded as follows:*

used immediately = 5
~2 -3 days = 5
~1 week = 4
10 days = 2
2 weeks or longer = 1
thaw amount needed each use = 4

**j)** How do you decide when to discard hot dogs or deli meats?

**Q23j-1. = smell**
**Q23j-2. = visual (slimy, etc.)**
**Q23j-3. = storage time:_____**
**Q23j-4. = when clean out frig**
**Q23j-5. = other**
**Q23j-6. = Comments:_____**

**24) <u>Deli Salads</u>**

**a)** How often do you purchase pre-made deli salads, such as tuna salad, potato or macaroni salad, coleslaw, etc.?

**Q24a-1. = never/once yearly**
**Q24a-2. = 2-6 times/year**
**Q24a-3. = monthly**

**Q24a-4. = weekly**
**Q24a-5. = Comments: _____**

*This question was included in the Risky Foods Procurement Index (RFPI) and coded as follows:*
never/once yearly = 5
2-6 times/year = 4
Monthly = 3
Weekly = 1

**b)** For how long do you generally store these items?
**Q24b-1. = used immediately**
**Q24b-2. = 2 -3 days**
**Q24b-3. = 1 week**
**Q24b-4. = 10 days**
**Q24b-5. = 2 weeks or longer**
**Q24b-6. = until spoiled**
**Q24b-7. = Comments:_____**

*This question was included in the Risky Foods Procurement Index (RFPI) and coded as follows:*
used immediately = 5
2 -3 days = 4
1 week = 3
10 days = 2
2 weeks or longer = 1
until spoiled = 0

**Food Storage**
**Next, I'd like to ask you a few questions related to food storage.**

**25)** For what foods (if any) do you regularly check the date labels for safety and quality?
**Q25a-1. = don't check**
**Q 25a-2. = milk**
**Q 25a-3. = yogurt**
**Q 25a-4. = cheese/butter**
**Q 25a-5. = eggs**
**Q 25a-6. = raw meat/fish/poultry**
**Q 25a-7. = luncheon/deli meats**
**Q 25a-8. = pre-packaged produce**
**Q 25a-9. = juices**
**Q 25a-10. = cereal**
**Q 25a-11. = other**
**Q 25a-12. = Comments:_____**

26) What foods (if any) are you most concerned about the safety of?
**Q 26a-1. = milk**
**Q 26a-2. = yogurt**
**Q 26a-3. = cheese/butter**
**Q 26a-4. = eggs**
**Q 26a-5. = raw meat/fish/poultry**
**Q 26a-6. = luncheon/deli meats**
**Q 26a-7. = pre-packaged produce**
**Q 26a-8. = juices**
**Q 26a-9. = other**
**Q 26a-10. = Comments:**_____

**Thermometer Use**
27) Do you keep a thermometer in your refrigerator?
**Q27. = No (0) Yes (1)**

28) Do you know what temperature your refrigerator is running at?
**Q28a-1. = No (0) Yes (1)**
**Q28a-2. = List:** _____
**Q28a-3. = Comments:** _____

**Food Preparation**

**Now, I have a few questions to ask about usual food preparation.**

29) Do you generally do any clean up before you start meal preparation?
**Q29a-1. = No (0) Yes (1)**
**Q29a-2. = already cleaned previously**

30) If so, what kinds of things do you clean?
**Q30a-1. = wipe surfaces w/ wet sponge/cloth**
**Q30a-2. = wash w/ soap**
**Q30a-3. = wash w/ disinfectant**
**Q30a-4a. = Other**
**Q30a-4b. = List other:** _____
**Q30a-5. = Comments:**_____

**Produce**
31) Do you usually wash your fruits/vegetables?
**Q31a-1. = No (0) Yes (1)**
If yes, how?
**Q31a-2.** = rinse in colander under running water
**Q31a-3.** spray w/ produce wash/rinse
**Q31a-4.**soak in sink
**Q31a-5.** scrub while rinsing

**Q31a-6a.** Other
**Q31a-6b.** List other: _____

32) What about foods such as melons?
**Q32a-1. = don't wash**
**Q32a-2. = rinse under running water**
**Q32a-3. = scrub w/ brush/rinse**

33) What about pre-packaged items such as lettuce?
**Q33. = No (0) Yes (1)**

34) Do you wash anything else that is pre-packaged?
**Q34a-1. = No (0) Yes (1)**
**Q34a-2. = What:** _____

35) What about other fresh veggies, such as head lettuce? Anything else?
**Q35a-1. = No (0) Yes (1)**
**Q35a-2. = What:** _____

**Food Preparation Surfaces**

**Meats**
36) Do you generally rinse your chicken (or other raw meats) in the sink before cooking them?
**Q36a-1. = No (0) Yes (1)**
**Q36a-2. = How:** _____
**Q36a-3. = Comments:**_____

37) What kind of surfaces do you usually prepare raw meat, fish or poultry on?
**Q37a-1. = cutting board**
**Q37a-2. = Plate**
**Q37a-3. = Countertop**
**Q37a-4. = place directly into pan/grill**
**Q37a-5a. = Other**
**Q37a-5b. = List other:**_____
**Q37a-6. = Comments:**_____

**Veggies**
38) What kind of surfaces do you usually prepare vegetables on?
**Q38a-1. = cutting board**
**Q38a-2. = Plate**
**Q38a-3. = Countertop**
**Q38a-4. = place directly into pan/grill**
**Q38a-5a. = Other**
**Q38a-5b. = List other:**_____
**Q38a-6. = Comments:**_____

39) What kind of cutting board(s) do you use?
**Q39a-1a. = plastic**
**Q39a-1b. = used for: _____**
**Q39a-2a. = wood**
**Q39a-2b. = used for: _____**
**Q39a-3. = Comments:_____**

40) Do you use a different cutting board for raw meat than you do for fresh veggies?
**Q40a-1. = No (0) Yes (1)**
**Q40a-2. = Comments:_____**
41) How do you clean your cutting board(s) after use?
**Q41a-1. = rinse w/ water**
**Q41a-2. = wash w/ soap and water**
**Q41a-3. = dishwasher**
**Q41a-4a. = Other**
**Q41a-4b. = List other:_____**
**Q41a-5. = Comments: _____**

*This question was included in the Indoor Cross-contamination Index (ICCI) and was coded as follows:*
rinse w/ water = 1
wash w/ soap and water = 3
dishwasher = 5
Other = missing value (.)

**Handwashing**
**Next, are a few questions about handwashing. I'd like you to think for a moment about when and how often you usually wash your hands in a typical day.**
42) How many times a day do you generally wash your hands?

_____
**Q42a-1. = 0-1 times/day**
**Q42a-2. = 2-3 times/day**
**Q42a-3. = 4-5 times/day**
**Q42a-4. = 6-8 times/day**
**Q42a-5. = 7-9 times/day**
**Q42a-6. = 10 or > times/day**
**Q42a-7. = Comments: _____**

43) Give me some examples of when you wash your hands. (Note all mentioned)
**Q43a-1. = before eating**
**Q43a-2. = after restroom**
**Q43a-3. = after diaper changes**
**Q43a-4. = when dirty**
**Q43a-5. = after taking out garbage**
**Q43a-6. = after sneezing**

**Q43a-7. = after gardening/being outdoors**
**Q43a-8a. = other**
**Q43a-8b. = List other:_____**
**Q43a-9. = Comments:_____**

**44)** For how long do you typically wash?
**Q44a-1. = <5 sec.**
**Q44a-2. = 5-10 sec.**
**Q44a-3. = 10-20 sec.**
**Q44a-4. = >20 sec.**
**Q44a-5. = Comments:_____**

**45)** Do you always use soap?
**Q45a-1. = No (0) Yes (1)**
**Q45a-2. = Comments:_____**

**46)** What do you dry your hands on?
Bathroom
**Q46a-1. = cloth towel**
**Q46a-2. = paper towel**
**Q46a-3. = don't dry**
**Q46a-4. = Comments:_____**

Kitchen:
**Q46b-1. = cloth towel**
**Q46b-2. = paper towel**
**Q46b-3. = don't dry**
**Q46b-4. = Comments:_____**

