USE OF HOUSEHOLD STEAM CLEANERS TO CONTROL MICROBIAL QUALITY OF ANIMAL CARCASSES IN SMALL AND VERY SMALL MEAT PROCESSING PLANTS

by

SUVANG UTPALKUMAR TRIVEDI

(Under the direction of JINRU CHEN)

ABSTRACT

This study investigated the efficacy of three household steam cleaners, S1, S2 and S3, in reducing the populations of artificially-inoculated *Escherichia coli* O157:H7 or *Listeria monocytogenes* on surfaces of pork skin as well as naturally-occurring pathogenic and spoilage bacterial populations on freshly-slaughtered beef (n = 72) and hog (n = 72) carcasses in four small or very small meat-processing plants in Georgia. The application of steam generated by S1, S2 or S3 for 180 s significantly reduced the population of *L. monocytogenes*, *E. coli* O157:H7, total aerobes and thermoduric bacteria on the surface of pork skin ($P \leq 0.05$). The treatment for 60 s with S1 significantly reduced the microflora at rump, midline, and neck regions of beef carcasses as well as ham, belly, and jowl regions of hog carcasses ($P \leq 0.05$). Results suggest that household steam cleaners could be used as a critical control measure in small or very small meat-processing plants.

INDEX WORDS: *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella*, total aerobes, thermoduric bacteria, *Enterobacteriaceae*, steam pasteurization, household steam cleaning systems, pork skin, beef and hog carcasses, small and very small meat processors.

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DEDICATION

I dedicate this work to everyone who has made a difference in my life.

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CHAPTER 1

INTRODUCTION

The microbiological safety of food products is an increasing public health concern worldwide. According to an estimate by Center for Disease Control and Prevention (CDC) there are approximately 76 million foodborne illnesses resulting in approximately 325,000 hospitalizations and 5,000 deaths each year in the United States (Mead et al. 1999). Mead et al. (1999) also reported that approximately 90% of the estimated food-related deaths involve *Salmonella* (31%), *Listeria monocytogenes* (28%), *Toxoplasma* (21%), Norwalk-like viruses (7%), *Campylobacter* (5%), and *Escherichia coli* O157:H7 (3%). The economic losses associated with product recalls, disease treatment, and loss of productivity were estimated to be approximately $6.9 billion in the year 2000 (Mead et at. 1999).

With a view to reduce the health risks and economic losses associated with the presence of bacterial pathogens on animal carcasses, the USDA promulgated regulatory changes in the processing of meat and meat products in 1996, mandating meat-processing plants of all sizes to follow the Hazard Analysis Critical Control Point (HACCP) system and Sanitation Standard Operating Procedures (SSOPs) (USDA FSIS 1996). Since then, extensive research has been conducted on methods for reducing bacterial contamination of slaughtered animal carcasses (Smulders and Greer, 1998; Sofos and Smith, 1998). Amongst these technologies, steam pasteurization (Pipek *et al*., 2005) has been recognized as one of the most effective methods for decreasing bacterial populations on animal carcasses (Sofos and Smith, 1998). Several meat-processing plants have used an automated commercial steam pasteurization system (SPS Frigoscandia) (Federal register, 1996).

The system is designed for medium and large beef slaughter operations and Model SPS 400 can ‘‘pasteurize’’ up to 400 carcasses per hour (Retzlaff *et al*., 2004).

Although effective in eliminating spoilage and pathogenic microorganisms, steam pasteurization has not been widely used in small and very small meat processing operations. The high cost of installation and operation of steam generators is a huge burden to small and very small meat processors. A system, which can provide effectiveness against pathogens and spoilage microorganisms, ease of installation and operation along with cost effectiveness, would help the small and very small meat processors to face their current challenges.

The objectives of this project are:

1. To evaluate, in a laboratory setting, the practicability of using household steam cleaners to reduce the populations of *E. coli* O157:H7, *L. monocytogenes*, and spoilage microorganisms associated with meat and animal carcasses; and
2. To determine the effectiveness and feasibility of using a commercial household steam cleaner for reducing the natural microflora on the surfaces of beef and hog carcasses commercially slaughtered by small and very small meat processing facilities.

CHAPTER 2

LITERATURE REVIEW

MEATBORNE ILLNESS AND OUTBREAKS

Foodborne illnesses are defined as diseases, either infectious or toxic or both, in nature caused by agents that enter the human intestinal track through the ingestion of food (WHO, 2002). Since 1973, the Center for Disease Control and Prevention (CDC) has maintained a collaborative surveillance program for collection and periodic reporting of data on the occurrence and causes of foodborne disease outbreaks in the United States. According to the report of this program, bacterial pathogens were responsible for 75% of the foodborne outbreaks and 86% of the total number of cases of foodborne illnesses in the United States during the period between 1993 and 1997 (CDC, 2000c). The report also identified beef and pork as the source of transmission in a total of 66 (2.4%) and 27 (0.9%) of the 2,751 reported cases of foodborne illness, respectively (CDC, 2000c). During the period of 1998 and 2002 the total number of reported foodborne outbreaks decreased as compared to previous data collected between 1993 and 1997. Out of the 6,647 reported cases of foodborne illness during the period of 1998 and 2002, beef and pork were identified as the vehicle of transmission in 208 (3.1%) and 138 (2.0%) instances, respectively (CDC, 2006c). Out of the 183 reported outbreaks associated with *E. coli* O157:H7 beef was the vehicle of transmission in 75 (41%) outbreaks (Rangel et al., 2005). Mead et al., (1999) reported that 95% of deaths resulting from foodborne disease were attributable to *Salmonella* (31%); *Listeria* (28%); *Toxoplasma* (21%); Norwalk-like viruses (7%); *Campylobacter* (5%); and *Escherichia coli* O157:H7 (3%).

The first outbreak of *E. coli* O157:H7 was reported in 1982 associated with consumption of beef petty (Rangel et al., 2005). Since then beef has been one of the most common vehicles associated with the foodborne outbreaks of *E. coli* O157:H7. An outbreak of *E. coli* O157:H7 infection, occurred in the Pacific Northwest in late 1992 and early 1993, was caused by undercooked hamburgers sold by a fast food chain (CDC, 1993; Bell et al., 1994; Dorn 1993). A total of 501 cases were reported, with 151 hospitalizations, 45 cases of hemolytic uremic syndrome (HUS), and 4 deaths (CDC, 1993; Bell et al., 1994; Dorn 1993). In August of 1994, an outbreak of *E. coli* O157:H7 associated with undercooked ground beef was reported to the Virginia Department of Health (CDC, 1995c). Soon after that in November of 1994 a total of 20 laboratory-confirmed cases of diarrhea were reported in the Washington State which were caused by the consumption of commercial dry-cured salami contaminated with *E. coli* O157:H7 (CDC, 1995a). In 1995 three cases of *E. coli* O157:H7 infection among residents of north Georgia were reported to the Georgia Department of Human Resources (GDPH), transmitted through consumption of unfrozen ground beef patties (CDC, 1996b). In 1997, the Colorado Department of Public Health and Environment (CDPHE) identified an outbreak of *E. coli* O157:H7 infections associated with eating a nationally distributed commercial brand of frozen beef patties and burgers (CDC, 1997a). Between June and July 2002 a multistate outbreak of *E. coli* O157:H7 was identified by the CDPHE. In seven states 28 illnesses and 7 hospitalizations were reported of which 5 developed HUS. The epidemiological studies of the outbreak identified ground beef as the vehicle of transmission (CDC, 2002b).

The transmission of *Salmonella* to humans can occur via various food vehicles, including eggs, meat, poultry, and produce (CDC, 2006b). In late December of 1994, 17 patients with acute gastrointestinal illness characterized by diarrhea and abdominal cramps were diagnosed as

infected by *Salmonella* serotype Typhimurium and the infection was transmitted via consumption of raw ground beef (CDC, 1995b). During 1995 and 2002, pork has been one of the most frequently implicated vehicles in transmitting foodborne salmonellosis in Chicago (Jones et al., 2004). During this period a total of 108 cases and 11 hospitalizations were reported with *Salmonella* being identified as an etiological agent in four outbreaks (Jones et al., 2004). From January to April of 2002, *Salmonella* serotype Newport was isolated from 47 persons in five states in northeastern United States (CDC, 2002a). The isolates resisted multiple antibiotics and the epidemiological investigation of the outbreak identified raw or undercooked ground beef as the vehicle of transmission (CDC, 2002a). An outbreak of *S.* Typhimurium definitive type 104 infections linked to commercial ground beef was detected in December 2003 by Maine Bureau of Health laboratory (Dechet et al., 2006). The outbreak caused more than 2,200 illnesses in seven states including Maine, Massachusetts, New Hampshire, Vermont, Connecticut, Rhode Island, and New York (Dechet et al., 2006). In the same year a multistate outbreak of *S.* Typhimurium was reported in nine states in western United States and the outbreak implicated 31 patients due to the consumption of contaminated ground beef (CDC, 2006a).

A substantial portion of sporadic listeriosis is foodborne (Schuchat et al., 1992; Pinner et al., 1992; Schwartz et al., 1988) and associated with consumption of ready-to-eat hot dogs and undercooked chicken (Schuchat et al., 1992; Pinner et al., 1992; Schwartz et al., 1988). Association of *L. monocytogenes* with ready-to-eat meats is one of the major concerns for the processed meat industry. In early 1998 40 cases of listeriosis was reported in ten states across United States and the outbreak was later traced to the consumption of hot dogs contaminated with *L. monocytogenes* (CDC, 1998). In May 2000, 29 cases of listeriosis were linked to the

consumption of deli turkey meat in 10 states in United States (CDC, 2000a). A multistate outbreak of *L. monocytogenes* infections with culture-confirmed cases, 7 deaths, and 3 miscarriages in eight states was linked to consumption of sliceable turkey deli meat in 2002 (CDC, 2002c). Based on PFGE pattern analysis one ready-to-eat meat processing plant located in Pennsylvania was linked to the outbreak (CDC, 2002c).

IMPORTANT BACTERIAL PATHOGENS ASSOCIATED WITH ANIMAL CARCASSES

L. MONOCYTOGENES

L. monocytogenes is a gram positive, non-spore-forming, non-capsulating, non-acid-fast, facultatively anaerobic, rod-shaped bacterium measuring 1.0-2.0 μm by 0.5 μm. The bacterium is flagellated and motile at 20-25^{o}C but not at 37^{o}C (Cliver, 1990; Holt et al., 1994; Swaminathan, 2001). On nutrient agar, colonies of *L. monocytogenes* are smooth with a translucent blue-gray tint. Under a transmitted oblique light, colonies appear with blue-green iridescence. Beta hemolysis can be observed on blood agar (Cliver, 1990; Holt et al., 1994; Swaminathan, 2001).

Based on results of DNA-DNA hybridization, multilocus enzyme analysis, and 16S rRNA sequencing, the genus *Listeria* is divided into 6 species with 3 lines of descendants. The first line of descendants consists of *L. monocytogenes, L. innocua, L. invanoii, L. welshimeri* and *L. seeliger*i, the second consists of *L. grayi* and the third includes environmental isolates closely related to *L. innocua* (Cliver, 1990; Holt et al., 1994; Swaminathan, 2001). Within the genus of *Listeria*, only *L. monocytogenes* and *L. ivanovii* are known human pathogens. There are 13 serotypes of *L. monocytogenes*, but approximately 95% of the *L. monocytogenes* isolated from

humans belong to 3 serotypes, 1/2a, 1/2b and 4b (Cliver, 1990; Holt et al., 1994; Swaminathan, 2001).

L. monocytogenes has an optimum growth temperature of 30-37°C however; they are also able to grow at temperatures as low as -1.5°C (Haugland, 2002; Helke and Wong, 1994). The pathogen can not tolerate high temperatures and it is readily killed at temperatures above 50^{o}C (Helke and Wong, 1994; Miettinen et al., 2001). The pH range for the growth of *L. monocytogenes* was from 5.6 to 9.6, with optimum growth pH at 6 to 8. Lou and Yousef (1999) however, reported that the pathogen can initiate its growth in laboratory media with a pH value as low as 4.4. *L. monocytogenes* is holotolerant and it grows in brain heart infusion broth with 6% of NaCl for 33 days (Hudson 1992) and survives in tryptic soy broth with 25.5% of NaCl at 22ºC for more than 24 days (Lou and Yousef, 1999). Cheroutre-Vialette et al. (1998) reported that *L. monocytogenes* tolerated 8% KCl better than the same concentration of NaCl. The optimum water activity (a_w) for *L. monocytogenes* growth is 0.97 or higher; however, growth at a_w as low as 0.90 has been reported (Lou and Yousef, 1999). The generation time of *L. monocytogenes* in tryptic meat broth at 20^{o}C is 1.37 h and this generation time can be prolonged under acidic and osmotic stresses (Cheroutre-Vialette et al., 1998).

L. monocytogenes is ubiquitous in nature and present in the intestinal tract of many animals, including humans. It has been found in soil, animal feces, sewage, silage, vegetation and water (Fenlon, 1985). In the food environment, *L. monocytogenes* has been isolated form raw milk, soft cheeses, fresh and frozen meats, poultry, seafood, fruits, and vegetables and processed foods (Fenlon, 1985). It has also been isolated from the feces of many healthy animals and birds (Swaminathan, 2001) which may explain why the organism can be found in sewage and soil (Swaminathan, 2001). Food contact surfaces, shoes, clothing, floors, drains, condensate,

stagnant water, hide, and soiled surface are vehicles for introducing *L. monocytogenes* into a food processing facility (Fenlon, 1985; Swaminathan, 2001). The pathogen is difficult to control in food processing facilities since the environment provides suitable conditions for its survival and growth (Henning and Cutter, 2001).

L. monocytogenes primarily causes clinical condition known as listeriosis which results in severe consequences in immunocompromised individuals, elderly, pregnant women, fetuses and newborn infants. In healthy individuals however, there might be flu-like or complete lack of symptoms (Salyers and Whitt, 1994). The organism can cross the placenta and causes stillbirths, spontaneous abortion, fetal death, severe neonatal septicemia, and meningitis. In nonperinatal patients with predisposing factors, bacteremia is more frequent with central nervous system infections being the predominant syndrome. The overall fatality rate for systemic or invasive listeriosis is usually about 20 to 30% for both epidemic and sporadic cases (Frederiksen and Samuelsson, 1992; Bula et al., 1995). Like in humans with listeriosis, the most common serotypes of *L. monocytogenes* isolated from foods are 1/2a, 1/2b and 4b (Farber and Petrkin, 1991).

Outbreaks of listeriosis have been associated with consumption of coleslaw, meat products, pasteurized milk, and milk products (Swaminathan, 2001). Epidemiological evidence has shown that ready-to-eat deli meats, non-reheated frankfurters, unpasteurized fluid milk as well as smoked sea food fall within the highest risk food categories for outbreaks of listeriosis for total population of United States on per serving per annum basis in 2003 (Sofos, 2006). The first documented outbreak of meat-borne listeriosis occurred in December 1988. The outbreak was linked to the consumption of ready-to-eat turkey frank and *L. monocytogenes* with serotype 1/2a was isolated from the infected patient (CDC, 1989). *L. monocytogenes* has been involved in

several large, multi-state outbreaks in the past decade. One such outbreak occurred in the summer of 1998 to early 1999 and it was linked to ready-to-eat hot dogs and luncheon meat. According to the estimate made by CDC a total of 15 deaths and 6 miscarriages/stillbirths as well as approximately 100 illnesses resulted from the outbreak (CDC, 1999).