Utility room:
**Q46c-1. = cloth towel**
**Q46c-2. = paper towel**
**Q46c-3. = don't dry**
**Q46c-4. = N/A**
**Q46c-5. = Comments:_____**

## Food Cooking/Serving

**Now let's talk about meal time-cooking and serving of food and clean-up.**
**47)** How do you determine when meat, hamburger or poultry is done, so that you don't overcook it?
**Q47a-1. = sight (outside appearance)**
**Q47a-2. = cut open-no longer pink in middle**
**Q47a-3. = time**
**Q47a-4. = food thermometer**

**Q47a-5. = Comments:**_____

**48)** When you grill out, do you use the same or a different plate/platter and utensils for cooked and uncooked foods?
**Q48a-1. = same**
**Q48a-2. = clean plate/platter/utensils**
**Q48a-3. = Comments:**_____

**49)** Do you ever use a food thermometer to test for doneness of meat, hamburger, fish or poultry?
**Q49a-1. = No (0) Yes (1)**
For what foods?
**Q49a-2. = whole turkey/chix**
**Q49a-3. = roasts**
**Q49a-4. = ham**
**Q49a-5. = grilled foods (steak, hamburgers)**
**Q49a-6. = chicken breasts**
**Q49a-7. = pork chops**
**Q49a-8. = fish**
**Q49a-9. = Comments:**_____

b. If yes, what kind of thermometer:
**Q49b-1. = bimetal stem thermometer**
**Q49b-2. = digital**
**Q49b-3a. = other**
**Q49b-3b. = list other:** _____

c.If yes, how do you decide what temperatures to cook the foods to?
**Q49c-1. = List:** _____
**Q49c-2. = Comments:** _____

**Leftover Storage and Cleanup**

**50)** Approximately how long after the meal do you generally put away leftovers and clean up?
**Q50a-1. = right away**
**Q50a-2. = within 30 minutes**
**Q50a-3. = 30 min.to 1 hour**
**Q50a-4. = 1-2 hours**
**Q50a-5. = > 2hrs**
**Q50a-6a. = depends on the occasion**
**Q50a-6b. = list:**_____
**Q50a-7. = Comments:**_____

**51)** For how long do you generally store your leftovers?
don't keep leftovers

**Q51a-1. = 1-2 days**
**Q51a-2. = 3-5 days**
**Q51a-3. = 6-7 days**
**Q51a-4. = 7-10 days**
**Q51a-5. = up to 2 wks**
**Q51a-6. = Comments:**_____

52) How do you initially dispose of leftover or spoiled foods? (Check all that apply.)
**Q52a-1. = sink disposal.**
**Q52a-2. = What foods?** _____
**Q52a-3. = compost bin- indoors**
**Q52a-4. = compost bin- outdoors**
**Q52a-5. = compost bin- both (when full-take out)**
**Q52a-6. = Comments:** _____

53) Is your compost bin accessible to animals?
**Q53a-1. = No (0) Yes (1) N/A (2)**
**Q53a-2.= trash container inside house.**
**Q53a-3.= what foods?** _____
**Q53a-4.= outdoor trash receptacle**
**Q53a-5 = what foods?** _____
**Q53a-6 = leftovers fed to animals**
**Q53a-7. = Where:** _____
**Q53a-8. = Comments:** _____

54) Where is your indoor trash receptacle kept?
**Q54a-1. = under sink**
**Q54a-2. = free-standing in kitchen**
**Q54a-3. = N/A**
**Q54a-4a. = Other**
**Q54a-4b. = List other:**
**Q54a-5. = Is it covered? No = (0) Yes = (1)**
**Q54a-6. = Is it open? No = (0) Yes = (1)**
**Q54a-7. = Comments:**_____

55) Where is your outdoor trash receptacle kept?
**Q55a-1. = Location:** _____
**Q55a-2. = Is it covered? No = (0) Yes = (1)**
**Q55a-3. = Is it open? No = (0) Yes = (1)**
**Q55a-4. = Comments:**_____

<u>Kitchen Cleaning Procedures</u>

**56) How often do you generally clean your kitchen countertops?**

**Q56a-1. = after meals**
**Q56a-2. = when needed**
**Q56a-3. = before meal prep if dirty**
**Q56a-4. = don't clean regularly**
**Q56a-5a. = other**
**Q56a-5b. = List other: _____**
**Q56a-6. = Comments: _____**

**57)** What do you use to clean with?
**Q57a-1. = wet sponge**
**Q57a-2. = wet dish cloth**
**Q57a-3. = wet paper towel**
**Q57a-4. = soapy sponge**
**Q57a-5. = soapy dish cloth**
**Q57a-6. = disinfectant/ paper towel**
**Q57a-7a = Other**
**Q57a-7b = List other: _____**
**Q57a-8. = Comments: _____**

**58)** How often do you clean your kitchen sink? What are some examples?
**Q58a-1. = after meals**
**Q58a-2. = when needed**
**Q58a-3. = before meal prep if dirty**
**Q58a-4. = don't clean regularly**
**Q58a-5a. = other**
**Q58a-5b. = list other: _____**
**Q58a-6. = Comments: _____**

**59)** How do you clean your kitchen sink?
**Q59a-1.** = rinse w/ water
**Q59a-2.** = wash w/ soapy sponge or cloth
**Q59a-3.** = wipe w/ disinfectant or cleanser
**Q59a-4a. = other**
**Q59a-4b. = list other: _____**
**Q59a-5. = Comments: _____**

**60)** a. How (if at all) do you clean your garbage disposal?
**Q60a-1. = rinse w/ water**
**Q60a-2. = wash w/ soapy sponge or cloth**
**Q60a-3. = wipe w/ disinfectant or cleanser**
**Q60a-4. = don't have disposal**
**Q60a-5a. = other**
**Q60a-5b. = list other: _____**
**Q60a-6. = Comments: _____**

b. When or how often?

**Q60b-1. = daily**
**Q60b-2. = weekly**
**Q60b-3. = monthly**
**Q60b-4. = as needed**
**Q60b-5a. = other**
**Q60b-5b. = list other:_____**
**Q60b-6. = Comments: _____**

**61)** a. How (if at all) do you clean the shelves in your refrigerator?
**Q61a-1. = don't clean**
**Q61a-2. = wipe w/ wet sponge or cloth**
**Q61a-3. = wash w/ soapy sponge or cloth**
**Q61a-4. = wipe w/ disinfectant or cleanser**
**Q61a-5a. = other**
**Q61a-5b. = list other: _____**
**Q61a-6. = Comments: _____**

b. How often?
**Q61b-1. = rarely**
**Q61b-2. = if something spills**
**Q61b-3. = 2-3 times/year**
**Q61b-4. = every 1-2 months**
**Q61b-5. = weekly**
**Q61b-6a. = other**
**Q61b-6b. = list other: _____**
**Q61b-7. = Comments: _____**

**62)** How often do you clean your kitchen floors?
**Q62a-1. = weekly**
**Q62a-2. = monthly**
**Q62a-3. = every 2-3 months**
**Q62a-4. = rarely**
**Q62a-5. = if something spills (as needed)**
**Q62a-6a. = other**
**Q62a-6b. = list other:_____**
**Q62a-7. = Comments: _____**

**63)** What cleaning products do you use?
**Q63a-1. = water only**
**Q63a-2. = vinegar/water**
**Q63a-3. = commercial cleaning products**
**Q63a-4a. = other**
**Q63a-4b. = list other: _____**
**Q63a-5. = Comments: _____**

**<u>Food Safety Information Needs</u>**

**Lastly, because we plan to include an education component in year 2 of this study, I'd like to find out what kinds of information you would like to learn more about.**

**64)** Where do you typically get information about foods and nutrition?
**Q64a-1. = news media**
**Q64a-2. = magazines/print**
**Q64a-3. = internet**
**Q64a-4. = communication**
**Q64a-5a. = other**
**Q64a-5b. = list other:** _____
**Q64a-6. = Comments:** _____

**65)** What sources would you turn to for information about how to safely handle and prepare foods?
**Q65a-1. = have never sought out information**
**Q65a-2. = magazines/books**
**Q65a-3. = internet**
**Q65a-4. = gov't publications**
**Q65a-5a. = other**
**Q65a-5b. = list other:** _____
**Q65a-6. = Comments:**_____

**66)** What is (or would be) your preferred format for receiving food-related information?
**Q66a-1. = printed brochure**
**Q66a-2. = web-based module**
**Q66a-3. = attend a class**
**Q66a-4a. = other**
**Q66a-4b. = list other:** _____
**Q66a-5. = Comments:**_____

**67)** Does your household currently have a computer?
**Q67a-1. = No (0) Yes (1)**
**68)** Do you have internet access?
**Q68a-1. = No (0) Yes (1)**

**69)** Would you be willing to complete a brief (~30 minutes) web-based educational module about prevention of listeriosis and other potential foodborne illnesses?
**Q69a-1. = No (0) Yes (1)**
**Q69a-2. = somewhat**
**Q69a-3. = Comments:**_____

**70)** What food safety-related topics would you like to learn more about?
**Q70a-1. = general food safety tips for preventing FBI**
**Q70a-2. = listeria/other pathogens for high risk individuals**
**Q70a-3. = temperatures for cooking meats**
**Q70a-4a. = other**

**Q70a-4b. = list other: _____**
**Q70a-5. = Comments:_____**

This concludes our interview. Thank you for your time. Schedule follow-up visit for sampling.