***E. COLI* O157:H7**

E. coli are gram-negative, asporogenous, and facultative anaerobic rods (Nataro and Kaper, 1998). Most *E. coli* strains confined to the intestinal lumen are nonpathogenic (Nataro and Kaper, 1998). Those known to cause human and animal diseases are categorized as Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC), Enteroinvasive *E coli* (EIEC), Enterotoxigenic *E. coli* (ETEC), Enteroaggregative *E. coli* (EAggEC), Diffuse-adhearing *E. coli* (DAEC), cytolethal distending toxin (CDT)-producing *E. coli*, cell-detaching *E. coli* (CDEC) or cytotoxin nacrotizing factor (CNF) producing *E. coli*, (DuPont et al., 1971; Riley et al., 1983; Karmali et al., 1983; Nataro et al., 1987; Vial et al., 1988; Gunzberg et al., 1993; Scott and Kaper, 1994; Pickett et al., 1997).. *E. coli* isolates are serologically differentiated based on three major surface antigens: somatic (O), flagella (H), and capsule (K) (Kauffman, 1947). At present there are 167 O antigens, 53 H antigens, and 72 K antigens (Lior, 1994; Meng et al., 2001).

E. coli serotype O157:H7 belongs to the family of EHEC that produces protein toxins similar to the toxin produced by *Shigella dysenteriae* type 1 (Calderwood et al., 1996), causes attaching and effacing (A/E) lesions, and possess a 60 MDa virulence plasmid (Knutton et al., 1989). *E. coli* O157:H7 can cause a serious systemic complication, known as HUS, characterized by hemolytic anemia, thrombocytopenia, and renal failure in young children and the elderly (Nataro and Kaper, 1998). Shiga toxins (Stxs) are the major virulence factor, and a

defining characteristic of EHEC (Nataro and Kaper, 1998). Other virulence factors of EHEC include hemolysin, intimin, serine protease, type III secretion proteins, and O157 lipopolysaccharide O-side chain. EHEC produces more than one type of Stxs (Nataro and Kaper, 1998). The two major classes of Stxs produced by EHEC are Stx1 and Stx2 (Scotland et al., 1985). Stxs have five B monomeric subunits surrounding one A subunit (Calderwood et al., 1987). The A subunit acts as N-glucosidase and removes a specific adenine from the 60S ribosomal subunit of mammalian cells. As a consequence, Stxs disrupt the function of the 28 S rRNA and inhibit peptide chain elongation in the ribosome (O'Brian et al., 1992**).** The B subunits of Stxs bind to the membrane receptor, globotriaosylceramide (Gb3) (Jacewicz et al., 1986; Keusch et al., 1991; O'Brian et al., 1992**).** The Gb3 facilitates the endocytosis and intracellular trafficking of the toxin. (O'Brian et al., 1992**)**. The cortex of the human kidney carries abundance of receptors for Stxs (Sandvig and van Deurs, 1996; Schmidt et al., 1996) hence, the toxins cause sever kidney damage and even kidney failure.

The formation of A/E lesion caused by *E. coli* O157:H7 involves the degeneration of the epithelial brush border microvilli as well as the formation of actin-rich pedestals within the host cell beneath the adherent bacteria (Moon et al., 1983; Knutton et al., 1989). Intimin is the adherence factor for *E. coli* O157:H7 while the translocated intimin receptor (Tir) protein triggers host-signaling and involves in the development of A/E lesions. The type III secretion proteins and lipopolysaccharide are involved in the adhering of EHEC to the host epithelial cells and exports virulence factors directly to eukaryotic cells (Bilge et al., 1996; Jarvis and Kaper, 1996). The genetic elements responsible for the formation of the A/E lesion and encoding intimin, Tir, and Type III secretion system are located on the locus of enterocyte effacement (LEE) pathogenicity-island (Donnenberg et al., 1997).

E. coli O157:H7 is similar to other serotypes of *E. coli* however, this serotype lacks the enzyme β-glucuronidase (Griffin, 1995), and produces enterohemolysin (Griffin, 1995). It generally does not grow well at temperatures above 44.5°C, and show unusual tolerance to an acidic environment (Griffin, 1995). Three different systems, acid-induced oxidative system, an acid-induced arginine system, and a glutamate dependent system are reported as the mechanisms for acid resistance in *E. coli* O157:H7 (Lin et al., 1996). Studies have shown that *E. coli* O157:H7 in ground beef has no unusual resistance to heat (Doyle and Schoeni, 1984). However, the presence of animal fat reportedly provides protection to the pathogen (Line et al., 1991). It has been recommended that thermal treatments at 68.3°C effective against *Salmonella* are also effective against *E. coli* O157:H7 (Goodfellow and Brown, 1987).

Cattle are a major reservoir for *E. coli* O157:H7, with the organism being more prevalent and persisting in calves than adult cattle (Beutin et al., 1993; Rahn et al., 1997). A variety of foods have been implicated in outbreaks of *E. coli* O157:H7 infections, including undercooked ground beef, salami (Blanco et al., 1996; CDC, 1995b; Duffy et al., 2000), roast beef (Rodrigue et al., 1995), deer jerky (Keene et al., 1997b), pre-cooked ground beef (Belongia et al., 1991), raw milk (Keene et al., 1997a; CDC, 1996a), raw goat milk (Bielaszewska et al., 1997), and goat cheese (Belongia et al., 1991). Water (municipal water supply and swimming waters), person to person contact and contact with infected bovine feces have also been cited as sources of *E. coli* O157:H7 transmission. Foodborne outbreaks of *E. coli* O157:H7 infections are seasonal, with most occurring from May to October (Rangel et al., 2005).

SALMONELLA

More than 2,000 serotypes of *Salmonella* have been identified, all of which are known to be pathogenic to human (Popoff et al., 2000). *Salmonella* are gram negative, non-sporulating,

facultative anaerobic rods. *Salmonella* have an optimal growth temperature at 37°C but can grow at an elevated temperature of 54°C and as low as 2-4°C. The organisms can survive in pH 4.5 to pH 9.5, with an optimal pH growth range of 6.5 to 7.5. Water activity levels below 0.93 and NaCl concentrations of 3-4% are inhibitory to *Salmonella* (Bailey and Maurer, 2001).

According to Mead et al. in 1999, non-typhoid strains of *Salmonella* were responsible for an estimated 1,412,498 illnesses each year in United States, of which 1,341,873 (95%) were foodborne. This accounts for 9.7% of all foodborne illnesses, 25.6% of hospitalized patients from foodborne illnesses, and 30.6% of foodborne illness deaths (Mead et at., 1999). It has been estimated that 50%–75% of human *Salmonella* infections are attributable to the presence of the organism on meat and poultry products (USDA-FSIS, 1995a). According to a report by the USDA National Microbiological Baseline Data Collection Program, during 1993 and 2000, *Salmonella* was isolated from 22.8% of 1,297 broiler chickens, 1.8% of 2,100 beef carcasses, and 8.7% of 2,112 market hogs sampled (USDA-FSIS, 1993; 1996b; 1996c). The 5 most common *Salmonella* serotypes isolated from broiler chickens are Heidelberg, Kentucky, Hadar, Typhimurium, and Thompson (Sarwari et al., 2001). *Salmonella* serotypes most common to beef carcasses were Typhimurium, Montevideo, Anatum, Kentucky, and Thompson and those most commonly isolated from market hogs were Derby, Typhimurium, Heidelberg, Anatum, and Hadar (Sarwari et al., 2001).

Salmonellosis, characterized by fever, abdominal cramps, and diarrhea, usually onsets approximately 12 to 72 h after consumption of a contaminated food or beverage. After ingestion, *Salmonella* travels through the gastrointestinal tract. In the small intestine, the organism is able to colonize and invades the intestinal columnar epithelial cells. The motility of *Salmonella* is believed to increase the frequency of contacts between the pathogen and target

host cells (epithelial cells), increasing the occurrence and rate of attachment. When *Salmonella* contacts the surface of epithelial cells, proteinaceous appendages develop on the surface of the pathogen and appear to be essential for entry into the epithelial cells (Finlay, 1994). Once inside the epithelial cells, the pathogen multiplies, and releases a thermostable cytotoxic factor. The production of the cytotoxic factor increases in the human GI tract. This cytotoxic factor lyses the host cells and releases thermolabile enterotoxin. The host responds to the release of the enterotoxin by increasing fluid secretions and electrolytes and decreasing fluid absorption (Finlay, 1994; Polotsky et al., 1994).

The duration of the illness is generally 4 to 7 days (D'Aoust, 1989). In some cases, the diarrhea may be severe and require hospitalization. Investigations of foodborne outbreaks of *Salmonella* infections indicate that 1 to 10 cells of the pathogen can constitute a human infectious dose (Kappaerud et al., 1990; D'Aoust et al., 1985). Those with weakened or compromised immune systems (extremes of age, inherent or chemotherapy suppressed) are more susceptible to the disease, have more severe symptoms that may also lead to septicemia, and can be fatal. Those with mild forms of the disease generally recover completely. Some long-term effects of *Salmonella* infections do occur, including Reiter's syndrome (painful joints, eye irritation, and painful urination) and chronic arthritis (D'Aoust, 1989).

Oosterom (1991) reported in his epidemiological study that farm animals are the major source of human salmonellosis. Bailey and Maurer (2001) noted that poultry products are the principle reservoir, but *Salmonella* can also be found in beef, pork, and mutton. Rodents, insects, and birds are also carriers. Sewage and effluent water used to fertilize fields, improper worker hygiene, and contaminated wash water can cause contamination of fresh fruits and vegetables (D'Aoust, 1994). *Salmonella* are ubiquitous in the environment and in animals, and

therefore are widely disseminated in various sectors of the food production and processing industries.

Out of more than 2,000 serotypes, *S*. Typhimurium and *S*. Enteritidis are responsible for 50% of the foodborne salmonellosis illnesses in the United States (CDC, 2000b). *S.* Typhimurium accounted for 24% of *Salmonella* outbreaks in 1995. Antibiotic-resistance was exhibited by 28% of these isolates, of which 83% were typed as DT104 (CDC, 1997b). Outbreaks of foodborne salmonellosis caused by *S*. Typhimurium have been associated with consumption of ground beef. In late 1994 to early 1995, 107 confirmed cases of *S*. Typhimurium infections were reported in Wisconsin, which were later linked to consumption of ground beef (CDC, 1995b). Recently in September 2004 a multistate outbreak of salmonellosis was reported in United States involving *S*. Typhimurium. The cause of infection was determined to be the consumption of contaminated ground beef (CDC, 2006a). *S*. Enteritidis is unique from other serotypes of *Salmonella* in its ability to infect the interior of an egg prior to shell formation (St. Louis et al., 1988). This protects the organism from sanitizing agents applied to the surface of shell eggs. The presence of *S*. Enteritidis inside of an intact egg has provided an effective means for the organism to be transferred to the human population through the consumption of raw or undercooked eggs, food dishes or beverages made with raw or undercooked eggs, and/or cross-contamination of ready-to-eat foods (Thiagrajan et al., 1994). Most of the salmonellosis outbreaks involving infection of *S*. Enteritidis are traced back to consumption of raw or contaminated eggs or poultry meats (Bailey and Maurer, 2001).

PREVALENCE OF PATHOGENIC AND SPOILAGE BACTERIA ON COMMERCIALLY PROCESSED ANIMAL CARCASSES

In 1992-1993, the USDA-FSIS initiated the Nationwide Microbiological Baseline Data Collection Program for Steers and Heifers to obtain a general microbiological profile of fed cattle carcasses (USDA-FSIS, 1993). The objective of the program was to evaluate new prevention programs and to reduce the risk of foodborne outbreaks (USDA-FSIS, 1993). It was found that the mean populations of aerobic plate counts (APCs), total coliforms, and *E. coli* (Biotype I) recovered from the 2,089 samples of steers and heifers were 2.68, 1.55, and 1.55 Log CFU/cm^2, respectively (USDA-FSIS, 1993). *Salmonella* was recovered from 1.0% of the carcasses (mean level of 0.1 MPN/cm^2), *E. coli* O157:H7 from 0.2% (mean level of 0.6 MPN/cm^2), and *L. monocytogenes* was recovered from 4.1% (mean level of 0.2 MPN/cm^2) of the carcasses (USDA-FSIS, 1993). All the samples were collected from the processing plants which had an average operating capacity of ≥ 40 heads per week or ≥ 2100 heads per year, and were under federal inspection at the time of study (USDA-FSIS, 1993). The pathogens were recovered from the rump, flank, and brisket regions of carcasses using the sponge swabbing technique. Since the objective the investigation was to provide data for estimating the distribution and population of important foodborne microorganisms it did not give details on other factors that might affect the microbial quality of animal carcasses such as geographic location of plants, operating capacity of the plant, slaughtering stage at which the samples are collected as well as processing environment sources contributing to the contamination (USDA-FSIS, 1993).

A study conducted by Rivera-Betancourt et al., (2004) evaluated the prevalence of *E. coli* O157:H7, *Listeria* spp., *L. monocytogenes*, and *Salmonella* on hide, carcass, and processing

environment in two geographically distant large beef processing plants over the course of 5 months. One plant located in the southern region of the United States (plant A) and the other one in the northern region of the country (plant B). The microbial prevalence on animal hides was 68.1 and 55.9%, respectively for *E. coli* O157: H7, and 91.8 and 50.3%, respectively for *Salmonella*. The incidences of *E. coli* O157:H7 and *Salmonella* at plant A was higher ($P < 0.05$) than those at plant B. The prevalence of *Listeria* spp. was 37.7 and 75.5%, respectively and of *L. monocytogenes* 0.8 and 18.7%, respectively. The incidences for *Listeria* spp. and *L. monocytogenes* were lower ($P < 0.05$) at plant A than at plant B. The prevalence of *E. coli* O157:H7 (3.1 versus 10.9%), *Listeria* spp. (4.5 versus 14.6%), and *L. monocytogenes* (0.0 versus 1.1%) was lower ($P < 0.05$) for pre-evisceration carcasses sampled at plant A compared to at plant B. The results of the study indicated that the differences in the geographical locations and slaughtering practice can affect the prevalence of pathogenic microorganisms on animal hides as well as carcasses (Rivera-Betancourt et al., 2004).

Upton (1995) stated that the microbial flora of a meat surface is largely determined by the processing environment and the microbiological status of the animal. Common spoilage flora isolated from meat products included *Pseudomonas* spp., *Moraxella* spp., *Enterobacteriaceae*, and *Brochothrix thermosphacta*. In a study by Sofos et al. (1999), microbiological populations on beef carcasses at different processing stages, from slaughtering to chilling, were evaluated in seven (four steer/heifer and three cow/bull) plants in the Unites states. Each facility was visited twice, once in November through January (wet season) and again in May through June (dry season). Carcasses were sampled by aseptic excision of surface tissue (100 cm^2) from the brisket, flank, and rump (30 samples each) after hide removal (pre-evisceration), after final carcass washing, and after 24-h carcass chilling. The samples were analyzed individually by

standard procedures for APCs, total coliform counts, *E. coli* (Biotype I) counts, and presence of *Salmonella.* The effects of carcass site sampled, slaughtering stage of sample collection and season were significant ($P \leq 0.05$) on the bacterial counts obtained from carcasses. The population of total coliforms exceeded 10^3 CFU/cm^2 in 2.5% (wet season) and 1.5% (dry season) of the samples indicating seasonal variation in microbial populations on beef carcasses. The population of *E. coli* (Biotype I) exceeded 10^2 CFU/cm^2 in 8.7%, 0.3%, and 1.5% of the pre-evisceration, final carcass-washing, and 24-h carcass-chilling samples, respectively, during the wet season whereas the corresponding populations during the dry season were 3.5%, 2.2%, and 3.0%, respectively indicating the variations in microbial populations at different stages of slaughter (Sofos et al., 1999).

During 1995-1996, USDA-FSIS presented the microbiological data for pork carcasses collected under the Nationwide Microbiological Data Collection Program. The results showed that mean populations of APCs, total coliforms, and *Escherichia coli* (Biotype I) recovered from 2,112 samples of market hogs were 3.69, 1.88, and 1.88 Log CFU/cm^2, respectively (USDA-FSIS, 1996b). *Salmonella* was recovered from 8.7% of carcasses, *E. coli* O157:H7 from 0%, and *L. monocytogenes* was recovered from 7.4% of the pork carcasses (USDA-FSIS, 1996b). Saide-Albornoz et al., (1995) evaluated the prevalence of *Staphylococcus aureus*, *Salmonella*, *L. monocytogenes*, and *Yersinia enterocolitica* on pork carcasses during commercial slaughter and fabrication (Saide-Albornoz et al., 1995). *S. aureus* was detected on 7.4% of all carcasses sampled, with the prevalence increasing from singeing through the process to chilled storage. The prevalence of *Salmonella* was 1.7% and was found to decrease from slaughter to chilled storage. The prevalence of *L. monocytogenes* and *Y. enterocolitica* were 4.4% and the pathogens persisted for 36 days during chilled storage (Saide-Albornoz et al., 1995).

Microbial quality of animal carcasses may vary depending upon the size of a processing plant. Hogue et al., (1993) attempted to correlate APCs on beef briskets and ground beef with the slaughter volume of the establishments. They determined that APCs were lower in establishments with larger slaughter volumes compared to establishments with smaller slaughter volumes. APCs of 3.6 Log CFU/cm^2 were observed on steer and heifer carcasses processed in establishments with annual slaughter volumes of 10,000 or less, whereas APCs of 2.2 Log CFU/cm^2 were observed in establishments with annual slaughter volumes of 1 to 1.5 million (Hogue et al., 1993). Similar findings have been reported by Hanson in Sweden (Hansson, 2001). The results of the study identified significantly greater populations of APCs on carcasses slaughtered at low-capacity slaughterhouses compared to those were slaughtered at high capacity slaughterhouses (Hansson, 2001).

ANIMAL CARCASS DECONTAMINATION TECHNOLOGIES

A. Water washing

Anderson et al. (1981) treated beef carcass sides with a 15°C water, applied either by hand with tap water (15 s) or in a prototype beef carcass washer. Overall mean APCs reductions on hand- and machine- washed carcasses were 0.99 and 1.07 Log CFU/cm^2, respectively. No significant differences between the two types of carcasses were observed judging by the percentage dirt, smear, or hair left on the carcasses.

In laboratory experiments, Dickson (1995) compared the effect of presence or absence of a pre-evisceration water wash on the susceptibility of beef tissue to contamination by *E. coli* O157: H7 and *Salmonella*. Results showed that the control tissue, receiving a final wash only, had APCs, and *E. coli* O157:H7 and *Salmonella* populations of 5.5, 5.3, and 5.6 Log CFU/cm^2, respectively whereas, the pre-evisceration washed tissue had APCs, and *E. coli* O157:H7, and

Salmonella populations of 4.9, 4.7, and 4.7 Log CFU/cm^2, respectively (Dickson, 1995). Thus, repeated washing of the animal carcass at different stages of slaughter may help reduce the contamination by great extent.

However, washing treatment may spread the microbial contamination from one location to another on carcasses. Prasai et al., (1995) evaluated the effect of trimming plus washing (final carcass wash) on the microbial quality of carcasses, and concluded that the greatest reduction in APCs, 3.0 Log CFU/cm^2, was achieved for samples that were trimmed but not washed followed by a reduction of 0.9 Log CFU/cm^2 on samples that were trimmed and washed. This is because of the trimming process in this particular study involved the removal of a pre-defined surface area (a single cut of approximately 400 cm^2 and 1.5 mm thickness) using sterile knife and scalpel. This observation indicates that the subsequent washing may have spread microbial contamination from one location of carcass to another (Prasai et al., 1995).

B. Hot water washing

Using a hot water wash cabinet, Davey and Smith (1989) treated beef carcass sides, inoculated with *E. coli* at neck, thoracic cavity, rump, midback, brisket, and shoulder, for 10 or 20 s at temperatures of 83.5, 74.2, 66.0, and 44.5°C. Carcasses were sampled at the inoculated sites before washing, after washing, and after chilling. The hot water washing at 83.5, 74.2, 66.0, and 44.5°C for 10 s reduced the *E. coli* populations by 2.23, 1.40, 0.91, and 0.2 Log CFU/cm^2, respectively and for 20 s the reductions in *E. coli* populations were 2.98, 2.14, 1.17, and 0.1 Log CFU/cm^2, respectively. The reductions achieved by 20 s hot water treatment were significantly greater than those achieved at 10 s. However, a bleaching effect was noticed on the surface color of the carcasses after the treatment with hot water, particularly when the temperatures of the carcasses were raised to 83.5° and 74.2°C. The appearance of the carcass

usually returned to near normal after 10-12 min at room temperature. However, on the carcasses treated at 83.5°C, the bleaching appearance persisted, even after 48 h at room temperature.

Barkate et al., (1993) used meat samples excised from randomly selected areas on freshly slaughtered beef carcasses and sprayed them with hot, sterile distilled water with a temperature of 95°C. Each meat sample was treated for 40 s with 2 liters of hot water. The results indicated that meat samples treated with hot water had mean APCs that were 1.3 Log CFU/cm^2 lower than the samples that did not receive any hot water treatment (Barkate et al., 1993).

Gill et al., (1995) sampled the back, waist, belly, and foreleg of naturally contaminated pork carcasses treated with hot water wash in a cabinet for 40 s at 60, 75, 80, 85, or 90°C. The results indicated that the washing treatment for 40 s at 85^oC was optimal as it resulted in a maximum overall bacterial reduction of 2 Log CFU/cm^2 at the back and front leg regions. Control carcasses had a mean bacteria level of 3 Log CFU/cm^2. Populations of generic *E. coli* on untreated carcasses were as high as 6.2 Log CFU/cm^2 (Gill et al., 1995).

Using a hot water spray cabinet Castillo et al. (1998) examined the microbial reductions on pre-rigor beef carcass surfaces at inside round, outside round, brisket, flank, and clod inoculated with bovine feces containing 10^6 CFU/g of *S.* Typhimurium and *E. coli* O157:H7. The inoculated beef samples were either treated by water wash or a water wash followed by a hot water spray of 95°C. All treatments significantly reduced populations of inoculated pathogens from initial level of 5.0 Log CFU/cm^2. Washing alone reduced the populations of *S.* Typhimurium at inside round, outside round, brisket, flank, and clod by 2.0, 2.6, 2.3, 2.0, and 1.9 Log CFU/cm^2, whereas washing followed by hot water washing reduced the pathogen by 2.7, 4.3, 3.8, 3.9, and 4.1 Log CFU/cm^2, respectively. The reduction in the populations of *E. coli* O157:H7 at inside round, outside round, brisket, flank, and clod by washing alone was 2.1, 2.7,

1.7, 1.9, and 2.0 Log CFU/cm^2, respectively, whereas treatment with hot water reduced the inoculated bacterial pathogen by 2.9, 4.0, 3.9, 3.8, and 4.1 Log CFU/cm^2, respectively (Castillo et al., 1998). The effectiveness of hot water wash was affected by the anatomical locations where the treatments were applied. This could be because of the varying degree of microbial contamination at different anatomical locations attributed to dressing practices currently used in slaughterhouses.

Bosilevac et al., (2006) evaluated the effect of replacing lactic acid wash with hot water wash on pre-evisceration beef carcasses in reducing the populations of aerobic bacteria and *Enterobacteriaceae* and prevalence of *E. coli* O157:H7. They treated pre-evisceration carcasses either with hot water wash (74°C), lactic acid spray (2%, 42°C) or hot water wash followed by 2% lactic acid spray. The results showed that a commercial hot water carcass wash cabinet applying 74°C (165°F) water for 5.5 s reduced both APCs and *Enterobacteriaceae* counts by 2.7 Log CFU/100 cm^2 on pre-evisceration carcasses. Application of 2% L-lactic acid spray at approximately 42°C (105 to 110°F) to pre-evisceration carcasses resulted in a reduction of APCs by 1.6 Log CFU/100 cm^2 and *Enterobacteriaceae* counts by 1.0 Log CFU/100 cm^2. When hot water wash was followed by lactic acid spray, the APCs were reduced by 2.2 Log CFU/100 cm^2 and *Enterobacteriaceae* counts were reduced by 2.5 Log CFU/100 cm^2. The surface of the carcasses sprayed with lactic acid after the hot water wash was significantly cooler than the carcass surface received only hot water treatment, thereby reducing the effect of hot water. The prevalence of *E. coli* O157:H7 was reduced by 81% and 35% using hot water wash and lactic acid spray, respectively, but the two treatments in combination produced only a 79% reduction in *E. coli* O157:H7 prevalence (Bosilevac et al., 2006).

C. Chlorinated water wash

Chlorine is a common sanitizing agent for disinfecting equipment, utensils and water supplies as well as poultry carcass chilling water (Sofos and Smith, 1998). Previous studies have reported that chlorinated water (200ppm) is effective in reducing microbial populations from beef forequarters by 1 to 2 log cycles after 45 min of treatment (Kotula et al., 1974). Conversely, in 1995 Kenny et al. used the same concentration of chlorine to spray beef carcasses at 21°C (69.8°F) followed by a repeat chlorine spray after chilling of carcasses for 8 h and found that chlorine was ineffective in reducing bacterial populations. Prior to treatment (immediately following slaughter), the total bacterial populations on beef carcasses were ca. 3.5 Log CFU/cm^2. After application of the second chlorine spray (following spray chill), the total bacterial populations found on beef carcasses was approximately 3.1 Log CFU/cm^2 which was a reduction of mere 0.4 Log CFU/cm^2 (Kenny et al., 1995).

The effectiveness of chlorine treatments is largely affected by total available chlorine. Once applied to beef tissue the reduction of available chlorine is dependent on exposure time, initial chlorine concentration, as well as the volume of aqueous chlorine per unit of organic materials and the type of organic materials present in the treatment system (Kotula et al., 1997). These findings are similar to those reported by Cutter and Siragusa (1995) who showed that in the presence of nitrogenous organic materials, free chlorine is readily bound and converted to chloramines, decreasing the antimicrobial activity of the solution. Cutter and Siragusa (1995) treated beef carcass tissue inoculated with either *E. coli* (generic) or *E. coli* O157:H7 to levels of ca. 5 Log CFU/cm^2 with a chlorine spray of 50, 100, 250, 500, and 800 ppm in a model carcass washer with spray nozzle oscillation speed at 80 cycles/min, chain speed at 14 m/min, nozzle pressure at 60 psi, flow rate at 4.2 litre/min and nozzle distance from sample at 17.8 cm.

Statistically significant reductions were observed only at the 800 ppm level. Generic *E. coli* counts were 5.4 Log CFU/cm^2 on non-treated tissue and the *E. coli* counts on tissue treated at 800 ppm were 4.1 Log CFU/cm^2. The non-treated tissue had ca. 5.0 Log CFU/cm^2 and the tissue treated at 800 ppm had 3.9 Log CFU/cm^2 of *E. coli* O157:H7.

Chlorine dioxide is a water-soluble compound that is considered to be more effective than chlorine, with no interference by pH as well as no reaction with organic nitrogeneous materials. In recent years chlorine dioxide has been presented as an alternative to traditional chlorine disinfectants (Sofos and Smith, 1998). Villarreal et al. (1990) evaluated a chlorine dioxide solution for rinsing and chilling poultry carcasses in a commercial slaughter facility. Carcasses were divided into different test groups and rinsed/chilled in either chlorine or chlorine dioxide solutions by soaking carcasses in tanks of the solutions for 4 h. Untreated and naturally contaminated control carcasses had a *Salmonella* incidence of approximately 70%. Birds rinsed/chilled in chlorine solutions had approximately 10-25% lower incidence than the controls, and birds rinsed/chilled in chlorine dioxide solutions had either no or a very low incidence of *Salmonella* (Villarreal et al., 1990).

In a later study by Cutter and Dorsa (1995) chlorine dioxide sprays, at concentrations of up to 20 ppm and durations of up to 60 s, were as effective as water at reducing microbial populations on fecally inoculated pre-rigor beef tissue. Untreated controls had populations of approximately 5.8 Log CFU/cm^2, whereas, samples washed with either water or chlorine dioxide had populations of approximately 4.0 Log CFU/cm^2.

Thus, chlorine dioxide may be useful as a microbiological decontamination method in beef carcass spray-chilling applications and in poultry chilling baths where it has a longer time for action. Concerns associated with the use of chlorine in meat decontamination are related to

potential effects on corrosion of metals and formation of harmful chemical by products with organic residue materials (Sofos and Smith, 1998).

D. Trisodium phosphate wash

Solutions of trisodium phosphate (TSP) have been approved by USDA for the treatment of beef, pork and poultry carcasses in the United States (Sofos and Smith, 1998). Dorsa et al., (1997) evaluated the effects of washing with TSP on bacterial populations of beef tissue both immediately after washing and during refrigerated storage of beef tissues for 21 days (Dorsa et al., 1997). The initial population of ca. 6.0 Log CFU/cm^2 of total aerobic bacteria were reduced to ca. 3.5 Log CFU/cm^2 following treatment with 12% TSP (32°C) solution for 15 s. Populations on TSP treated samples (ca. 6.7 Log CFU/cm^2) were lower than populations on controls (ca. 8.5 Log CFU/cm^2) at the end of a 21 day storage period (Dorsa et al., 1997). The study however evaluated the effect of only single concentration of TSP. To understand the effect of different concentrations of TSP, Dickson et al., (1994) treated sliced beef tissue artificially inoculated with *S.* Typhimurium*, L. monocytogenes* and *E. coli* O157:H7 by immersing them in 8, 10 and 12% solutions of TSP at 25, 40 and 55°C with contact times of up to 3 min. There were no significant differences noted in the effectiveness of TSP at different concentrations however, significant differences were noted between different temperatures at which the treatment was applied. Greater reductions in bacterial populations were observed on adipose tissue, with maximum reductions of 2 to 2.5 $\log_{10}$ cycles and 1 to 1.5 $\log_{10}$ cycles for the gram-negative and the gram-positive pathogens, respectively with increasing temperatures (Dickson et al., 1994).

The influence of sample type (i.e., excised versus non-excised chicken skin) on the efficiency of TSP solutions in reducing *L. monocytogenes* populations and inhibiting their

growth during refrigerated storage was studied by Capita et al., (2003). Whole chicken legs and excised chicken leg skin fragments were inoculated by dipping into solution containing 10^8 CFU/ml of *L. monocytogenes* for 5 min. After inoculation samples were dipped for 15 min in sterile tap water (control) or in a solution containing 8, 10, or 12% of TSP. Samples were analyzed for enumeration of *L. monocytogenes* counts on 0, 1, 3, and 5 days of refrigerated storage (2°C). The *L. monocytogenes* counts recovered on 0 day from whole leg samples treated by tap water (control), 8, 10, and 12% of TSP were 6.98, 5.75, 5.52, and 5.24 Log CFU/g of skin, respectively, whereas on excised skin samples 7.36, 5.48, 5.18, and 4.67 Log CFU/g of skin, respectively. Significant differences were noticed between counts of *L. monocytogenes* recovered from control samples and samples treated with TSP.

Efficacy of TSP has also been compared with efficacy of lactic acid spray. The inoculated lamb carcass breasts inoculated with a lamb fecal paste were applied with a 12% TSP dip at 55°C for 60 s or a 9 s 2% lactic acid spray at 55°C followed by a 90 s water rinse (Ramirez et al., 2001). The results indicated that treatment with TSP reduced *E. coli* and APCs by 1.8 Log CFU/cm^2 and 0.7 Log CFU/cm^2, respectively, whereas, lactic acid reduced *E. coli* counts and APCs by 1.6 Log CFU/cm^2.

Use of 10% TSP has been reported to be effective on chicken wings by Rodriguez de Ledesma et al., (1996). The inoculated chicken wings were dipped into 10% TSP at 10°C for 15 s, which resulted in reduction of the prevalence of viable *S.* Typhimurium, *L. monocytogenes* and *S. aureus* by 93.45%, 39.04%, and 80.33%, respectively (Rodriguez de Ledesma et al., 1996).