**Appendix 7. Kitchen Safety Checklist**

**Q.1. General Observations**
**Overall Cleanliness of kitchen: (Not clean) 1 2 3 4 5 (Very clean)**
(clean dishes/utensils, clean counters, clean appliances, clean floors, clean garbage area)
Comments:

**Q.2. Check all that apply:**

| | | | |
|---|---|---|---|
| a. Microwave | __2_ clean | __1_ dirty | __0_Not observable |
| b. Stove/oven | __2_ clean | __1_ dirty | |
| c. Dishes/utensils: | __2_ clean | __1_ dirty/piling up | |
| d. Counter top/table: | __2_ clean | __1_ dirty | |
| e. Hand soap by sink: | __2_ yes | __1_ no | |
| f. Sponge/dishcloth: | __2_ clean | __1_ dirty | |
| g. Cutting boards | __2_ safe | __1_ unsafe (grooves, split,etc.) | |
| Describe: | | | |
| h. Trash/garbage area | __2_ clean | __1_dirty | |
| i. Perishable food at room temp. | __2_ yes | __1_ no | |
| Describe: | | | |

*Part of this question was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

| | | |
|---|---|---|
| Stove/oven | __5_ clean | __0_ dirty |
| Dishes/utensils: | __5_ clean | __0_ dirty/piling up |
| Counter top/table: | __5_ clean | __0_ dirty |
| Hand soap by sink: | __5_ yes | __0_ no |
| Sponge/dishcloth: | __5_ clean | __0_ dirty |
| Cutting boards | __5_ safe | __0_ unsafe |
| Trash/garbage area | __5_ clean | __0_ dirty |
| Perishable food at room temp. | __0_ yes | __5_ no |

**Refrigerator**
**Q3. Refrigerator Temperature:** __a.___°F (__b.___ °C)
≤40 = 5
41-49 = 3
≥50 = 0
*Part of this question was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*
Below 40°F = 5
Between 40 – 50°F = 3

Above 50°F = 0

(c. ) **0= no thermometer**
**1= door**
**2= front, high shelf or drawer**
**3= back, high shelf or drawer**
**4= front, low shelf or drawer**
**5= back, low shelf or drawer**

| **Q4. Type of Refrigerator/Freezer:** | **Yes** | **No** |
|---|---|---|
| a. ____ side-by-side (2 door) | **1** | **0** |
| b. ____ top-bottom (freezer on top or bottom?) | **1** | **0** |
| c. ____ stand alone freezer (deep freeze) | **1** | **0** |

**Q.5. Cleanliness of Refrigerator:** (Not clean) **1 2 3 4 5** (Very clean)

| **Q6. Observations:** | **Yes** | **No** | **not observable** |
|---|---|---|---|
| a. _____ meat items are stored below RTE foods | **1** | **0** | **(.)** |
| b. _____ visibly spoiled food | **1** | **0** | |
| List: _____ | | | |
| c. _____ items past "use-by" date | **1** | **0** | |
| List: _____ | | | |
| d. _____ leftovers: 1. _____uncovered | **0** | **1** | **(.)** |
| 2. _____covered | **1** | **0** | **(.)** |
| e. _____ odor | **1** | **0** | |
| f. _____ unsafe food storage | **1** | **0** | |
| Describe:_____ | | | |
| g. _____ spills/juices dripping | **1** | **0** | |
| h. _____ any items labeled and dated? | **0** | **1** | **(.)** |

**3= either/or**

**One or the other=3**

*Part of this question was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*

visibly spoiled food: Yes = 0, No = 5

items past "use-by" date: Yes = 0, No = 5

leftovers: uncovered = 0, covered = 5

odor : Yes = 0, No = 5

unsafe food storage: Yes = 0, No = 5

spills/juices dripping: Yes = 0, No = 5

**Q.7a.** How full is the refrigerator?

**1= less than 1/3 full**
**2= 1/3 to 2/3 full**
**3= more than 2/3 full**

*This question was included in the Kitchen and Househols Cleanliness Index (KHCI) and was coded as follows:*

less than 1/3 full = 5
1/3 to 2/3 full = 3
more than 2/3 full = 0

**Q7b.** Adequate air circulation? _2__yes _1__no

*This question was included in the Kitchen and Household Cleanliness Index (KHCI) and was coded as follows:*
Yes = 5, No = 0