However, studies have also been reported on ineffectiveness of 10% TSP solutions for inactivating pathogens present on beef tissue. Kim and Slavik (1994) used an immersion treatment for beef tissue (post-rigor) inoculated with either *S.* Typhimurium or *E. coli* O157:H7

with a 10% TSP solution at 10°C (50°F) for 15 s. Samples washed only with sterile water were used as controls and had populations of approximately 6.5 to 7.0 Logs whereas the TSP treated samples had populations of approximately 5.5 to 6.1 Logs. Because pathogen levels greater than 5.0 Logs remained on the beef tissue samples after treatment, statistically TSP was fairly ineffective as a decontaminating compound under the conditions used in the study (Kim and Slavik, 1994).

E. Organic Acid wash

Organic acids are typically applied as a rinse to the entire surface of the carcass. Acetic and lactic acids are most widely accepted organic acids for carcass decontamination (Smulders, 1995). Additionally, it widely accepted that the effectiveness of organic acids is best achieved shortly after hide removal when the carcass is still warm (Smulders, 1995). USDA-FSIS (1996a) has approved the use of organic acid solutions such as acetic, lactic and citric acids at concentrations of 1.5–2.5%. Many researchers have evaluated the efficacy of organic acids as decontaminants for meat animal carcasses and tissue. Difficulty arises when attempting to compare results from these evaluations due to the numerous treatment parameters involved, including acid type, concentration, temperature, and pH as well as sample type, application method, and exposure time.

Acetic Acid Treatments

In a model spray chilling cycle, Dickson (1991) incorporated acetic acid and determined reductions in *S.* Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7 on 4 cm^2 pieces of lean and adipose beef tissue. Beef tissue pieces inoculated with *S.* Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7 at an initial population of ca. 5.8 to 6.0 Log CFU/cm^2, were dipped into 2% acetic acid for 30 min intervals over a period of 4 h. To compare the effectiveness of the

treatment control beef tissues were dipped into water for the same period of time. Reductions in *S.* Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7 at the end of the 4 h period were 0.7, 1.5, and 1.1 Log CFU/cm^2, respectively, on lean tissue and 2.5, 2.1, and 1.3 Log CFU/cm^2, respectively, on adipose tissue. The reduction observed in the populations of inoculated pathogens was higher in adipose tissue compared to lean tissue. The reduction in the populations of pathogenic or spoilage microorganisms are affected by the type of tissue they are inoculated on. This inference is also supported by another study by Bell et al. (1997) who used 1% acetic acid at a pH of 2.92 and a temperature of 25°C in a pilot scale model carcass washer to decontaminate beef lean and adipose tissue contaminated with fecal materials containing *E. coli*, *L. innocua*, or *S. wentworth* at ca. 5 Log CFU/cm^2. The acetic acid spray was applied for 15 s, the tissue was held for 90 s, and the spray was applied a second time for 15 s. The acetic acid treatment resulted in 2.47 and 3.12 Log CFU/cm^2 reductions in populations of *E. coli* for adipose and lean tissue, respectively, compared to spraying with distilled water which resulted in reductions in *E. coli* populations by 1.96 and 2.33 for adipose and lean tissue, respectively. For *L. innocua*, reductions of 1.94 and 2.85 (acetic acid) logs and of 2.07 and 2.45 (distilled water) logs were observed for adipose and lean tissue, respectively. The reductions in populations of *E. coli* and *L. innocua* were higher on lean tissue compared to adipose tissue. However, for *S. wentworth*, log reductions observed were higher on adipose tissue compared to lean tissue. The reductions in *S. wentworth* populations were 3.51 and 3.47 logs when treated with acetic acid and 2.48 and 2.77 logs when treated with distilled water on adipose and lean tissue, respectively (bell et al., 1997). The lower reduction of pathogenic bacteria on lean tissue indicates that due to fibrous structure of muscle tissue microorganisms may be present as a sandwich between two muscle tissues and hence receive protection against antimicrobial intervention.

Tinney et al., (1997) examined the effectiveness of an acetic acid spray (2% at 37°C) for reducing *E. coli* O157:H7 populations artificially inoculated on beef steaks. The acetic acid treated steak samples had 3.4 Log CFU/cm^2 of a residual population of *E. coli* O157:H7, compared to non-treated controls, which had significantly higher populations of 3.9 Log CFU/cm^2. For *S.* Typhimurium, populations on acetic acid treated samples (3.8 Log CFU/cm^2) were significantly lower than those of non-treated controls (4.6 Log CFU/cm^2).

Kochevar et al., (1997b) evaluated the effectiveness of a single water wash (temperatures of 16, 35, or 74°C and pressures of 2.76, 13.79, 20.68, and 27.58 bar) and a water wash followed by spraying with acetic acid (2%, 16°C, 1.38 bar, 18 s) for decontaminating adipose tissue from the breast region of freshly slaughtered lamb carcasses inoculated with lamb feces. Control samples were inoculated but not treated with any wash had initial APCs of 5.9 Log CFU/cm^2. The water wash alone resulted in residual populations of 2.7 Log CFU/cm^2, whereas the water wash followed by the application of acetic acid resulted in residual APCs of 2.1 Log CFU/cm^2.

Lactic Acid Treatments

Lactic acid has also been used in commercial trials on beef carcasses and reported to be effective as a single treatment. Application of lactic acid after dehiding and after evisceration is more effective compared to application at any other stage of slaughtering (Prasai et al., 1991). In a commercial evaluation beef carcass sides were sprayed with 1% lactic acid (55°C, pH 2.8, 500 ml/35 s, 40 psi, distance of 80 cm) either after dehiding, after evisceration, or both (Prasai et al., 1991). Compared to untreated control carcasses with initial APCs of ca. 3.7 Log CFU/cm^2, all treated carcasses had APCs counts of approximately 2.0 Log CFU/cm^{2}immediately after treatment.

In a later study, Prasai et al. (1997) evaluated the effect of lactic acid treatment on beef sub-primal just prior to vacuum packaging and/or after 14, 28, 56, 84, and 126 days of storage at refrigeration temperatures. In general, application of acid prior to packaging and storage was more effective than application of acid after storage. For all treatments in which acid was applied before storage, aerobic populations were lower than controls at each sampling point; however the difference was not always statistically significant.

The aroma profile of beef subprimals were evaluated as affected by spraying carcasses with 3% lactic acid or 200 ppm chlorine (Garcia Zepeda et al., 1994b). Lactic acid on beef subprimals could not suppress the off flavors during subsequent cold storage while chlorine was more effective in preserving the aroma of beef subprimals (Garcia Zepeda et al. 1994b).

Multiple Organic Acids

Fu et al., (1994) sprayed pork carcasses with either 1.5% acetic, citric, or lactic acid solutions. Immediately after acid treatment APCs were significantly lower on loins treated with acetic and citric acid, however no significant reduction was achieved on pork loins treated with lactic acid. Acetic acid treatment reduced the populations of coliforms and *E. coli* immediately after treatment, however, no significant reductions in these populations were noticed when loins were treated with citric or lactic acid. None of the acid treatment extended the shelf-life or changed the spoilage pattern of pork chops during the post treatment refrigerated storage for 14 to 35 days. Dickson and Siragusa (1994) showed that storage of acid treated beef tissues at lower RH may improve the microbial quality of the beef tissues. Beef lean and adipose tissue were washed with water, 1% acetic acid, or 1% lactic acid, and stored the tissue at 5°C (41°F) and 99% or 26% RH for 3 days. The treatment of washing with 1% lactic acid resulted in a reduction of ca. 0.5 Log CFU/cm^2 in the populations of S. Typhimurium, *E. coli* O157:H7, and

L. monocytogenes, from an initial populations of ca. 5.0 Log CFU/cm^2 following the 3 day storage period. The reductions of over 4 Log CFU/cm^2 were observed when treated samples were stored at 26% RH rather than 99% RH (Dickson and Siragusa, 1994).

Brackett et al., (1994) used hot organic acid sprays (acetic, citric, or lactic) at three concentrations (0.5, 1.0 and 1.5%) and reported that they were ineffective at reducing populations of *E. coli* O157:H7 inoculated at ca. 3.5 or 6.8 Log CFU/g on post-rigor beef tissue discs. Regardless of acid type or concentration, no differences in *E. coli* O157:H7 populations were observed on samples treated with sterile distilled water (controls) and those treated with 55°C (131°F) acid when the initial inoculation level was ca. 3.5 Log CFU/g. At an inoculation level of ca. 6.8 Log CFU/g, samples treated with 1.5% lactic acid at 55°C (131°F) had a residual population of 6.6 Log CFU/g, which was statistically lower than the control population of 6.8 Log CFU/g. Although significant, this difference was of no practical significance.

Garcia Zepeda et al. (1994a) demonstrated the effectiveness of gluconic acid and lactic acid, alone or in combination, in reducing the populations of aerobic psychrotrophs and lactic acid bacteria on lean beef tissue. After 56 days, the untreated controls had aerobic psychrotrophic populations of ca. 6.0 Log CFU/cm^2, whereas acid treated samples had populations of ca. 3.5 to 5.5 Log CFU/cm^2, with the lowest population being observed on the beef tissues treated with the 3% gluconic acid and 1.5% lactic acid. Conversely, Podolak et al. (1996) showed the ineffectiveness of use of combined acid treatments. Lean beef tissues inoculated with either *L. monocytogenes* (8.6 Log CFU/cm^2) or *E. coli* O157:H7 (5.1 Log CFU/cm^2) were treated using fumaric acids (0.5, 1.0, 1.5, or 2.0%) individually or in combination with lactic (1 %) and acetic acids (1 %), respectively. After a 90 day storage period there was less than 1 Log CFU/cm^2 reductions in *L. monocytogenes* populations observed for all

types of treatments at all sampling times. The 1.5 and 2.0% fumaric acid treatments resulted in the largest reductions of 0.9 Log CFU/cm^2 after 7 days of storage. Similar trend was observed in *E. coli* O157: H7, with the largest reduction of 1.3 Log CFU/cm^2 resulting from 1.0% fumaric acid treatment after 14 days of storage. Fumaric acid at concentrations of 1.0% and 1.5% was more effective than any of the combined solutions of acids (Podolak et al., 1996).

Organic acids must be used with some degree of caution due to concern of acid adaptation of pathogens. It has been reported that acid adaptation of *E. coli* O157:H7 and other pathogens may occur in dilute decontamination acid fluids in meat packing plants. In laboratory studies Samelis et al. (2002), showed that a previously acid adapted *E. coli* O157:H7 strain survived for 14 days at 4^{o}C or 10^{o}C in acid containing waste fluids from meat decontamination. In a separate study, Stopforth et al. (2004) evaluated the effect of lactic acid decontamination treatment in reducing the populations of acid-adapted or acid-non-adapted *E. coli* O157:H7 cells artificially inoculated on beef tissues at 10^4 or 10^5 CFU/cm2. Following the 2% lactic acid decontamination treatment, populations of the acid-adapted (incubated in 2% lactic acid solution at pH 4.12 for 48 h at 15^{o}C) and acid-non-adapted (water, pH 6.05) *E. coli* O157:H7 cells were reduced to 0.7 and 1.4 Log CFU/ml from an initial population of 4.0 and 4.2 Log CFU/ml, respectively. Thus, acid-adapted *E. coli* O157:H7 cells were more acid resistant ($P < 0.05$) than were the non–acid-adapted cells (Stopforth et al., 2004).

Castillo et al. (1999) examined the effectiveness of citric acid-activated acidified sodium chlorite spray (CASC) for reducing inoculated *E. coli* O157:H7 on beef carcasses. The compound was applied at room temperature, to surfaces that had been inoculated with *E. coli* O157:H7 at 5.5 Log CFU/cm^2 and a reduction of 4.5 Log CFU/cm^2 was observed. CASC effectively reduced the populations of pathogens spread to areas beyond the initial contaminated

area of cuts to levels close to or below the detection limit of the assay (0.5 Log CFU/cm^2). Although significant reductions in the populations of *E. coli* O157:H7 were achieved on beef carcasses, the treatment with CASC did not eliminate the pathogen completely as 22–50% of the treated carcasses still had viable *E. coli* O157:H7 cells by direct plating.

F. Spot-Cleaning (Knife trimming or steam/hot water vacuuming)

One of the most commonly accepted method to remove visible contamination on beef carcasses is knife trimming (CFR, 1993). The effectiveness of physically removing bacteria associated with visible fecal contamination has been investigated by several research groups.

In 1995, Gorman et al. reported a reduction of 1.96 Log CFU/cm^2 of total aerobic bacteria, and 2.19 Log CFU/cm^2 of generic *E. coli* using knife trimming alone (Gorman et al., 1995). In another study, Gorman et al. (1995) observed that trimming reduced bacterial populations by at least 1 Log. However, there was still a significant amount of bacterial contamination after trimming, and there was a potential for cross-contamination when knife trimming was used. Hardin et al., (1995) found that knife trimming significantly reduced pathogens associated with fecal contamination and the spread of pathogens outside of the inoculated area was minimal. The authors also reported that trimming with knife was as effective as organic acid treatment at the inside round area of the beef carcasses. Prasai et al. (1995) reported trimming alone reduced total aerobic bacteria by 0.9 Log CFU/cm^2. Saide-Albomoz et al. (1995) reported that knife trimming of fat from pork loins decreased the number of *S. aureus* positive samples. However, Gill et al. (1996a, 1996b) found that trimming of the neck and brisket did not significantly reduce *E. coli* populations, but was effective in reducing both total aerobic bacteria and *E. coli* populations at the rump area of beef carcasses.

According to Delmore et al. (1997) knife trimming of beef carcasses effectively reduced total coliforms associated with fecal contamination. In 1997, findings of a study by Phebus et al. infer that knife trimming alone and in combination with other treatments is effective in reducing the inoculated pathogenic populations on beef carcasses (Delmore et al., 1997).

In April of 1996, the FSIS approved the use of steam/hot water vacuuming equipment in commercial slaughtering beef operations (Kochevar et al., 1997a). The process of spot-cleaning/decontamination of carcasses (<2.5 cm diameter spots) with hand-held equipment uses hot water and/or steam to loosen soil and inactivate bacteria, followed by application of vacuum to remove the contaminants. Kochevar et al. (1997a) evaluated the efficacy of decontamination achieved by steam-vacuuming and knife-trimming of soiled carcasses (≤2.5 cm in diameter spots of fecal material) in seven beef slaughtering plants. The results indicated that treatment of steam vacuuming removed physical materials from beef carcasses similar to those obtained by knife-trimming, as indicated by visual evaluation. Steam-vacuuming and knife-trimming reduced mean APCs on treated carcass spots by 1.7–2.0 and 1.4–1.6 Log CFU/cm^2, respectively. Corresponding reductions in total coliform counts were 1.7–2.2 and 1.6–1.8 Log CFU/cm^2, respectively. Steam-vacuuming also reduced mean bacterial counts on carcass surfaces that had no visible contamination by 0.6–0.7 and 0.2–0.3 Log CFU/cm^2 for APCs and total coliform counts, respectively (Kochevar et al., 1997a).

G. Steam pasteurization

Steam has been used effectively in food processing industry for many years. Heat is one of the most effective and safest interventions to inactivate pathogenic and spoilage microorganisms present in raw as well as processed foods. One of the major advantages of using

steam as a decontamination intervention is that it uses a condensable form of gas (Kozempel et al., 2003). The gaseous steam condensing at atmospheric pressure facilitates rapid heat transfer which can easily reach the hidden parts of a carcass surface and uniformly heats the entire surface of a carcass to inactivate microorganisms (Kozempel et al., 2003).

A patented process of applying pressurized steam to carcasses was developed by Frigoscandia and Cargill, Inc. (The Frigoscandia Steam Pasteurization System™), and it was approved for commercial application by the FSIS in the United States (USDA-FSIS, 1995b). The system consists of an entrance section where air is blown over the sides of beef to dry surface water remaining from carcass washing; this is followed by the pasteurization chamber, which is sealed and filled with steam under pressure (105°C); and an exiting section where the beef sides are sprayed with cold water. The exposure of carcasses to steam lasts for approximately 6.5–8.0 s. This high cost process system has been approved by FSIS and has reportedly been installed and used in several meat processing plants (Sofos and Smith, 1998).

The Frigoscandia process was evaluated by Phebus et al., (1997) who inoculated cutaneous trunci muscles from pre-rigor beef with feces containing 5 Log CFU/cm^2 each of *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium. A variety of decontamination treatments alone as well as in combination of the following: knife trimming, water washing (35^{o}C), steam vacuuming, spraying 2% lactic acid (54^{o}C, pH 2.25), and steam pasteurization (Frigoscandia Steam Pasteurization™) were evaluated. The combination treatments of knife trimming with washing, trimming with washing and steam pasteurization, washing with steam pasteurization, steam vacuuming with washing, steam vacuuming with washing and steam pasteurization, trimming with washing, lactic acid wash and steam pasteurization, and steam vacuuming with washing, lactic acid wash and steam pasteurization reduced all three pathogen populations, with

reductions ranging from 3.5 to 5.3 Log CFU/cm^2. When knife trimming, steam vacuuming, and steam pasteurization were used as an individual treatment, it resulted in pathogen reductions ranging from 4.2 to 5.3 Log CFU/cm^2. Steam pasteurization™ consistently produced numerically greater reductions than knife trimming or steam vacuuming treatments.

Using Frigoscandia Steam pasteurization™ system, Nutsch et al. (1997) tested 140 randomly selected freshly slaughtered beef sides in a commercial facility over ten days by treatment with steam for 8 s at temperature range of 90.5° to 94.0°C. Beef sides were sampled before and after treatment and after 24 h of chilling, and samples were enumerated for APCs, coliforms, *E. coli* and *Enterobacteriaceae*. Mean APCs of 2.2 Log_{10} CFU/cm^2 before treatment were reduced to 0.8 and 0.9 Log_{10} CFU/cm^2 immediately after steam pasteurization and 24 h chilling, respectively. Before steam pasteurization, 16.4% of the samples were positive for generic *E. coli* (0.6-1.5 log_{10} cfu/cm^2), 37.9% for coliforms (0.6-2.3 log_{10} cfu/cm^2), and 46.4% for *Enterobacteriaceae* (0.6-2.3 log_{10} cfu/cm^2). After exposure to steam, the results were 0% positive for *E. coli*, 1.4% for coliforms (0.6-1.5 log_{10} cfu/cm^2), and 2.9% for *Enterobacteriaceae* (0.6-2.0 log_{10} CFU/cm^2). One carcass tested positive for *Salmonella* before steam pasteurization treatment however no *Salmonella* were detected after steam pasteurization. In another study by the same research group, effect of using Frigoscandia Steam Pasteurization System on the microbial quality of beef carcasses at five anatomical locations were studied in a commercial slaughtering facility (Nutsch et al., 1998). To determine microbial reductions at five anatomical locations sponge samples were collected from each location before and after steam pasteurization treatment at 82.2°C for 6.5 s. Significant reductions in the populations of total aerobic bacteria were observed at all five anatomical locations following the steam pasteurization treatment. The steam pasteurization treatment was least effective at the neck area in spite of significant

reductions at this region. The temperature data indicated that neck area was not heated to temperatures as high as the other locations. The other probable reason could be the dressing practice currently used by the meat processing industry. Carcasses in a commercial slaughtering practice are hanged upside-down which exposes neck region to contamination by internal organs upon evisceration. Before steam treatment 68% of beef carcass samples were detected with *E. coli* populations however after the steam pasteurization only 15% of the samples had detectable levels of *E. coli* populations.

Another steam pasteurization apparatus uses an ultra-high temperature, ultra-short time surface pasteurization treatment (Morgan et al., 1996). Using a four step treatment of vacuum (air was removed from around the meat), flush with steam (low temperature steam), treat with steam (meat surface saturated by pure, saturated steam), and cool with vacuum (evaporative cooling) chicken carcasses inoculated with *L. innocua* at 7 log cycles were treated for 25 milliseconds at 145^{o}C which resulted in a 4 log cycle reduction in *Listeria* counts. This process was also found effective in reducing bacterial counts on fresh beef and pork (Morgan et al., 1996).

Dorsa et al., (1996) used a wash-air dry-steam treatment to decontaminate un-inoculated and fecally inoculated areas of freshly slaughtered sheep carcasses. Carcasses were exposed to low pressure steam for 30s in a custom built plywood cabinet, and the treatment caused a reduction of 1.5 log cycles in APCs on un-inoculated surfaces from the initial APCs of ca. 2.5 Log_{10} CFU/cm^2). The APCs on carcass tissue inoculated with feces was reduced by ca. 3 Log_{10} CFU/cm^2 from the initial level of 6 Log CFU/cm^2.

Davidson et al. (1985) used a steam chamber to decontaminate whole chicken carcasses and chicken pieces by exposing them to a continuous flow of steam for 20 s at atmospheric

pressure with internal steam chamber temperatures ranging from 180° to 200°C. The APCs were reduced by 1.0 - 3.0 Log CFU/cm^2 for both types of samples. The variations observed in the populations of APCs after 20 s steam treatment between different lots of chicken carcasses supplied by the same supplier were attributed to the uneven surfaces of the chicken carcasses. A slight discoloration of the treated samples was believed to be caused by longer exposure of the chicken samples to high temperatures.

In a recent study Corantin et al. (2005) examined the effectiveness of steam pasteurization on microbial quality of cull cow carcasses. Sponge swab samples were collected from randomly selected sites of carcasses before and after pasteurization and after chilling to enumerate total aerobic bacteria, total coliforms, and generic *E. coli*. The results of the study indicated that mean populations of total aerobic counts, total coliform counts, and *E. coli* counts before pasteurization were 2.18, 0.16, and 0.06 Log CFU/cm^2, respectively, 1.17, 0.03, and 0.01 Log CFU/cm^2, respectively after pasteurization; and 0.89, 0.02, and 0.01 Log CFU/cm^2, respectively after chilling.

IMPLEMENTATION OF HACCP IN SMALL AND VERY SMALL MEAT PROCESSING PLANTS

HACCP is a preventive-based food safety system that is now the regulatory requirement for food safety in slaughter and processing establishments in the red meat and poultry industries (Dreesen, 1998). HACCP is defined as a "systematic approach to the identification, evaluation, and control of food safety hazards" (NACMCF 1998). In response to a request by the National Aeronautics and Space Administration for providing safe, hazard-free foods to the astronauts Pillsbury Company created the concept of the HACCP in 1960 (Dreesen, 1998). On March 1992, a formal report on implementation of HACCP in processed food industry was issued by

National Advisory Committee on Microbiological Criteria for Foods (NACMCF), a panel organized by the USDA FSIS and the Food and Drug Administration (NACMCF 1998). On July 25, 1996 FSIS issued the HACCP Systems Final Rule which required meat and poultry-processing establishments of all sizes in the United States to implement the system in order to increase food safety and reduce risks of illnesses associated with consumption of foods (USDA-FSIS 1996a). The seven fundamental principles included under the HACCP system are: conducting a hazard analysis, determining critical control points, establishing critical limits, establishing monitoring procedures, establishing corrective actions, and establishing record-keeping and documentation procedures (Dreesen, 1998). The final rule uses *Salmonella* as criterion for pathogen control and prevention in animal products. Foods will be withheld or suspended and the right of the manufacturer to distribute is curtailed if *Salmonella* contamination is above the criteria limit (USDA-FSIS 1996a).

The strategic goals of FSIS included implementation of the HACCP plan for all State and federally inspected meat and poultry slaughter plants by 2000 (USDA-FSIS, 2000a). These plants must develop and implement written sanitation standard operating procedures. The FSIS will test for *Salmonella* on raw meat and poultry products to verify standards for *Salmonella* pathogen reduction are within the set limits. Slaughter plants must test for generic *E. coli* on carcasses to verify prevention and removal of fecal contamination (USDA-FSIS, 1996a). On January 25, 2000, FSIS achieved its goal when 3,159 Federal and approximately 2,300 State-inspected very small plants were operated under HACCP (USDA-FSIS, 2000c).

Small meat processing plants are the establishments with more than 10 but less than 500 employees while very small meat processing plants are the establishments with fewer than 10 employees or annual sales of less than 2.5 million US dollars (SEMA, 2003). As of January

2000, 100% of cattle, swine, and chicken are subject to testing for *Salmonella* incidence at the slaughter plant. Data from a year of testing in small plants show a decline in the prevalence of *Salmonella* from the pre-HACCP baseline studies. Of broiler carcasses, 20% tested positive for *Salmonella* before HACCP implementation, compared to 16.3% since HACCP implementation. In ground beef, 7.5% of the national baseline samples tested positive for *Salmonella* prior to HACCP implementation versus 4.3% since HACCP implementation and for cow and bull carcasses, 2.7% tested positive before HACCP implementation while 2.3% tested positive after HACCP implementation (USDA-FSIS, 2000b).

While the implementation of HACCP has resulted into decreased incidence of foodborne pathogens, it has been determined that cost of implementing HACCP system is higher for small plants than for large plants (Hooker et al., 2002). The small meat and poultry processors may have to absorb higher costs for food safety regulations compared to the larger plants.

DECONTAMINATION TECHNOLOGY ADOPTED OR ADOPTABLE BY SMALL AND VARY SMALL MEAT PROCESSING PLANTS

Small and very small establishments represent approximately 70% of the total slaughter plants in the United States. (USDA FSIS, 2003). This large amount of establishments represents an important field of work where implementation of affordable carcass decontamination technologies is needed to ensure consumer safety from foodborne pathogens (Gabriel et al., 2004). Many carcass decontamination technologies have been developed but only few have been implemented in large commercial meat processing plants. Most of these technologies, although effective in eliminating spoilage and pathogenic microorganisms have not been widely used in small and very small meat processing operations. The high cost of installation and operation of these technologies is huge burden to small and very small meat processors. A system which can provide effectiveness against pathogens and spoilage microorganisms, ease of installation and

operation along with cost effectiveness would help the small and very small meat processors to face their current challenges (Gabriel et al., 2004).

Carcass decontamination utilizing organic acids is a sanitation process that is widely used in the industry, and has been studied deeply. Spraying with organic acid solutions and/or hot or cold water is increasingly applied as sequential interventions for meat decontamination (Stopforth et al., 2003). One of the economic methods for spraying carcasses with organic acid washes is conventional garden sprayer. Household vinegar (usually 5% acetic acid) is mixed with water in 1:1 ratio (2.5% acetic acid) and sprayed on meat carcasses using hand sprayer (Kerth and Braden, 2003). No systematic study on effectiveness of such system has been documented in literature. However reports on use of such systems in small and very small establishments are available. Recently, Gabriel et al. (2004) have proposed and validated a lactic acid spray system to be used by small and very small meat processing plants. The effectiveness of the proposed system has been compared with the conventional garden hand sprayer. Populations of mesophilic counts, APCs and coliforms recovered from carcasses treated using the proposed system were lower than those treated with conventional hand sprayer (Gabriel et al., 2004).

More research is required on development of new technologies that could be implemented by small and very small meat processors. .The objectives of the present study were:

1. To compare the efficacy of three household steam cleaners in reducing the pathogenic and spoilage bacteria from artificially inoculated pork skins under laboratory conditions.
2. To evaluate the feasibility of using one of the steam cleaners for control of naturally occurring pathogenic and spoilage microorganisms on beef and pork carcasses commercially processed by small or very small meat processors.

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CHAPTER 3

Effectiveness of Commercial Household Steam Cleaning Systems in Reducing the Populations of *Listeria monocytogenes* and Spoilage Bacteria on Pork Skin Surfaces[1]

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Abstract

The goals of this study were to determine the maximum levels of bacterial reduction caused by treatments using the steam cleaner, and to compare the efficacy of this cleaner with the efficacies of two other commercial household steam cleaning systems in reducing bacterial populations on the surface of pork skin artificially inoculated with ca. 7.61 or 5.75 $\log_{10}$ CFU/cm^2 of *L. monocytogenes*. The contaminated pork skin were treated with the three stream cleaners for 30 - 180 s in 30 s intervals, and assayed for the populations of *L. monocytogenes* as well as total aerobic and thermoduric bacteria. The application of steam significantly reduced the population of *L. monocytogenes* (ca. 7.61 – 3.23 $\log_{10}$ CFU/cm^2), as well as total aerobic (ca. 5.68 – 4.04 $\log_{10}$ CFU/cm^2) and thermoduric bacteria (ca. 6.12 – 2.57 $\log_{10}$ CFU/cm^2) on the surface of pork skin ($P \leq 0.05$). Significant differences in microbial reductions were observed between different inoculation levels, treatment times, and types of cleaning systems used in the study ($P \leq 0.05$). The results suggest that the commercial household cleaning systems could be effectively used by meat-processing facilities, especially small and very small meat-processing facilities as an essential part of the HACCP system.

Keywords: *Listeria monocytogenes*, Thermoduric bacteria, Total aerobic bacteria, Steam pasteurization, Household steam cleaning systems, Pork skin

1. Introduction

Listeria monocytogenes is an important bacterial pathogen that potentially causes fatal illnesses as listeriosis. According to an estimate made by the Centers for Disease Control and Prevention (CDC), there are approximately 2,500 severe cases of listeriosis and 500 deaths each year in the United States (Mead et al., 2000). A relatively high resistance to heat and salt, an ability to proliferate at 0^{o} to 50^{o}F, and its ubiquitous nature makes *L. monocytogenes* a potential threat to the meat processing industry and the consumers (Murphy, Duncan, Johnson, Davis, Wolfe, & Brown, 2001; USDA-FSIS, 2003). To reduce the level of microbial contamination on raw meats and animal carcasses, meat processing facilities of all sizes in the United States are currently required to establish Sanitation Standard Operating Procedures (SSOP) as well as the Hazard Analysis Critical Control Point (HACCP) program (Sofos & Smith, 1998). Although large scale meat processors have adequate personnel, resources, and technology to comply with the regulations, small and very small meat processors are in dearth of technical resources. This in some degree increases the risk of foodborne illnesses associated with pathogens such as *L. monocytogenes* on meat products processed by small and very small meat processing facilities. Thus, a special need for identifying a suitable carcass decontamination technology for small and very small scale meat processing operations has been recognized by regulating authorities.

Several carcass decontamination methods such as, steam/hot water vacuuming, spray washing, and steam pasteurization, have been validated for use as critical control points to reduce bacterial populations on meat and poultry carcasses (Dorsa, Cutter, Siragusa, & Koohmaraie, 1996; Nutsch et al., 1997; Phebus et al., 1997; Retzlaff, Phebus, Nutsch, Reimann, Kastner, & Marsden, 2004; Sommers, Kozempel, Fan, & Radewonuk, 2002). Among these, steam pasteurization has been proven to be one of the most effective methods for inactivating bacterial

pathogens. An automated steam pasteurization system, Frigoscandia™, has been developed and commercially validated for reducing microbial populations on beef carcasses (Federal register, 1996). The system is designed for medium and large beef slaughter operations and Model SPS 400 can ‘‘pasteurize’’ up to 400 carcasses per h (Retzlaff et al., 2004). Phebus et al. (1997) evaluated the efficacy of the equipment in reducing the populations of *L. monocytogenes* Scott A, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium from contaminated muscles of freshly slaughtered steers, and found that a 15 s steam treatment caused a mean *L. monocytogenes* reduction of 4.6 Log CFU/cm^2.

The primary goal of the meat decontamination practice is to reduce the risk to consumers from pathogenic organisms like *L. monocytogenes*. Although many attempts have been made in recent years to develop effective technologies to reduce the level of meat contamination by pathogenic bacteria, only some of them have been successfully implemented in meat processing plants. Some of these decontamination systems are not viable for small and very small scale operations due to the high cost of installation, operation, and maintenance. A system which can offer an ease of operation and installation, reusability, effectiveness against pathogens, and economic affordability would meet the needs of small and very small meat processors (USDA-FSIS, 1998).

The objective of the present study was to compare the efficacy of three commercial household steam cleaners in reducing the populations of *L. monocytogenes* as well as total aerobic and thermoduric bacteria on artificially contaminated pork skin surfaces.