**Q7c. Comments:**

# APPENDIX VII
## Data tables

Appendix Table 1 (Figure 4.1). *L. monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with lactate/diacetate after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Watts (Power level) | Time (s) | 18/0 | 18/3 | 18/7 |
| Control (no microwave heating, 120 s) | | 1.0±0.1$^{a}$ | 1.0±0.2$^{a}$ | 1.0±0.2$^{a}$ |
| 1100 | 30 | 0.8±0.2$^{a}$ | 0.8±0.2$^{a}$ | 0.7±0.1$^{ab}$ |
| (High) | 45 | 0.3±0.1$^{a}$ | 0.2±0.0$^{b}$ | 0.5±0.1$^{b}$ |
| | 60 | -0.2±0.3$^{bc}$ | <-0.4±0.0$^{c}$ | <-0.4±0.0$^{c}$ |
| | 75 | <-0.4±0.0$^{d}$ | <-0.4±0.0$^{c}$ | <-0.4±0.0$^{c}$ |
| 550 | 60 | 0.9±0.3$^{a}$ | 0.6±0.4$^{ab}$ | 0.8±0.4$^{ab}$ |
| (Medium) | 75 | 0.6±0.1$^{a}$ | 0.5±0.2$^{ab}$ | 0.4±0.2$^{b}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 2 (Figure 4.2). *L. monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with lactate/diacetate after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Watts (Power level) | Time (s) | 36/0 | 36/3 | 36/7 |
| Control (no microwave heating, 120 s) | | 0.9±0.1$^{a}$ | 0.7±0.0$^{a}$ | 1.0±0.1$^{a}$ |
| 1100 | 30 | 0.6±0.3$^{ab}$ | 0.8±0.0$^{a}$ | 0.7±0.1$^{abc}$ |
| (High) | 45 | 0.3±0.0$^{b}$ | 0.4±0.0$^{a}$ | 0.3±0.2$^{bcd}$ |
| | 60 | <-0.4±0.1$^{c}$ | -0.3±0.2$^{b}$ | -0.3±0.1$^{ed}$ |
| | 75 | <-0.4±0.0$^{c}$ | <-0.4±0.0$^{b}$ | <-0.4±0.0$^{f}$ |
| 550 | 60 | 0.4±0.1$^{ab}$ | 0.7±0.3$^{a}$ | 0.8±0.2$^{ab}$ |
| (Medium) | 75 | 0.4±0.1$^{ab}$ | 0.4±0.0$^{a}$ | 0.1±0.3$^{cde}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 3 (Figure 4.3). *L. monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with lactate/diacetate after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating Watts (Power level) | Time (s) | Storage time in days (sealed at 4°C/aerobic at 7°C) 54/0 | 54/3 | 54/7 |
|---|---|---|---|---|
| Control (no microwave heating, 120 s) | | 0.7±0.0$^{a}$ | 0.9±0.3$^{a}$ | 0.8±0.2$^{a}$ |
| 1100 | 30 | 0.6±0.3$^{a}$ | 0.6±0.1$^{a}$ | 0.6±0.0$^{abc}$ |
| (High) | 45 | 0.2±0.4$^{ab}$ | 0.5±0.1$^{a}$ | 0.0±0.0$^{abc}$ |
| | 60 | <-0.4±0.0$^{c}$ | <-0.4±0.1$^{c}$ | -0.2±0.4$^{bc}$ |
| | 75 | <-0.4±0.0$^{c}$ | <-0.4±0.0$^{c}$ | <-0.4±0.0$^{de}$ |
| 550 | 60 | 0.7±0.2$^{a}$ | 0.0±0.2$^{b}$ | 0.5±0.2$^{ab}$ |
| (Medium) | 75 | 0.4±0.2$^{a}$ | 0.4±0.2$^{ab}$ | 0.4±0.2$^{ab}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 4 (Figure 4.4). *L. monocytogenes* counts (mean LogCFU/cm$^2$ ± sd) on frankfurters formulated without lactate/diacetate after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating Watts (Power level) | Time (s) | Storage time in days (sealed at 4°C/aerobic at 7°C) 18/0 | 18/3 | 18/0 |
|---|---|---|---|---|
| Control (no microwave heating, 120 s) | | 1.5±0.1$^{a}$ | 1.7±0.1$^{a}$ | 3.7±0.8$^{a}$ |
| 1100 | 30 | 1.4±0.5$^{a}$ | 2.1±0.2$^{a}$ | 1.4±0.5$^{a}$ |
| (High) | 45 | 1.2±0.4$^{a}$ | 1.7±0.1$^{a}$ | 1.2±0.4$^{a}$ |
| | 60 | 0.0±0.2$^{b}$ | <-0.4±0.0$^{b}$ | 0.0±0.2$^{b}$ |
| | 75 | 0.0±0.1$^{b}$ | <-0.4±0.0$^{b}$ | 0.0±0.1$^{b}$ |
| 550 | 60 | 1.4±0.2$^{a}$ | 1.9±0.2$^{a}$ | 1.4±0.2$^{a}$ |
| (Medium) | 75 | 0.8±0.3$^{ab}$ | 1.7±0.4$^{a}$ | 0.8±0.3$^{ab}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 5 (Figure 4.5). *L. monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without lactate/diacetate after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Watts (Power level) | Time (s) | 36/0 | 36/3 | 36/7 |
| Control (no microwave heating, 120 s) | | 3.3±0.6$^{a}$ | 4.1±0.4$^{a}$ | 3.8±0.0$^{a}$ |
| 1100 | 30 | 3.4±0.9$^{ab}$ | 4.6±1.1$^{a}$ | 5.8±0.8$^{a}$ |
| (High) | 45 | 2.9±0.6$^{a}$ | 4.1±0.2$^{a}$ | 5.5±0.9$^{a}$ |
| | 60 | 1.0±1.2$^{bc}$ | 2.9±0.4$^{a}$ | 4.5±0.4$^{a}$ |
| | 75 | <-0.4±0.0$^{d}$ | 0.3±0.3$^{b}$ | -0.1±0.0$^{b}$ |
| 550 | 60 | 3.0±0.7$^{a}$ | 4.1±0.1$^{a}$ | 5.7±0.9$^{a}$ |
| (Medium) | 75 | 2.8±0.0$^{a}$ | 4.3±0.3$^{a}$ | 5.9±1.0$^{a}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 6 (Figure 4.6). *L. monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without lactate/diacetate after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Watts (Power level) | Time (s) | 54/0 | 54/3 | 54/7 |
| Control (no microwave heating, 120 s) | | 5.9±0.4$^{a}$ | 6.2±0.0$^{ab}$ | 7.2±0.5$^{a}$ |
| 1100 | 30 | 5.0±0.4$^{ab}$ | 7.0±0.3$^{a}$ | 6.8±1.4$^{a}$ |
| (High) | 45 | 4.4±0.3$^{b}$ | 5.6±0.2$^{ab}$ | 7.5±0.6$^{a}$ |
| | 60 | 3.3±0.1$^{c}$ | 4.3±1.3$^{b}$ | 4.8±2.0$^{ab}$ |
| | 75 | 1.3±0.3$^{d}$ | 0.3±0.1$^{c}$ | 2.3±1.4$^{b}$ |
| 550 | 60 | 5.0±0.1$^{ab}$ | 6.0±0.1$^{ab}$ | 6.7±1.3$^{a}$ |
| (Medium) | 75 | 5.1±0.4$^{ab}$ | 5.5±0.5$^{ab}$ | 6.9±0.3$^{a}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 7 (Figure 4.7). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with lactate/diacetate after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating Watts (Power level) | Time (s) | Storage time in days (sealed at 4°C/aerobic at 7°C) 18/0 | 18/3 | 18/7 |
|---|---|---|---|---|
| Control (no microwave heating, 120 s) | | 1.2±0.3$^{a}$ | 1.3±0.3$^{a}$ | 1.3±0.4$^{a}$ |
| 1100 | 30 | 0.9±0.3$^{ab}$ | 1.0±0.2$^{a}$ | 0.8±0.1$^{a}$ |
| (High) | 45 | 0.8±0.2$^{ab}$ | 0.7±0.2$^{a}$ | 0.8±0.2$^{ab}$ |
| | 60 | 0.0±0.4$^{bc}$ | 0.3±0.3$^{a}$ | -0.1±0.2$^{bc}$ |
| | 75 | <-0.4±0.1$^{d}$ | -0.2±0.5$^{a}$ | <-0.4±0.0$^{d}$ |
| 550 | 60 | 1.1±0.3$^{ab}$ | 0.8±0.6$^{a}$ | 1.0±0.2$^{a}$ |
| (Medium) | 75 | 1.0±0.3$^{ab}$ | 0.9±0.2$^{a}$ | 1.0±0.8$^{a}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 8 (Figure 4.8). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with lactate/diacetate after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating Watts (Power level) | Time (s) | Storage time in days (sealed at 4°C/aerobic at 7°C) 36/0 | 36/3 | 36/7 |
|---|---|---|---|---|
| Control (no microwave heating, 120 s) | | 1.5±1.0$^{a}$ | 1.2±0.8$^{a}$ | 0.8±0.2$^{a}$ |
| 1100 | 30 | 0.8±0.1$^{a}$ | 0.9±0.3$^{ab}$ | 1.2±0.4$^{a}$ |
| (High) | 45 | 0.4±0.2$^{ab}$ | 0.4±0.5$^{ab}$ | 0.7±0.3$^{a}$ |
| | 60 | -0.1±0.4$^{bc}$ | -0.2±0.3$^{ab}$ | -0.1±0.3$^{a}$ |
| | 75 | <-0.4±0.0$^{d}$ | <-0.4±0.0$^{c}$ | -0.2±0.6$^{a}$ |
| 550 | 60 | 0.3±0.5$^{ab}$ | 0.6±0.6$^{ab}$ | 1.2±0.4$^{a}$ |
| (Medium) | 75 | 0.6±0.2$^{ab}$ | 0.5±0.3$^{ab}$ | 0.6±0.5$^{a}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 9 (Figure 4.9). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with lactate/diacetate after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating Watts (Power level) | Time (s) | Storage time in days (sealed at 4°C/aerobic at 7°C) 54/0 | 54/3 | 54/7 |
|---|---|---|---|---|
| Control (no microwave heating, 120 s) | | 0.8±0.2$^{a}$ | 1.1±0.2$^{a}$ | 1.0±0.3$^{a}$ |
| 1100 | 30 | 0.8±0.1$^{a}$ | 0.8±0.2$^{a}$ | 0.8±0.1$^{ab}$ |
| (High) | 45 | 0.5±0.5$^{ab}$ | 0.9±0.8$^{a}$ | 0.3±0.5$^{bc}$ |
| | 60 | 0.0±0.4$^{ab}$ | 0.0±0.5$^{a}$ | -0.3±0.3$^{cd}$ |
| | 75 | <-0.4±0.1$^{c}$ | <-0.4±0.0$^{b}$ | <-0.4±0.0$^{e}$ |
| 550 | 60 | 0.9±0.2$^{a}$ | 1.1±0.8$^{a}$ | 0.8±0.2$^{ab}$ |
| (Medium) | 75 | 0.7±0.3$^{a}$ | 0.7±0.3$^{a}$ | 0.8±0.1$^{ab}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 10 (Figure 4.10). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without lactate/diacetate after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating Watts (Power level) | Time (s) | Storage time in days (sealed at 4°C/aerobic at 7°C) 18/0 | 18/3 | 18/7 |
|---|---|---|---|---|
| Control (no microwave heating, 120 s) | | 1.8±0.3$^{a}$ | 1.8±0.2$^{a}$ | 3.4±0.3$^{a}$ |
| 1100 | 30 | 1.7±0.5$^{a}$ | 2.0±0.3$^{a}$ | 2.5±1.2$^{ab}$ |
| (High) | 45 | 1.2±0.2$^{a}$ | 1.8±0.4$^{a}$ | 2.6±0.8$^{ab}$ |
| | 60 | 1.3±0.9$^{a}$ | 0.1±0.4$^{b}$ | 0.6±1.2$^{ab}$ |
| | 75 | 0.1±0.8$^{a}$ | 0.2±0.5$^{b}$ | -0.2±0.5$^{b}$ |
| 550 | 60 | 1.7±0.6$^{a}$ | 1.7±0.3$^{a}$ | 3.4±0.5$^{a}$ |
| (Medium) | 75 | 1.1±0.4$^{a}$ | 1.8±0.4$^{a}$ | 2.9±0.5$^{ab}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 11 (Figure 4.11). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without lactate/diacetate after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating Watts (Power level) | Time (s) | Storage time in days (sealed at 4°C/aerobic at 7°C) 36/0 | 36/3 | 36/7 |
|---|---|---|---|---|
| Control (no microwave heating, 120 s) | | 3.5±1.0[a] | 4.3±0.7[a] | 6.1±1.1[a] |
| 1100 | 30 | 3.2±0.8[a] | 4.9±0.9[a] | 5.5±0.3[a] |
| (High) | 45 | 3.2±0.8[a] | 3.9±0.4[a] | 5.5±0.9[a] |
| | 60 | 1.8±0.9[a] | 3.4±0.6[a] | 4.7±0.8[a] |
| | 75 | -0.3±0.2[b] | 0.3±0.7[b] | <-0.4±0.0[b] |
| 550 | 60 | 2.6±0.6[a] | 4.3±0.7[a] | 5.5±0.7[a] |
| (Medium) | 75 | 2.9±0.8[a] | 4.4±0.3[a] | 6.0±0.8[a] |