2. Materials and methods

2.1. Bacterial cultures

Five *L. monocytogenes* cultures from our laboratory collection were grown at 37°C for 24 h on Oxford medium base supplemented with modified Oxford antimicrobial supplements (MOX) (Difco Lab., Sparks, MD, USA). A single colony of each culture was transferred into 9 ml tryptic soy broth and the inoculated broth was incubated for 18 h at 37°C. The resulting liquid cultures were used to prepare the *L. monocytogenes* inocula needed for artificial inoculation of the pork skin samples.

2.2. Preparation of L. monocytogenes inocula

On the day of trial, fresh fecal materials were collected from a private farm located near the Georgia Agriculture Experiment Station in Griffin, GA. Nine ml of each *L. monocytogenes* culture were transferred to a 50 ml centrifuge tube and mixed thoroughly using a Minivortexer (VWR Scientific products). The *L. monocytogenes* mixture was centrifuged at 4,000 *g* for 15 min. The pellet of the cell mixture was washed twice and then re-suspended into an appropriate volume of maximum recovery diluent (MRD). Accurately weighed fecal materials were inoculated with an appropriate volume of the *L. monocytogenes* cell suspension to obtain an inoculation level of ca. 9 $\log_{10}$ CFU/g. For the preparation of the fecal materials containing approximately 7 $\log_{10}$ CFU/g of *L. monocytogenes*, a similar procedure was employed except that the aforementioned *L. monocytogenes* suspension was diluted 100 fold before being mixed with the same amount of fecal materials.

2.3. Pork skin

Fresh pork skin was purchased one day prior to each trial from the International Farmers' Market in Lake City, GA. The skin was rinsed with tap water for 1 min and was allowed to dry

at room temperature. The flesh and muscles on the other side of the skin were trimmed using a knife in a way that only the upper most layer of the skin remained at the end. The pork skin was cut into equal blocks of 7 X 3.5 cm using a pair of scissors with each block of the skin being placed into a Petri-plate. Using a sterile spatula and sterile card board template, an accurately weighed 0.5 g of the inoculated fecal materials mentioned above was evenly spread on a randomly selected 5 X 2 cm area of the pork skin blocks. The contaminated pork skin blocks were allowed to stand at room temperature for 15 min before steam treatment. The *L. monocytogenes* inoculation level on the pork skin surface was approximately 7.61 or 5.75 $\log_{10}$ CFU/cm^2.

2.4. Steam treatment

Three commercial household steam or steam/vacuum cleaning systems, S1, S2, and S3, were purchased from two different suppliers. The systems differed in their technical parameters. S1 was a household steam cleaner with a high steam capacity power of 1,500 W, S2 used a combination of steam (1,000 W) and vacuum (800 W), producing a high temperature steam (248^{o}F) at 58 psi (4 bars), whereas, S3 was a vapor cleaning system with the highest capacity of 1,600 W producing low moisture steam at high pressure (85-95 psi). Two separate trials were conducted and the experiments in each trial had duplications. The inoculated pork skin samples were divided, in each trial, into three groups with 12 inoculated pork skin samples in each group. The samples were treated with S1, S2, or S3 for 30, 60, 90, 120, 150, or 180 s with two samples being treated at each treatment interval. The Petri-plates containing the pork skin samples were transferred to a bucket of ice for immediate chilling after the steam treatment. During each trial, two inoculated pork skin samples were run as positive controls which were inoculated with *L.*

monocytogenes but not subjected to any steam treatment. Skin samples inoculated with natural fecal materials were included in the study as negative controls.

2.5. Microbiological analysis

After the completion of steam treatment, the treated pork skins were sampled by aseptically excising the 5 X 2 cm inoculated area with a pair of sterile scissors. Each pork skin sample was aseptically transferred to a sterile sampling bag (4.5" X 9", Fisher Scientific Inc., Pittsburgh, PA, USA) containing 10 ml of sterile MRD solution. The contents of the bag were stomached for 1 min at normal speed using a Stomacher 400 lab blender (Seward Ltd., London, UK). Serial dilutions were prepared using sterilized MRD solution. A 50 µl of appropriate dilutions was spirally plated in duplicate on MOX agar plates for the enumeration of *L. monocytogenes*, on tryptic soy agar (TSA) plates for the enumeration of total aerobic bacteria, and on MacConkey (MAC) agar plates for the enumeration of thermoduric bacteria, using the Autoplate® 4000 automated spiral plater (Spiral Biotech, Bethesda, MD, USA). The MOX and TSA plates were incubated at 37°C for 24 h while the MAC plates were incubated at 45°C for 24 h. The colonies on each plate were counted using a **Q***COUNT*™ automatic colony counter (**Q***COUNT*™ software, Spiral Biotech, Bethesda, MD, USA).

2.6. Enrichment

A qualitative estimation of heat injured *L. monocytogenes* was carried out according to a procedure outlined in Compendium of Methods for the Microbiological Examination of Foods (Hitchins, 2003). Pre-enrichment was performed by transferring 5 ml of the sample to 45 ml of sterile MRD and then incubating for 24 h at 37°C. One ml of the resulting culture was transferred to 9 ml of sterile *L. monocytogenes* enrichment broth (LEM) and incubated for 24 h at 37°C. After incubation, a loop full of the enriched culture was inoculated onto the MOX agar

plates and incubated at 37°C for 24 h. For the recovery of heat injured thermoduric bacteria and total aerobic bacteria, a loop full of the pre-enriched culture was inoculated directly onto MAC or TSA agar plates followed by incubation for 24 h at 45 and 37°C, respectively.

2.7. Temperature profile

The temperature increase of pork skin surfaces, while receiving the steam treatment, was recorded using a Fisherbrand traceable dual channel thermometer with offsets (Fisher Scientific Inc., Pittsburgh, PA, USA). Each channel was connected *via* K-type beaded probes which are designed to measure the surface temperatures. Both probes were attached to the pork skin surfaces and temperature readings were recorded at each treatment interval for all three steam systems. The temperature readings were then plotted as a function of treatment time.

2.8. Data analysis

Data was transformed into Log_{10} CFU/cm^2 before comparison of means. Analysis of data was accomplished using the Fisher's least significant difference of means of bacterial populations calculated with the General Linear Model (GLM) procedure of SAS (windows version 5.1.2600, 2000, SAS institute, Inc., Cary, NC) based on a 95% confidence level.

3. Results

When the *L. monocytogenes* inoculation level was at ca. 7.61 log_{10} CFU/cm^2, the mean total aerobic bacteria on pork skin samples treated by S1, S2, and S3 were ca. 1.91, 2.47, and 2.56, log_{10} CFU/cm^2, respectively, after 180 s of treatment (Table 3.1), whereas on samples inoculated with ca. 5.75 log_{10} CFU/cm^2 of *L. monocytogenes*, the mean total aerobic populations after the same length of treatment by the three steam cleaners were ca. 2.16, 2.80, and 2.45 log_{10} CFU/cm^2, respectively (Table 3.3). None of the three systems however, was able to reduce the total aerobic bacteria to the undetectable level (< 20 CFU/cm^2) at any treatment time (Table 3.1

and Table 3.3). At both levels of *L. monocytogenes* inoculation, no significant differences ($P > 0.05$) were observed between the overall mean populations of total aerobic bacteria on pork skin samples treated by S2 and S3 (Table 3.2 and Table 3.4). On samples inoculated with ca. 7.61 $\log_{10}$ CFU/cm^2 of *L. monocytogenes*, the overall mean of total aerobic bacteria on pork skin samples treated by S1 was 4.02 $\log_{10}$ CFU/cm^2, which was significantly lower than the overall mean total aerobic counts on the samples treated by S2 (4.60 $\log_{10}$ CFU/cm^2) and S3 (4.75 $\log_{10}$ CFU/cm^2) (Table 3.2). A similar reduction in total aerobic counts was observed on samples with an initial *L. monocytogenes* population of ca. 5.75 $\log_{10}$ CFU/cm^2 (Table 3.4), and among these samples 3 treated by S1 for 120 s tested negative for total aerobes by direct plating. Only 1 out of these 3 samples was however, positive for total aerobes after enrichment (Table 3.5).

Table 3.1 shows the mean populations of *L. monocytogenes* on pork skin samples inoculated with ca. 7.61 $\log_{10}$ CFU/cm^2 of *L. monocytogenes* and treated by three steam systems for six different lengths of time. After 180 s of steam treatment, the population of *L. monocytogenes* on pork skin samples treated by S2, and S3 were ca. 0.70, and 1.82 $\log_{10}$ CFU/cm^2, respectively, whereas on samples treated by S1 for 150 s, the population of *L. monocytogenes* became undetectable by direct plating (Table 3.1). One sample each treated for 30 and 60 s, 2 samples treated for 90 s, 3 samples treated for 120 s, and 4 samples each treated for 150 and 180 s by S1 tested negative for *L. monocytogenes* by direct plating. These negative samples were examined further using the enrichment procedure for *L. monocytogenes* (Table 3.5). Viable *L. monocytogenes* cells were recovered from 1 sample each treated for 30, 60, and 180 s, 2 samples each treated for 90 and 150 s, and 3 samples treated for 120 s. No significant difference ($P \leq 0.05$) was observed in the overall mean populations of *L. monocytogenes* on pork skin samples that were inoculated with ca. 7.61 $\log_{10}$ CFU/cm^2 of *L. monocytogenes* and treated

by S2 and S3 (Table 3.2). Among the samples treated by S2, 2 samples each treated for 150 and 180 s tested negative for *L. monocytogenes* by direct plating, however, only 1 sample treated for 150 s remained negative after enrichment (Table 3.5). One sample treated for 180 s by S3 also tested negative for *L. monocytogenes* before enrichment (Table 3.5). Viable *L. monocytogenes* cells were however recovered from this sample with the aid of the enrichment procedures.

When treated by S2 and S3 for 180 s, the mean counts of surviving *L. monocytogenes* on pork skin samples with an initial *L. monocytogenes* level of ca. 5.75 $\log_{10}$ CFU/cm^2 were 1.71 and 2.52 Log CFU/cm^2, respectively (Table 3.3). The *L. monocytogenes* counts were approximately 1.55 $\log_{10}$ CFU/cm^2 on pork skin samples treated for 90 s by S1. Any treatment longer than 90 s with the same equipment reduced the *L. monocytogenes* populations to the undetectable level (Table 3.3). The enrichment results however, showed that 1 sample treated for 90 s, 4 samples treated for 120 s, 3 samples treated for 150 s, and 1 sample treated for 180 s had viable *L. monocytogenes* cells (Table 3.5). At this level of inoculation, the performance of S1 was significantly better than S2 and S3 ($P > 0.05$), and the performance of S2 was significantly better than S3 ($P > 0.05$) (Table 4) regarding the inactivation of *L. monocytogenes*.

On pork skin samples inoculated with ca. 7.61 $\log_{10}$ CFU/cm^2 of *L. monocytogenes,* the populations of thermoduric bacteria on some of the skin samples were reduced to the undetectable level after a 60 s or longer treatment by S1 and S2 (Table 3.1), whereas, the treatment by S3 did not reduce the levels of thermoduric bacteria to the undetectable level until treatment time reached 180 s (Table 3.1). One sample treated for 60 s, and 4 samples each treated for 90 s or longer by S2 were found to be negative for thermoduric bacteria by direct plating. Viable thermoduric bacterial cells were however, detected after enrichment from 1 sample each treated for 60 and 180 s, 4 samples treated for 90 s, 3 samples treated for 120 s, and

2 samples treated for 150 s (Table 3.5). Among samples treated by S1, 1 sample treated for 60 s, 2 samples treated for 90 s, 3 samples treated for 150 s, and 4 samples each treated for 120 and 180 s tested negative for thermoduric bacteria by direct plating. Following enrichment, 1 sample each treated for 60, 90, and 120 s became positive for thermoduric bacteria whereas, the samples treated for 150 and 180 s remained negative after enrichment (Table 3.5). Two samples tested negative for thermoduric bacteria on pork skin samples treated by S3 for 180 s before enrichment, however, both were found to have viable thermoduric bacterial cells with the aid of the enrichment procedures (Table 3.5). At this inoculation level, the performance of S3 with regard to inactivation of thermoduric bacteria was significantly lower ($P > 0.05$) than S1 and S2 but, no significant difference was observed between the performance of S1 and S2 (Table 3.2).

From the initial mean population of ca. 4.05 $\log_{10}$ CFU/cm^2, thermoduric bacteria on pork skin samples inoculated with *L. monocytogenes* at ca. 5.75 $\log_{10}$ CFU/cm^2 were reduced to the undetectable level when treated by S1 for 60 s or longer (Table 3.3). With the aid of enrichment, 4 samples treated for 60 s, 2 samples each treated for 90 and 120 s, and 3 samples treated for 150 s tested positive for thermoduric bacteria (Table 3.5). All 4 samples treated for 180 s by S1 however, remained negative for thermoduric bacteria after the enrichment procedures (Table 3.5). On 3 samples treated for 60 s, 2 samples treated for 120 s, and 4 samples each treated for 90, 150, and 180 s with S2, the thermoduric bacteria were not detectable by direct plating (Table 3.5). The enrichment results showed that 3 samples each treated for 60 and 150 s, 4 samples treated for 90 s, and 2 samples each treated for 120 and 180 s were positive for thermoduric bacteria (Table 3.5). Four samples each treated for 90, 120, and 150 s, and 2 samples treated for 180 s by S3 were negative for thermoduric bacteria (Table 3.5). With the aid of the enrichment procedures, 4 samples each treated for 90 and 120 s, 3 samples treated for 150 s, and 2 samples

treated for 180 s were found to contain thermoduric bacterial cells (Table 3.5). The performance of S1 was significantly better than ($P > 0.05$) S2 and S3 (Table 3.4), while no difference was observed between the performance of S2 and S3 (Table 3.4) with regard to the inactivation of thermoduric bacteria.

Figure 3.1 illustrates the temperatures of pork skin surfaces while receiving treatments from S1, S2, or S3 for 0, 30, 60, 90, 120, 150, and 180 s. The highest temperatures of pork skin surfaces were 87, 71, and 66^{o}C, when treated for 180 s by S1, S2, and S3, respectively (Fig. 3.1). The increases in temperature of pork skin surfaces were 45, 33, and 27^{o}C when the samples were treated for 30 s with S1, S2, and S3, respectively (Fig. 3.1). It was noticed that after 90 s of steam exposure, the subsequent increase in temperature of the pork skin surfaces is much smaller compared to the pork skin temperature increase during the initial 60 s of treatment (Fig. 3.1). The effectiveness of the three steam or steam/vacuum systems in inactivating *L. monocytogenes* as well as total aerobic and thermoduric bacteria may be attributed, in part to the ability of the individual system to impart the maximum increase in the temperature of the surface of pork skin.

4. Discussion

Beef muscle tissue was initially used as a test specimen in the preliminary stage of the project, and a high degree of inconsistency was encountered in the populations of the surviving pathogens and spoilage-causing bacteria after the steam treatment (Data not shown). This inconsistency could be attributed to the differences in the uniformity of the surfaces and fibrous structure of the beef tissues. In order to minimize the variability in the results caused by meat matrix, pork skin was selected as a model system in the later stage of the study. The use of pork skin was rationalized by the fact that the commercial hog slaughtering practices does not require

the removal of skin from hog carcasses. Thus, pork skin being an intact part of commercially slaughtered hog carcasses has the potential to harbor pathogens like *L. monocytogenes*.

None of the three systems evaluated in the present study were able to reduce the total aerobic bacteria to the undetectable level (< 20 CFU/cm^2) at any treatment time (Table 3.1 and Table 3.3). This observation is similar to those observed by Castillo, Lucia, Goodson, Savell, & Acuff (1999) and Dorsa et al. (1996), who demonstrated that 3 passes of steam-vacuum for 6 s reduced the total aerobes by 2.7 Log CFU/cm^2 from an initial level of approximately 6 Log CFU/cm^2 (1999). Neither study however, was able to reduce the total aerobes below the respective detection levels (Castillo et al., 1999; Dorsa et al., 1996). This could be attributed to the heat resistant mesophilic aerobic spore-formers naturally present in the systems of food animals as well as the processing environment.

It was noticed in this study that a 150 to 180 s steam treatment by S1 was required for a ca. > 6 log reduction (Table 3.1) whereas, a 120 s steam treatment was required for a ca. > 4 log reduction in the population of *L. monocytogenes* (Table 3.3) on artificially inoculated pork skins. This observation suggests that the intensity of the steam treatment needed to improve the microbial quality of meats and animal carcasses could very well be affected by the initial loads of bacterial populations on the surface of meats and animal carcasses. Thus, it is essential to have control over the populations of the pathogen in food animals in order to ensure the safety of meat and meat products.

It was also noticed in the present study that the numbers of *L. monocytogenes* positive enrichment results were not always correlated with the lengths of the steam treatment (Table 3.5). For instance, among the samples inoculated with ca. 7.61 $\log_{10}$ CFU/cm^2 of *L. monocytogenes* and treated by S2, 2 samples each treated for 150 and 180 s tested negative for *L.*

monocytogenes by direct plating (Table 3.5). After enrichment, 1 sample that received the shorter treatment (150 s) and 2 samples which received the longer treatment (180 s) tested positive for *L. monocytogenes* (Table 3.5). A similar phenomenon was observed with the populations of thermoduric bacteria on the pork skin samples inoculated with a ca. 5.75 $\log_{10}$ CFU/cm^2 of *L. monocytogenes* and treated for 120 and 150 s, respectively, by S1 (Table 3.5). Out of 4 samples tested at each interval, 2 treated for 120 s and 3 treated for 150 s were positive for thermoduric bacteria after enrichment (Table 3.5). Similar results has been reported by Muriana et al. in the inactivation of *L. monocytogenes* on ready-to-eat deli meat subjected to post-package pasteurization by submersion heating (Muriana, Quimby, Davidson, & Grooms, 2002). It was observed that when the deli meat was inoculated with 10^7 CFU/ml of *L. monocytogenes*, a relatively lower level of reduction was obtained at 205^{o}F (96.1^{o}C) compared with the levels of reduction obtained at 195^{o}F (90.6^{o}C) or 200^{o}F (93.3^{o}C) (Muriana et al., 2002). The authors attributed such inconsistencies to the imperfections of the product surfaces such as cuts, folds, deep grooves, wrinkles, or tears present on the surface of the deli meat (Muriana et al., 2002).

The effectiveness of the three steam/steam-vacuum cleaning systems evaluated in the study was determined by their abilities to reduce the populations of *L. monocytogenes*, total aerobes, and thermoduric bacteria on the surface of pork skins. The results indicated that system S1 was more effective than S2 and S3 in reducing the populations of *L monocytogenes* and total aerobes at both inoculation levels (Table 3.2, Table 3.4). System S2 was more effective than S3 in reducing the populations of *L. monocytogenes* when pork skins were inoculated with 5 Log_{10} CFU/cm^2 of *L. monocytogenes*, and the populations of thermoduric bacteria when pork skins were inoculated with 7 Log_{10} CFU/cm^2 of *L. monocytogenes* (Table 3.2, Table 3.4). The degree

of microbial reduction achieved in this study can be attributed to the quality of the steam generated by a given steam system. Such quality is generally governed by the technical design of that system, for example, the operating pressures, steam capacity, length of the steam hose, and water content of the steam (dryness fraction) being produced. Although these parameters were not studied in depth in the study, it was evident that S1 produced the steam that could attain the maximum temperature on the surface of the pork skins (Fig. 3.1). It was worthy to note, that treatment with high temperature steam for a relatively long time could adversely affect the sensory quality of meat to some extent. In the present study, slight discoloration and deformation of the pork skins were noticed when they were treated by S1 for longer than 150 s.

Several research groups have used steam and/or steam-vacuum as a decontamination treatment to reduce pathogenic microorganisms from meat and meat products, however, most of these studies employed large scale commercial systems. Phebus et al. used Vac-San®, a commercially available steam-vacuum system, to decontaminate prerigor beef tissue inoculated with *L. monocytogenes* Scott A, *E. coli* O157:H7, and *S.* Typhinurium (Phebus et al., 1997). The study demonstrated a mean reduction of 3.33, 3.11, and 3.37 Log CFU/cm^2 in the populations of *L. monocytogenes* Scott A, *E. coli* O157:H7, and *S.* Typhinurium, respectively (Phebus et al., 1997). Kozempel et al. used, in two separate studies, a vacuum-steam-vacuum surface pasteurizer to inactivate *L. innocua* inoculated on the surfaces of hot dogs and ham skin (Kozempel, Goldberg, Radewonuk, Scullen, & Craig, 2000; Sommers et al., 2002). A single pass of vacuum-steam-vacuum cycle reduced the populations of *L. innocua* by >3 Log CFU/ml of hot dog rinse, and two passes of vacuum-steam-vacuum cycles resulted in a 2.35 Log CFU/cm^2 reduction in the population of *L. innocua* on the surface of ham skin (Kozempel et al., 2000; Sommers et al., 2002). Retzlaff et al. developed and tested a laboratory scale steam

pasteurization unit for inactivating *L. innocua*, *E. coli* O157:H7, and *S.* Typhinurium and achieved a mean reduction of 4.6, 4.7, and 4.8 Log CFU/cm^2, respectively, when the inoculated prerigor beef tissues were exposed to a 15 s steam treatment at 98.9^{o}C (Retzlaff et al., 2004).

Comparison of the results of microbial inactivation obtained from the aforementioned, with those of the present study is somewhat difficult because most, if not all, of the steam and/or steam-vacuum systems used by the other research groups are designed for continuous operation with more than one stage involved in a single treatment, e.g. the SPS Frigoscandia system involves three steps: water removal, steam application, and cold water spray. All of the carcasses are exposed to a high temperature (90^{o}C) - high pressure (90 kPa) steam for a short period of time (6.5 to 15 s) in an enclosed pressurized chamber (Phebus et al., 1997). The laboratory-scale steam pasteurizer used by Phebus et al. is similar to the SPS Frigoscandia system, except that the cooling of carcasses are done manually once the carcasses leave the steam chamber (Retzlaff et al., 2004). Clearly, these systems are designed for medium or high scale (400 heads per day) meat processing plants.

The S1 and S3 systems used in the present study use a low pressure steam which is released onto the pork skins kept at atmospheric pressure. The steam condensing at atmospheric pressure is in a gaseous form, which facilitates rapid heat transfer (Kozempel et al., 2003). However, air at the surface of pork skin may act as an insulating medium (Kozempel et al., 2003), and as a result, a longer steam time is required for attaining similar surface temperature to that achieved by a large scale steam pasteurizer. Nutsch et al. have reported that the surface temperature of the beef carcasses reached 82.2^{o}C within 1.5 to 6.5 s during treatment by the SPS Frigoscandia steam pasteurizer (Nutsch et al., 1997). In the present study, the pork skin temperature reached 83^{o}C after 90 s of steam treatment by S1 (Fig. 3.1). However, when pork

skins were treated by S3, the maximum attainable temperature was 67^{o}C after 180 s of steam treatment (Fig. 3.1). Although cleaner S2 (1,000 W-steam capacity and 800 W-vacuum capacity, producing 248^{o}F steam at 58 psi/4 bars) is a steam-vacuum system, its design and technical parameters are different from those of the Vac-San® system which delivers a continuous stream of 7 to 10 psi at 88 to 94^{o}C while simultaneously vacuuming the area (Castillo et al., 1999).

Use of the large commercial steam pasteurization systems in small and very small scale meat processing plants is not viable. This is because establishment of such systems require installation of other utilities such as steam generators, chilled water, and a regeneration plant, which increases the capitol cost and maintenance overheads and is therefore, not economical for small meat processing establishments. Secondly, small meat processors may not need continuous operation systems since the throughput of such plants ranges from approximately 30 to 200 heads per week (USDA-FSIS, 1998). The steam cleaning systems used in the present study could be an effective solution to the challenges faced by small and very small meat processing facilities as they can offer an ease of operation, reusability, effectiveness against pathogens, and affordability. These cleaning systems are inexpensive ($150-$1,500) and portable, and do not require any additional external utilities like steam generators or chilled water. The results of the present study demonstrate the effectiveness of such systems in inactivating pathogens like *L. monocytogenes* and spoilage-causing bacteria.

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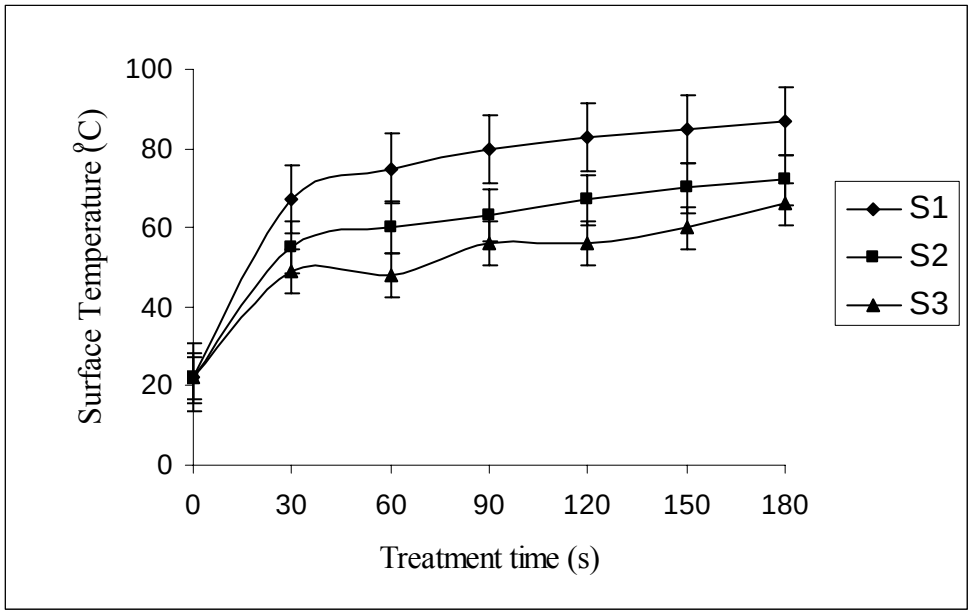

Figure 3.1. Temperature (^{o}C) of pork skin surfaces as a function of treatment time (s) when treated for 0, 30, 60, 90, 120, 150, or 180 s by steam or steam/vacuum cleaning system S1(♦), S2(■), or S3(▲).

Table 3.1. Mean populations (Log_{10} CFU/cm^2) of *L. monocytogenes*, total aerobes, and thermoduric bacteria recovered from pork skin samples, inoculated with ca. 7.61 Log_{10} CFU/cm^2 of *L. monocytogenes*, after treatment by steam or steam/vacuum for 0, 30, 60, 90, 120, 150, or 180 s using the household cleaning systems S1, S2, and S3.

	S1			S2			S3		
Time (s)	*Listeria monocytogenes*	Total aerobes	Thermoduric bacteria	*Listeria monocytogenes*	Total aerobes	Thermoduric bacteria	*Listeria monocytogenes*	Total aerobes	Thermoduric bacteria
0	7.61a*	7.59a	6.12a	7.61a	7.59a	6.12a	7.61a	7.59a	6.12a
30	3.02b	5.20b	2.47b	5.38b	5.68b	4.17b	4.95b	5.77b	4.02b
60	2.36b	3.56c	1.15d	3.76c	4.92bc	2.59c	4.01bc	5.23bc	3.75b
90	1.61bc	3.60c	1.88c	2.88d	4.47c	0.0d	3.75bcd	4.64cd	3.69b
120	0.35c	3.35c	0.00e	2.63d	4.02cd	0.0d	3.11bcd	3.83de	1.55c
150	0.0c§	2.92cd	0.35e	1.46e	3.08de	0.0d	2.58cd	3.67e	2.03c
180	0.0c	1.91d	0.00e	0.70f	2.47e	0.0d	1.82d	2.56f	1.15c

*Means within a column followed by same letters are not significantly different.
§The detection limit of the assay was < 20 CFU/ml

Table 3.2. Overall mean populations (Log_{10} CFU/cm^2) of *L. monocytogenes*, total aerobes, and thermoduric bacteria recovered from pork skin samples, inoculated with ca. 7.61 Log_{10} CFU/cm^2 of *L. monocytogenes*, after treatment with steam or steam/vacuum for 30, 60, 90, 120, 150, or 180 s using the three household cleaning systems S1, S2, and S3.

Cleaning System	*L. monocytogenes*	Total aerobes	Thermoduric bacteria
S1	2.13a*	4.02a	1.71a
S2	2.49b	4.60b	1.84a
S3	3.99b	4.75b	3.18b

* Means within a column followed by the same letters are not significantly different.

Table 3.3. Mean populations (Log_{10} CFU/cm^2) of *L. monocytogenes*, total aerobes, and thermoduric bacteria recovered from pork skin samples, inoculated with ca. 5.75 Log_{10} CFU/cm^2 of *L. monocytogenes*, after treatment by steam or steam/vacuum for 0, 30, 60, 90, 120, 150, or 180 s using the household cleaning system S1, S2, and S3.

	S1			S2			S3		
Time (s)	*Listeria. monocytogenes*	Total aerobes	Thermoduric bacteria	*Listeria monocytogenes*	Total aerobes	Thermoduric bacteria	*Listeria monocytogenes*	Total aerobes	Thermoduric bacteria
0	5.75a*	7.13a	4.05a	5.75a	7.31a	4.05a	5.75a	7.13a	4.05a
30	2.54b	5.82b	1.20b	3.86b	5.71b	1.52c	4.62b	6.09b	1.85b
60	1.85c	3.95c	0.00c	3.49bc	5.37b	0.00d	3.44dc	5.21bc	1.54b
90	1.55c	3.58cd	0.00c	3.11bcd	4.88c	0.00d	3.71c	4.86cd	0.00c
120	0.00d§	3.56cd	0.00c	2.78cd	4.27d	2.83b	2.88ef	3.95de	0.00c
150	0.00d	2.92de	0.00c	2.50d	3.10e	0.00d	3.25de	3.63e	0.00c
180	0.00d	2.16e	0.00c	1.71e	2.80e	0.00d	2.52f	2.45f	1.48b

*Means within a column followed by same letters are not significantly different.
§The detection limit of the assay was < 20 CFU/ml

Table 3.4. Overall mean populations (Log_{10} CFU/cm^2) of *L. monocytogenes*, total aerobes, and thermoduric bacteria recovered from pork skin samples, inoculated with ca. 5.75 Log_{10} CFU/cm^2 of *L. monocytogenes*, after treatment with steam or steam/vacuum for 30, 60, 90, 120, 150, or 180 s using the three household cleaning systems S1, S2, and S3.

Cleaning system	*L. monocytogenes*	Total aerobes	Thermoduric bacteria
S1	1.67a*	4.16a	0.75a
S2	3.31b	4.75b	1.20b
S3	3.74c	4.76b	1.27b

*Means within a column followed by the same letter are not significantly different.

Table 3.5. The numbers of samples enriched and the numbers of samples positive for *L. monocytogenes*, total aerobes, and thermoduric bacteria after enrichment.