[abc]Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 12 (Figure 4.12). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without lactate/diacetate after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating Watts (Power level) | Time (s) | Storage time in days (sealed at 4°C/aerobic at 7°C) 54/0 | 54/3 | 54/7 |
|---|---|---|---|---|
| Control (no microwave heating, 120 s) | | 5.7±0.2[a] | 6.2±0.5[a] | 7.3±0.6[a] |
| 1100 | 30 | 5.2±0.4[a] | 6.1±0.5[a] | 7.3±0.5[a] |
| (High) | 45 | 4.4±1.0[ab] | 5.6±0.3[a] | 7.1±0.2[a] |
| | 60 | 3.4±0.5[b] | 4.5±1.0[a] | 5.9±0.6[a] |
| | 75 | 1.7±0.9[c] | 1.1±1.0[b] | 3.8±0.7[b] |
| 550 | 60 | 5.1±0.5[a] | 6.0±0.7[a] | 7.3±0.4[a] |
| (Medium) | 75 | 5.1±0.3[a] | 5.5±0.7[a] | 6.9±0.3[a] |

[abc]Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 13 (Figure 4.13). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) in water used to reheat frankfurters formulated with lactate/diacetate after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Watts (Power level) | Time (s) | 18/0 | 18/3 | 18/7 |
| Control (no microwave heating, 120 s) | | -0.1±0.6$^{b}$ | -0.9±0.4$^{a}$ | -1.6±0.0$^{b}$ |
| 1100 | 30 | 0.6±0.1$^{a}$ | -1.5±0.3$^{ab}$ | -1.5±0.3$^{a}$ |
| (High) | 45 | 0.5±0.1$^{a}$ | -2.0±0.1$^{ab}$ | -2.1±0.1$^{c}$ |
| | 60 | 0.0±0.4$^{a}$ | <-2.4±0.0$^{c}$ | <-2.4±0.0$^{d}$ |
| | 75 | -0.4±0.0$^{b}$ | <-2.4±0.0$^{c}$ | <-2.4±0.0$^{d}$ |
| 550 | 60 | 0.5±0.0$^{a}$ | -1.1±0.9$^{ab}$ | -1.3±0.2$^{a}$ |
| (Medium) | 75 | 0.6±0.1$^{a}$ | -1.9±0.5$^{ab}$ | -1.8±0.2$^{bc}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 14 (Figure 4.14). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) in water used to reheat frankfurters formulated with lactate/diacetate after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Watts (Power level) | Time (s) | 36/0 | 36/3 | 36/7 |
| Control (no microwave heating, 120 s) | | -1.7±0.3$^{a}$ | -1.5±0.0$^{a}$ | -1.6±0.1$^{a}$ |
| 1100 | 30 | -1.7±0.1$^{a}$ | -1.7±0.4$^{ab}$ | -1.5±0.1$^{a}$ |
| (High) | 45 | -1.9±0.0$^{ab}$ | -1.8±0.4$^{ab}$ | -1.7±0.0$^{a}$ |
| | 60 | -2.2±0.2$^{ab}$ | <-2.4±0.0$^{c}$ | -2.3±0.1$^{b}$ |
| | 75 | <-2.4±0.0$^{c}$ | <-2.4±0.0$^{c}$ | -2.3±0.2$^{b}$ |
| 550 | 60 | -1.8±0.0$^{ab}$ | -1.4±0.1$^{a}$ | -1.7±0.3$^{a}$ |
| (Medium) | 75 | -1.7±0.2$^{a}$ | -2.0±0.1$^{ab}$ | -1.8±0.0$^{a}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 15 (Figure 4.15). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) in water used to reheat frankfurters formulated with lactate/diacetate after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Watts (Power level) | Time (s) | 54/0 | 54/3 | 54/7 |
| Control (no microwave heating, 120 s) | | -1.3±0.4$^{a}$ | -1.6±0.1$^{a}$ | -1.7±0.0$^{a}$ |
| 1100 | 30 | -1.4±0.0$^{a}$ | -1.5±0.1$^{a}$ | -1.9±0.1$^{a}$ |
| (High) | 45 | -1.5±0.0$^{a}$ | -1.7±0.0$^{ab}$ | -1.8±0.2$^{a}$ |
| | 60 | <-2.4±0.0$^{b}$ | -2.3±0.1$^{bc}$ | -2.3±0.1$^{a}$ |
| | 75 | <-2.4±0.0$^{b}$ | <-2.4±0.0$^{d}$ | <-2.4±0.0$^{b}$ |
| 550 | 60 | -1.6±0.1$^{a}$ | -1.6±0.3$^{a}$ | -1.7±0.4$^{a}$ |
| (Medium) | 75 | -1.8±0.1$^{a}$ | -1.9±0.0$^{abc}$ | -1.9±0.3$^{a}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 16 (Figure 4.16). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) in water used to reheat frankfurters formulated without lactate/diacetate after treatment in a household microwave oven at 18 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Watts (Power level) | Time (s) | 18/0 | 18/3 | 18/7 |
| Control (no microwave heating, 120 s) | | 1.5±0.1$^{ab}$ | -0.7±0.4$^{cb}$ | 1.4±0.8$^{a}$ |
| 1100 | 30 | 2.0±0.5$^{a}$ | 0.5±0.3$^{a}$ | 0.7±0.4$^{ab}$ |
| (High) | 45 | 0.9±0.1$^{bc}$ | 0.0±0.2$^{ab}$ | 0.8±0.5$^{a}$ |
| | 60 | 0.3±0.5$^{cd}$ | -2.1±0.4$^{de}$ | -1.3±1.6$^{ab}$ |
| | 75 | -0.1±0.2$^{d}$ | <-2.4±0.0$^{f}$ | <-2.4±0.0$^{c}$ |
| 550 | 60 | 1.7±0.1$^{ab}$ | -0.6±0.1$^{b}$ | 1.5±0.6$^{a}$ |
| (Medium) | 75 | 0.7±0.4$^{c}$ | -0.1±0.7$^{bc}$ | 0.9±0.1$^{a}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 17 (Figure 4.17). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) in water used to reheat frankfurters formulated without lactate/diacetate after treatment in a household microwave oven at 36 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Watts (Power level) | Time (s) | 36/0 | 36/3 | 36/7 |
| Control (no microwave heating, 120 s) | | 1.3±1.3$^{a}$ | 2.6±0.8$^{a}$ | 2.8±0.6$^{bc}$ |
| 1100 | 30 | 1.5±0.5$^{a}$ | 2.7±0.7$^{a}$ | 4.1±1.4$^{ab}$ |
| (High) | 45 | 1.6±1.1$^{a}$ | 2.6±0.3$^{a}$ | 3.6±1.2$^{ab}$ |
| | 60 | 0.0±1.9$^{ab}$ | 1.7±1.3$^{a}$ | 1.8±1.1$^{c}$ |
| | 75 | -1.7±1.0$^{b}$ | -1.8±0.3$^{b}$ | -1.5±0.1$^{d}$ |
| 550 | 60 | 2.0±0.7$^{a}$ | 3.6±1.3$^{a}$ | 3.9±0.9$^{ab}$ |
| (Medium) | 75 | 1.5±0.4$^{a}$ | 2.8±0.6$^{a}$ | 4.4±1.1$^{a}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 18 (Figure 4.18). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) in water used to reheat frankfurters formulated without lactate/diacetate after treatment in a household microwave oven at 54 days of storage in vacuum packages at 4°C followed by aerobic storage for 7 days at 7°C.

| Microwave heating | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Watts (Power level) | Time (s) | 54/0 | 54/3 | 54/7 |
| Control (no microwave heating, 120 s) | | 3.4±0.5$^{a}$ | 4.1±0.2$^{a}$ | 5.3±0.2$^{a}$ |
| 1100 | 30 | 3.5±0.4$^{a}$ | 4.6±0.0$^{a}$ | 4.6±1.5$^{a}$ |
| (High) | 45 | 3.1±0.7$^{ab}$ | 4.2±0.2$^{a}$ | 4.9±0.7$^{a}$ |
| | 60 | 1.8±0.2$^{b}$ | 1.9±2.6$^{ab}$ | 3.0±1.9$^{a}$ |
| | 75 | 0.1±0.2$^{c}$ | -1.9±0.8$^{b}$ | 1.3±0.7$^{a}$ |
| 550 | 60 | 3.5±0.3$^{a}$ | 4.2±0.4$^{a}$ | 4.8±0.9$^{a}$ |
| (Medium) | 75 | 3.5±0.3$^{a}$ | 3.9±0.8$^{a}$ | 5.1±0.6$^{a}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 19 (Figure 5.2). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 18 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 18/0 | 18/7 | 18/14 |
| Dry control | | 1.7±0.4$^{a}$ | 2.8±1.2$^{a}$ | 5.0±2.1$^{a}$ |
| Control, 25 | 300 | 1.1±0.3$^{a}$ | 2.4±0.9$^{a}$ | 3.6±1.0$^{a}$ |
| | 420 | 1.2±0.3$^{a}$ | 2.3±1.1$^{ab}$ | 4.3±1. 9$^{a}$ |
| 80 | 0 | 0.3±0.5$^{b}$ | 1.8±0.7$^{ab}$ | 3.5±1.2$^{ab}$ |
| | 30 | -0.2±0.5$^{bc}$ | 0.9±0. 9$^{bc}$ | 2.9±1. 8$^{abc}$ |
| | 60 | -0.4±0.0$^{d}$ | -0.3±0.2$^{c}$ | 1.1±0.9$^{bcde}$ |
| | 120 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.3±0.4$^{e}$ |
| 94 | 0 | 0.3±0.4$^{b}$ | 1.6±0.6$^{ab}$ | 2.6±1.4$^{abcd}$ |
| | 30 | -0.4±0.0$^{d}$ | -0.2±0.6$^{c}$ | 0.9±1.1$^{cde}$ |
| | 60 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.3±0.4$^{e}$ |
| | 120 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.1$^{de}$ |
| 94 and removed from heating source | 300 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{f}$ |
| | 180 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{f}$ |
| | 300 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{f}$ |
| | 420 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{f}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 20 (Figure 5.3). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 40 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 40/0 | 40/7 | 40/14 |
| Dry control | | 2.7±1.5$^{a}$ | 4.4±2.5$^{a}$ | 6.7±2.7$^{a}$ |
| Control, 25 | 300 | 1.9±0. 9$^{ab}$ | 3.5±2.0$^{a}$ | 4.3±2.3$^{ab}$ |
| | 420 | 2.3±1.2$^{ab}$ | 3.6±2.1$^{ab}$ | 5.1±2.3$^{ab}$ |
| 80 | 0 | 2.1±0.6$^{ab}$ | 3.0±1.8$^{abc}$ | 5.2±2.9$^{ab}$ |
| | 30 | 0.8±1.0$^{bc}$ | 2.3±1.7$^{abc}$ | 3.6±3.0$^{abc}$ |
| | 60 | -0.1±0.4$^{c}$ | 1.1±1.6$^{abc}$ | 1. 9±2.2$^{bc}$ |
| | 120 | -0.4±0.0$^{d}$ | -0.3±0.3$^{c}$ | -0.4±0.0$^{d}$ |
| 94 | 0 | 1.9±0.8$^{ab}$ | 3.1±2.5$^{abc}$ | 3.9±3.1$^{abc}$ |
| | 30 | -0.4±0.2$^{c}$ | 1.5±1.5$^{abc}$ | 2.3±2.2$^{abc}$ |
| | 60 | -0.4±0.0$^{d}$ | 0.0±0.7$^{bc}$ | 1.0±1.4$^{bc}$ |
| | 120 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | 0.4±0.9$^{bc}$ |
| 94 and removed from heating source | 300 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ |
| | 180 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ |
| | 300 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ |
| | 420 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 21 (Figure 5.4). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 60 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 60/0 | 60/7 | 60/14 |
| Dry control | | 4.5±2.1$^{a}$ | 6.1±1.8$^{a}$ | 5.5±1.8$^{a}$ |
| Control, 25 | 300 | 3.8±1.4$^{ab}$ | 4.4±2.1$^{ab}$ | 5.3±2.7$^{ab}$ |
| | 420 | 3.8±1.8$^{abc}$ | 4.7±1.9$^{ab}$ | 6.1±1.3$^{ab}$ |
| 80 | 0 | 3.3±1.8$^{abcd}$ | 5.3±1.8$^{ab}$ | 4.9±3.7$^{ab}$ |
| | 30 | 2.0±1.3$^{abcde}$ | 4.1±2.7$^{abcd}$ | 4.6±2.8$^{ab}$ |
| | 60 | 1.0±1.3$^{cde}$ | 2.2±2.1$^{bcd}$ | 2.9±2.8$^{ab}$ |
| | 120 | -0.2±0.0$^{e}$ | 0.4±1.2$^{d}$ | 1.0±1.1$^{b}$ |
| 94 | 0 | 2.9±1.6$^{abcd}$ | 4.4±3.1$^{abc}$ | 4.6±3.7$^{ab}$ |
| | 30 | 1.4±1.2$^{bcde}$ | 1.9±2.0$^{bcd}$ | 3.4±2.9$^{ab}$ |
| | 60 | -0.3±0.2$^{e}$ | 1.7±1.6$^{bcd}$ | 2.1±2.2$^{ab}$ |
| | 120 | -0.4±0.0$^{f}$ | -0.3±0.2$^{cd}$ | 0.9±0.8$^{ab}$ |
| 94 and removed from heating source | 300 | -0.4±0.0$^{f}$ | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ |
| | 180 | -0.4±0.0$^{f}$ | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ |
| | 300 | -0.4±0.0$^{f}$ | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ |
| | 420 | -0.4±0.0$^{f}$ | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$).

Appendix Table 22 (Figure 5.5). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 18 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 18/0 | 18/7 | 18/14 |
| Dry control | | 1.8±0.4$^{a}$ | 2.7±1.2$^{a}$ | 5.0±2.2$^{a}$ |
| Control, 25 | 300 | 1.2±0.3$^{a}$ | 2.6±0.9$^{a}$ | 4.2±1.2$^{a}$ |
| | 420 | 1.1±0.2$^{a}$ | 2.5±1.1$^{a}$ | 4.3±1. 9$^{a}$ |
| 80 | 0 | 0.8±0.4$^{a}$ | 2.3±0.8$^{a}$ | 4.4±0.9$^{a}$ |
| | 30 | 0.9±1.9$^{a}$ | 1.9±1.3$^{ab}$ | 3.1±0.6$^{ab}$ |
| | 60 | -0.0±0.6$^{a}$ | 0.9±0.8$^{abcd}$ | 1.7±1.6$^{ab}$ |
| | 120 | -0.4±0.1$^{a}$ | -0.2±0.3$^{a}$ | -0.1±0.6$^{b}$ |
| 94 | 0 | 0.3±0.5$^{a}$ | 1.8±0.4$^{abc}$ | 2.4±1.4$^{ab}$ |
| | 30 | -0.3±0.3$^{a}$ | 0.1±0.8$^{bcd}$ | 1.4±1.3$^{ab}$ |
| | 60 | -0.4±0.0$^{b}$ | -0.1±0.4$^{cd}$ | 0. 5±0.9$^{b}$ |
| | 120 | -0.4±0.0$^{b}$ | -0.4±0.0$^{e}$ | 0.1±0.8$^{b}$ |
| 94 and removed from heating source | 300 | -0.4±0.0$^{b}$ | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ |
| | 180 | -0.4±0.0$^{b}$ | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ |
| | 300 | -0.4±0.0$^{b}$ | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ |
| | 420 | -0.4±0.0$^{b}$ | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 23 (Figure 5.6). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 40 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 40/0 | 40/7 | 40/14 |
| Dry control | | 2.8±1.6$^{a}$ | 4.5±2.5$^{a}$ | 6.5±2.4$^{a}$ |
| Control, 25 | 300 | 2.6±1.6$^{a}$ | 3.9±2.0$^{a}$ | 5.7±1.9$^{a}$ |
| | 420 | 2.7±1.6$^{ab}$ | 3.2±2.7$^{abc}$ | 5.8±3.0$^{ab}$ |
| 80 | 0 | 2.6±0.5$^{a}$ | 4.0±1.8$^{ab}$ | 5.9±2.0$^{ab}$ |
| | 30 | 1.5±0.6$^{abc}$ | 3.3±1.2$^{abc}$ | 4.5±2.7$^{abc}$ |
| | 60 | 0.3±0.5$^{abc}$ | 1.9±1.3$^{abc}$ | 1.9±2.7$^{bc}$ |
| | 120 | -0.4±0.0$^{bc}$ | 0.1±0.6$^{c}$ | 0.1±0.7$^{c}$ |
| 94 | 0 | 1.6±1.1$^{abc}$ | 3.4±1.8$^{abc}$ | 4.2±3. 9$^{abc}$ |
| | 30 | 0.4±0.8$^{abc}$ | 1.7±1.6$^{abc}$ | 3.6±1.8$^{abc}$ |
| | 60 | -0.4±0.1$^{c}$ | -0.2±0.3$^{c}$ | 1.4±1.6$^{bc}$ |
| | 120 | -0.1±0.7$^{bc}$ | -0.4±0.0$^{d}$ | 0.7±1.2$^{bc}$ |
| 94 and removed from heating source | 300 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ |
| | 180 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ |
| | 300 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ |
| | 420 | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{d}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 24 (Figure 5.7). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 60 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 60/0 | 60/7 | 60/14 |
| Dry control | | 4.9±2.0$^{a}$ | 6.4±2.0$^{a}$ | 7.2±1.7$^{a}$ |
| Control, 25 | 300 | 4.2±1.3$^{a}$ | 5.3±2.4$^{ab}$ | 5.9±2.4$^{abc}$ |
| | 420 | 3.7±1.7$^{abc}$ | 5.5±2.5$^{ab}$ | 6. 6±1.2$^{ab}$ |
| 80 | 0 | 3.8±1.2$^{ab}$ | 6.1±2.2$^{ab}$ | 5.1±3.1$^{abcd}$ |
| | 30 | 2.3±1.0$^{abcd}$ | 4.8±2.6$^{abc}$ | 5.0±3.1$^{abcd}$ |
| | 60 | 1.2±1.3$^{cd}$ | 3.2±2.8$^{abc}$ | 3.6±2.2$^{abcd}$ |
| | 120 | 0.0±1.3$^{b}$ | 1.2±1.3$^{c}$ | 1.1±1.2$^{d}$ |
| 94 | 0 | 3.1±1.5$^{abc}$ | 5.0±2.9$^{abc}$ | 5.2±3.4$^{abcd}$ |
| | 30 | 1.7±1.3$^{bcd}$ | 2.7±2.4$^{abc}$ | 3.8±2.6$^{abcd}$ |
| | 60 | 0.3±0.9$^{d}$ | 1.8±1.6$^{bc}$ | 2. 5±2.5$^{bcd}$ |
| | 120 | -0.4±0.0$^{e}$ | -0.4±0.0$^{d}$ | 1.1±0.8$^{cd}$ |
| 94 and removed from heating source | 300 | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ | -0.4±0.0$^{d}$ |
| | 180 | -0.4±0.0$^{e}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{e}$ |
| | 300 | -0.4±0.0$^{e}$ | -0.4±0.0$^{d}$ | -0.4±0.0$^{e}$ |
| | 420 | -0.4±0.0$^{e}$ | -0.4±0.0$^{d}$ | -0.4±0.0e |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 25 (Figure 5.8). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 18 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 18/0 | 18/7 | 18/14 |
| Dry control | | 1.5±0.2$^a$ | 1.3±0.0$^a$ | 1.6±0.2$^a$ |
| Control, 25 | 300 | 0.8±0.2$^b$ | 0.9±0.1$^b$ | 0.7±0.3$^b$ |
| 80 | 0 | -0.1±0.3$^c$ | -0.4±0.0$^c$ | 0.1±0.4$^c$ |
| | 30 | -0.4±0.1$^c$ | -0.4±0.0$^e$ | -0.4±0.0$^e$ |
| | 60 | -0.4±0.0$^d$ | -0.4±0.0$^e$ | -0.4±0.0$^e$ |
| | 120 | -0.4±0.0$^d$ | -0.4±0.0$^e$ | -0.4±0.0$^e$ |
| 94 | 0 | -0.3±0.2$^c$ | -0.2±0.2$^d$ | -0.3±0.3$^d$ |
| | 30 | -0.4±0.0$^d$ | -0.4±0.0$^e$ | -0.4±0.0$^e$ |
| | 60 | -0.4±0.0$^d$ | -0.4±0.0$^e$ | -0.4±0.0$^e$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 26 (Figure 5.9). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 40 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 40/0 | 40/7 | 40/14 |
| Dry control | | 1.4±0.2$^a$ | 1.3±0.1$^a$ | 1.4±0.1$^a$ |
| Control, 25 | 300 | 0.9±0.7$^a$ | 0.8±0.3$^b$ | 0.9±0.6$^a$ |
| 80 | 0 | -0.1±0.2$^b$ | -0.1±0.3$^c$ | -0.1±0.3$^b$ |
| | 30 | -0.3±0.2$^b$ | -0.4±0.1$^c$ | -0.3±0.3$^b$ |
| | 60 | -0.4±0.1$^b$ | -0.4±0.0$^d$ | -0.4±0.0$^c$ |
| | 120 | -0.4±0.0$^e$ | -0.4±0.0$^d$ | -0.4±0.0$^c$ |
| 94 | 0 | -0.1±0.2$^b$ | -0.4±0.1$^c$ | -0.4±0.0$^c$ |
| | 30 | -0.4±0.0$^c$ | -0.4±0.0$^d$ | -0.4±0.0$^c$ |
| | 60 | -0.4±0.0$^c$ | -0.4±0.0$^d$ | -0.4±0.0$^c$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 27 (Figure 5.10). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 60 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 60/0 | 60/7 | 60/14 |
| Dry control | | 1.4±0.2$^{a}$ | 1.3±0.5$^{a}$ | 1.2±0.2$^{a}$ |
| Control, 25 | 300 | 0.7±0.3$^{b}$ | 0.8±0.3$^{a}$ | 0.6±0.7$^{a}$ |
| 80 | 0 | 0.3±0.7$^{bc}$ | -0.4±0.1$^{b}$ | -0.3±0.2$^{b}$ |
| | 30 | -0.4±0.0$^{e}$ | -0.1±0.5$^{b}$ | -0.4±0.0$^{c}$ |
| | 60 | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ | -0.4±0.0$^{c}$ |
| | 120 | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ | -0.4±0.0$^{c}$ |
| 94 | 0 | -0.3±0.2$^{cd}$ | -0.4±0.0$^{c}$ | -0.3±0.3$^{b}$ |
| | 30 | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ | -0.4±0.0$^{c}$ |
| | 60 | -0.4±0.0$^{e}$ | -0.4±0.0$^{c}$ | -0.4±0.0$^{c}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 28 (Figure 5.11). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 18 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 18/0 | 18/7 | 18/14 |
| Dry control | | 1.7±0.2$^{a}$ | 1.2±0.4$^{a}$ | 1. 7±0.2$^{a}$ |
| Control, 25 | 300 | 0.7±0.3$^{ab}$ | 1.1±0.2$^{a}$ | 1.2±0. 6$^{ab}$ |
| 80 | 0 | 0.3 ±0.6$^{b}$ | 0. 6±0.6$^{ab}$ | 1.0±0.8$^{abc}$ |
| | 30 | -0.1±0.6$^{b}$ | -0.1±0.4$^{b}$ | 0.6±0.7$^{abc}$ |
| | 60 | -0.4±0.1$^{b}$ | -0.3±0.2$^{b}$ | -0.2±0.4$^{c}$ |
| | 120 | -0.3±0.3$^{b}$ | -0.0±0.6$^{b}$ | -0.4±0.0$^{d}$ |
| 94 | 0 | 0.3±0.9$^{b}$ | -0.1±0.3$^{b}$ | 0.4±0.7$^{abc}$ |
| | 30 | -0.4±0.1$^{b}$ | -0.1±0.4$^{b}$ | 0.3±0.3$^{c}$ |
| | 60 | -0.4±0.1$^{b}$ | -0.2±0.4$^{b}$ | -0.1±0.5$^{c}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 29 (Figure 5.12). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 40 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 40/0 | 40/7 | 40/14 |
| Dry control | | 1.5±0.2$^{a}$ | 1. 6±0.3$^{a}$ | 1.5±0.2$^{a}$ |
| Control, 25 | 300 | 1.1±0.6$^{ab}$ | 1.1±0.4$^{a}$ | 1.0±0.3$^{a}$ |
| 80 | 0 | 0.8±0.5$^{abc}$ | 0.1±0.7$^{b}$ | 0.8±1.2$^{a}$ |
| | 30 | 0.1±0.1$^{bc}$ | -0.4±0.1$^{b}$ | 1.1±1.3$^{a}$ |
| | 60 | -0.2±0.4$^{bc}$ | -0.4±0.0$^{c}$ | -0.4±0.0$^{b}$ |
| | 120 | -0.1±0.5$^{bc}$ | -0.4±0.0$^{c}$ | -0.3±0.2$^{d}$ |
| 94 | 0 | 0.8±0.8$^{abc}$ | -0.2±0.3$^{b}$ | -0.3±0.3$^{a}$ |
| | 30 | -0.4±0.1$^{c}$ | -0.1±0.6$^{b}$ | -0.3±0.2$^{a}$ |
| | 60 | -0.2±0.2$^{bc}$ | -0.3±0.4$^{b}$ | 0.0±0.9$^{a}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 30 (Figure 5.13). Total microbial counts (mean log CFU/cm$^2$ ± sd) on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate after treatment with hot water at 60 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| Hot water treatment | | Storage time in days (sealed at 4°C/aerobic at 7°C) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 60/0 | 60/7 | 60/14 |
| Dry control | | 1.5±0.3$^{a}$ | 1.4±0.4$^{a}$ | 1.3±0.2$^{a}$ |
| Control, 25 | 300 | 0.8±0.3$^{ab}$ | 1.3±0. 6$^{a}$ | 1.2±0.8$^{a}$ |
| 80 | 0 | 0.3±0.2$^{ab}$ | 0.6±0.3$^{ab}$ | 0.5±0.3$^{ab}$ |
| | 30 | 0.2±0.9$^{ab}$ | 0.7±0.7$^{ab}$ | -0.1±0.4$^{bc}$ |
| | 60 | -0.4±0.0$^{c}$ | -0.4±0.1$^{b}$ | -0.4±0.0$^{d}$ |
| | 120 | -0.4±0.0$^{c}$ | 0.2±0.0$^{ab}$ | -0.4±0.8$^{c}$ |
| 94 | 0 | 0.5±1.2$^{ab}$ | 0.3±0.8$^{ab}$ | 0.3±0.6$^{bc}$ |
| | 30 | -0.4±0.0$^{c}$ | 0.0±0.7$^{ab}$ | -0.3±0.2$^{bc}$ |
| | 60 | -0.4±0.0$^{c}$ | -0.0±0.5$^{b}$ | -0.4±0.0$^{d}$ |

$^{abc}$Means with the same superscript within a column are not significantly different ($P \geq 0.05$)

Appendix Table 31 (Figure 5.14). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) in water used for ambient control treatments of frankfurters formulated with and without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD), 18 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| | Storage time in days (sealed at 4°C/aerobic at 7°C) | | | | | |
|---|---|---|---|---|---|---|
| | 18/0 | | 18/7 | | 18/14 | |
| | PL/SD | NoPL/SD | PL/SD | NoPL/SD | PL/SD | NoPL/SD |
| Control 300 s | -0.89±1.27 | -0.49±1.31 | -1.12±1.22 | 0.60±1.56 | -1.01±1.17 | 0.91±1.06 |
| Control 420 s | na | 0.59±0.31 | na | 1.57±1.16 | na | 1.87±0.36 |

na: treatment not applied

Appendix Table 32 (Figure 5.15). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) in water used for ambient control treatments of frankfurters formulated with and without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD), 40 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| | Storage time in days (sealed at 4°C/aerobic at 7°C) | | | | | |
|---|---|---|---|---|---|---|
| | 40/0 | | 40/7 | | 40/14 | |
| | PL/SD | NoPL/SD | PL/SD | NoPL/SD | PL/SD | NoPL/SD |
| Control 300 s | -1.43±1.18 | 1.16±1.87 | -1.14±1.32 | 1.91±2.59 | -0.89±1.54 | 2.59±2.91 |
| Control 420 s | na | 2.60±0.71 | na | 2.74±2.18 | na | 4.30±2.19 |

na: treatment not applied

Appendix Table 33 (Figure 5.16). *Listeria monocytogenes* counts (mean log CFU/cm$^2$ ± sd) in water used for ambient control treatments of frankfurters formulated with and without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD), 60 days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

| | Storage time in days (sealed at 4°C/aerobic at 7°C) | | | | | |
|---|---|---|---|---|---|---|
| | 60/0 | | 60/7 | | 60/14 | |
| | PL/SD | NoPL/SD | PL/SD | NoPL/SD | PL/SD | NoPL/SD |
| Control 300 s | -0.96±1.19 | 1.84±1.75 | -1.14±1.41 | 2.46±2.82 | -0.67±1.69 | 2.70±3.14 |
| Control 420 s | na | 4.01±2.16 | na | 3.95±2.07 | na | 5.20±1.38 |

na: treatment not applied

There was no significant difference ($P \geq 0.05$) between Control 300 s and Control 420 s for any of the storage period