Inoculation level of *L. monocytogenes*	10^7 CFU/cm^2						10^5 CFU/cm^2					
Steam time (s)	30	60	90	120	150	180	30	60	90	120	150	180
L. monocytogenes												
S1	1[a](1[b])	1(1)	2(2)	3(3)	4(2)	4(1)	-	-	1(1)	4(4)	4(3)	4(1)
S2	-[P cP]	-	-	-	2(1)	2(2)	-	-	1(1)	-	-	-
S3	-	-	-	-	-	1(1)	-	-	-	-	-	-
Total aerobes												
S1	-	-	-	-	-	-	-	-	-	3(1)	-	-
S2	-	-	-	-	-	-	-	-	-	-	-	-
S3	-	-	-	-	-	-	-	-	-	-	-	-
Thermoduric bacteria												
S1	-	1(1)	2(1)	4(1)	3(0)	4(0)	-	4(4)	4(2)	4(2)	4(3)	4(0)
S2	-	1(1)	4(4)	4(3)	4(2)	4(1)	-	3(3)	4(4)	2(2)	4(3)	4(2)
S3	-	-	-	-	-	2(2)	-	-	4(4)	4(4)	4(3)	2(2)

[a:] The numbers of samples enriched;
[b:] The numbers of samples positive for the respective microorganism after enrichment;
[c:] All four samples were positive by direct plating, and none of the samples was subjected to enrichment

CHAPTER 4

Effectiveness of Commercial Household Steam Cleaning Systems in Reducing the Populations of *Escherichia coli* O157:H7 and Spoilage Bacteria on Pork Skin Surfaces[1]

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ABSTRACT

The small and very small meat processing facilities in the United States are in need of a pathogen reduction technology which would be effective, operational, and economical to implement. This study investigated the efficacy of three household steam and vacuum/steam cleaners in reducing the populations of pathogenic and spoilage bacteria on the surfaces of pork skin inoculated with a five strain mixture of *E. coli* O157:H7 at approx. 7.39 or 5.01 $\log_{10}$ CFU/cm^2. The contaminated pork skin was treated with steam generated by three commercial household cleaning systems, S1, S2, and S3, for 30, 60, 90, 120, 150, and 180 s, respectively. A sampling area of 5 X 2 cm was excised and transferred into a sampling bag with 10 ml of Maximum Recovery Diluent, followed by stomaching and microbiological analysis in order to enumerate the populations of *E. coli* O157:H7, total aerobes, and thermoduric bacteria. The application of steam for 180 s by S1, S2, or S3 resulted in the *E. coli* O157:H7 counts up to < 1.30, 2.74, and 1.69 $\log_{10}$ CFU/cm^2 ($P < 0.05$), respectively at the ca. 7.39 $\log_{10}$ CFU/cm^2 inoculation level. On pork skin samples inoculated with the ca. 5.01 $\log_{10}$ CFU/cm^2 of *E. coli* O157:H7, steam treatment for 180 s by the three cleaning systems, S1, S2, or S3 resulted in surviving *E. coli* O157:H7 population of < 1.30, < 1.30, and 0.34 $\log_{10}$ CFU/cm^2 ($P < 0.05$), respectively. Significant differences were observed between different inoculation levels, treatment times, and types of steam and steam/vacuum systems used ($P<0.05$). A similar trend was observed in the reduction of populations of total aerobes and thermoduric bacteria at both inoculation levels.

Keywords: *Escherichia coli* O157:H7, total aerobes, thermoduric bacteria, steam pasteurization, household steam cleaning systems, pork skin, and small and very small meat processors.

INTRODUCTION

It is estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year in the United States. Among these only 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths are caused by known etiological agents (Mead *et al*. 2000). One such etiological agent is *Escherichia coli* O157:H7, a bacterial pathogen that was first identified in 1982 when implicated in outbreaks associated with undercooked ground beef patties (Riley *et al*. 1983). Studies have shown that cattle are the natural reservoir of *E. coli* O157:H7 and healthy cattle carriers may shed the pathogen through their feces (Chapman *et al*. 1993; Hancock *et al*. 1994; Bell, R.G. 1997; Hancock *et al*. 1997; Garber *et al*. 1999). Fecal materials present on the hide of such animals may be a potential source of carcass contamination during hide removal, evisceration as well as other steps of the slaughter (Bell, R.G. 1997; Hancock *et al*. 1997).

In order to minimize the level of microbial contamination on raw meats and animal carcasses, the Food Safety and Inspection Service of the United States Department of Agriculture (FSIS-USDA) established new regulations in 1996, mandating meat processing facilities of all sizes in the United States to establish Sanitation Standard Operating Procedures (SSOPs) as well as Hazard Analysis Critical Control Point (HACCP) systems, and to monitor microbiological performance criteria for *E. coli* and *Salmonella* (USDA-FSIS 1996; Sofos and Smith 1998). Since then several carcass decontamination technologies have been identified (Reagan *et al*. 1996; USDA-FSIS 1996). Among these technologies, steam pasteurization has been proven to be one of the most effective methods for inactivating bacterial pathogens on animal carcasses (Castillo *et al*. 1998). Phebus *et al*. (1997) compared the effectiveness of steam pasteurization with other carcass decontamination methods under laboratory conditions and found that a 15 s

steam treatment, using a commercial steam pasteurizer, caused a mean reduction of 3.53 $\log_{10}$ CFU/cm^2 in the populations of *E. coli* O157:H7 on cutaneus truncii muscles processed from freshly slaughtered steers. Although effective in eliminating spoilage and pathogenic bacterial populations on the surfaces of animal carcasses, steam pasteurization has not been widely used by small and very small meat processing operations because steam generators are generally expensive and consume large amounts of energy (Sofos and Smith 1998). Thus, a special need for identifying a suitable carcass decontamination technology for small and very small meat processing operations that offers an ease of operation and installation, reusability, effectiveness against pathogens, and economic affordability, has been recognized by regulating authorities.

The objective of the present study is to compare the efficacy of the three commercial household steam cleaners in reducing the populations of *E. coli* O157:H7, total aerobes and thermoduric bacteria on artificially contaminated pork skin surfaces.

MATERIALS AND METHODS

Bacterial cultures and growth media

Five *E. coli* O157:H7 cultures from our laboratory collection were retrieved from frozen storage and grown at 37°C for 24 h on Sorbitol MacConkey's agar supplemented with Cefixime – Tellurite (CT-SMAC). The medium was prepared by adding the Cefixime-Tellurite supplement (Dynal biotech, Oslo, Norway) to Sorbitol MacConkey's agar (Difco Lab., Sparks, MD, USA) as per the supplier's instructions with a final concentration of Cefixime 0.05 mg/l and of Tellurite 2.5 mg/l. A single colony of each culture was transferred into 9 ml tryptic soy broth (TSB) and the inoculated broth was incubated for 18 h at 37°C. The resulting liquid cultures were used to prepare the *E. coli* O157:H7 inocula needed for artificial inoculation of the pork skin samples.

Preparation of *E. coli* O157:H7 inocula

On the day of trial, fresh fecal materials were collected from a private farm located near the Georgia Agriculture Experiment Station in Griffin, GA. Nine ml of each *E. coli* O157:H7 culture were transferred to a 50 ml centrifuge tube and mixed thoroughly using a Minivortexer (VWR Scientific products). The *E. coli* O157:H7 mixture was centrifuged at 4,000 g for 15 min. The pellet of the cell mixture was washed twice and then re-suspended into an appropriate volume of Maximum Recovery Diluent (MRD). Accurately weighed fecal materials were inoculated with an appropriate volume of the *E. coli* O157:H7 cell suspension to obtain an inoculation level of ca. 9 $\log_{10}$ CFU/g. For the preparation of the fecal materials containing ca. 7 $\log_{10}$ CFU/g of *E. coli* O157:H7, similar procedure was employed except that the aforementioned *E. coli* O157:H7 suspension was diluted 100 fold before being mixed with the same amount of fecal materials.

Pork skin

Pork skin was purchased one day prior to each trial from the International Farmers' Market in Lake City, GA. The skin was rinsed with tap water for 1 min and was allowed to dry at room temperature. The flesh and muscles on the other side of the skin were trimmed using a knife in a way that only the upper most layer of the skin remained at the end. The pork skin was cut into equal blocks of 7 X 3.5 cm using a pair of scissors with each block of the skin being placed into a Petri-plate. Using a sterile spatula and sterile card board template, an accurately weighed 0.5 g of the inoculated fecal materials mentioned above was evenly spread on a randomly selected 5 X 2 cm area of the pork skin blocks. The contaminated pork skin blocks were allowed to stand at room temperature for 15 min before steam treatment. The *E. coli*

O157:H7 inoculation level on the pork skin surface was approximately 7.39 or 5.01 $\log_{10}$ CFU/cm^2.

Steam treatment

Three commercial household steam or steam/vacuum cleaning systems, S1, S2, and S3, were purchased from two different suppliers. The systems differed in their technical parameters. S1 was a household steam cleaner with a high steam capacity power of 1,500 W, S2 used a combination of steam (1,000 W) and vacuum (800 W), producing a high temperature steam (248^{o}F) at 58 psi (4 bars), whereas, S3 was a vapor cleaning system with the highest capacity of 1,600 W producing low moisture steam at high pressure (85-95 psi). Two separate trials were conducted to study the reduction of *E. coli* O157:H7 at each inoculation level, and each trial was duplicated. The inoculated pork skins were divided, in each experimental trial, into three groups with 12 inoculated pork skin samples in each group. The samples were treated with S1, S2, or S3 for 30, 60, 90, 120, 150, or 180 s with two samples being treated at each treatment interval. The pork skin samples were transferred to a bucket of ice for immediate chilling after the steam treatment. During each trial two inoculated pork skin samples were run as positive controls which were inoculated with *E. coli* O157:H7 but were not subjected to any steam treatment. Skin samples inoculated with natural fecal materials were included in the study as negative controls.

Microbiological analysis

After the completion of steam treatment, the treated pork skins were sampled by aseptically excising the 5 X 2 cm inoculated area with a pair of sterile scissors. Each pork skin sample was aseptically transferred to a sterile sampling bag (4.5" X 9", Fisher Scientific Inc., Pittsburgh, PA, USA) containing 10 ml of sterile MRD solution. The contents of the bag were

stomached for 1 min at normal speed using a Stomacher 400 lab blender (Seward Ltd., London, UK). Serial dilutions were prepared using sterilized MRD solution. A 50 µl of appropriate dilution was spirally plated in duplicate on CT-SMAC agar plates for the enumeration of *E. coli* O157:H7, on tryptic soy agar (TSA) plates for the enumeration of total aerobic bacteria, and on MacConkey (MAC) agar plates for the enumeration of thermoduric bacteria, using the Autoplate® 4000 automated spiral plater (Spiral Biotech, Bethesda, MD, USA). The CT-SMAC and TSA plates were incubated at 37°C for 24 h while the MAC plates were incubated at 45°C for 24 h. The colonies on each plate were counted using a **Q***COUNT*™ automatic colony counter (**Q***COUNT*™ software, Spiral Biotech, Bethesda, MD, USA).

Enrichment

A qualitative estimation of heat injured *E. coli* O157:H7 was carried out according to a procedure outlined by USDA-FSIS (Anderson *et al.* 1990). Pre-enrichment was performed by transferring 5 ml of the sample to 45 ml of sterile MRD solution and then incubating for 24 h at 37°C. One ml of the resulting culture was transferred to 9 ml tubes of sterile modified Tryptic Soy Broth (mTSB) for selective enrichment of *E. coli* O157:H7 and incubated for 24 h at 37°C. After incubation, a loop full was streaked onto the CT-SMAC agar plates and incubated at 37°C for 24h. For recovery of heat injured thermoduric bacteria and total aerobic bacteria, a loop full of the pre-enriched culture was inoculated directly onto MAC agar plates followed by incubation at 45°C for 24 h and TSA plates followed by incubation at 37°C for 24 h, respectively.

Data analysis

Data was transformed into Log_{10} CFU/cm^2 before comparison of means. Analysis of data was accomplished using the Fisher's least significant difference of means of bacterial

populations calculated with the General Linear Model (GLM) procedure of SAS, windows version 5.1.2600, SAS institute, Inc., Cary, NC (SAS, 2000) based on a 95% confidence level.

RESULTS

On pork skin samples initially inoculated with ca. 7.39 $\log_{10}$ CFU/cm^2 of *E. coli* O157:H7 and treated for 180 s by S2 and S3 the surviving populations of *E. coli* O157:H7 were ca. 2.74 and 1.69 $\log_{10}$ CFU/cm^2, respectively (Table 4.1). At this inoculation level, the majority of the pork skin samples treated by S2 and S3 were positive for *E. coli* O157:H7 by direct plating, except for 2 samples treated by S3 for 180 s. These two samples were however, found to be positive for *E. coli* O157:H7 after the enrichment (Table 4.5). The populations of *E. coli* O157:H7 fell below the detectable level (< 20 CFU/cm^2) on pork skin samples treated by S1 for 60 s or longer (Table 4.1). Following the enrichment viable *E. coli* O157:H7 cells were recovered from 1 sample each treated for 120 and 150 s, 3 samples treated for 90 and 180 s, and 4 samples treated for 60 s by S1 (Table 4.5).

When the *E. coli* O157:H7 inoculation level was ca. 5.01 $\log_{10}$ CFU/cm^2, the populations of *E. coli* O157:H7 on pork skin samples were reduced to the undetectable level when treated with S1 for 30 s, S2 for 90 s, and S3 for 180 s (Table 4.3). The enrichment results however, showed that 1 sample each treated for 30, 60, 90, and 180 s, 2 samples treated for 120 s, and 3 samples treated for 150 s by S1 as well as 1 sample each treated for 90 and 120 s, 4 samples treated for 150 s, and 2 samples treated for 180 s by S2 had viable *E. coli* O157:H7 cells (Table 4.5). When treated by S3 for 180 s the population of *E. coli* O157:H7 on pork skin samples changed from 5.01 to 0.34 $\log_{10}$ CFU/cm^2 (Table 4.3).

A vast majority of pork skin samples treated by the three cleaning systems were positive for total aerobes by direct plating at the end of 180 s treatment (Table 4.1 and Table 4.3). The

resulting mean populations of total aerobic bacteria on pork skin samples inoculated with ca. 7.39 $\log_{10}$ CFU/cm^2 of *E. coli* O157:H7 were 1.97, 3.23, and 2.87 $\log_{10}$ CFU/cm^2, when treated for 180 s by S1, S2 or S3, respectively (Table 4.1). From an initial inoculation level of ca. 5.01 $\log_{10}$ CFU/cm^2 of *E. coli* O157:H7, the populations of total aerobic bacteria were changed to 2.12, 2.24, and 2.57 $\log_{10}$ CFU/cm^2 following the 180 s steam treatment by S1, S2, and S3, respectively (Table 4.3). At both levels of *E. coli* O157:H7 inoculation, no significant differences ($P > 0.05$) were observed between the overall mean populations of total aerobic bacteria on pork skin samples treated by S2 and S3 (Table 4.2 and Table 4.4). The performance S1 was significantly better than ($P \leq 0.05$) S2 and S3 when pork skins were inoculated with ca. 7.39 $\log_{10}$ CFU/cm^2 (Table 4.2). However, no significant differences were observed between overall mean populations of total aerobes on pork skins treated by S1 and S2 when pork skins were inoculated with ca. 5.01 $\log_{10}$ CFU/cm^2 (Table 4.4).

On pork skin samples inoculated with ca. 7.39 $\log_{10}$ CFU/cm^2 of *E. coli* O157:H7, the populations of thermoduric bacteria were reduced to the undetectable level after a 60 sec or longer treatment by S1 and 180 s treatment by S3 (Table 4.1). The treatment with S2 did not reduce the levels of thermoduric bacteria to the undetectable level even when the treatment was extended to 180 s (Table 4.1). Viable thermoduric bacterial cells were however, detected after the enrichment from 1 sample each treated for 120 and 150 s, 3 samples treated for 60 s, and 2 samples treated for 180 s by S1 as well as 2 samples treated by S3 for 180 s (Table 4.5).

When pork skin samples were inoculated with ca. 5.01 $\log_{10}$ CFU/cm^2 of *E. coli* O157:H7, the surviving populations of thermoduric bacteria on pork skin samples treated by S1, S2, and S3 were 0.82, 0.82, and 1.41 $\log_{10}$ CFU/cm^2, respectively, after 180 s of steam treatment (Table 4.3). With the aid of enrichment, 3 samples each treated for 60 and 120 s, 2 samples

treated for 90 s, and 1 sample each treated for 30 and 150 s by S1 tested positive for thermoduric bacteria (Table 4.5). All 4 samples treated for 180 s by S1 however, remained negative for thermoduric bacteria even with the enrichment procedures (Table 4.5). On 3 samples treated for 180 s, 2 samples treated for 150 s, as well as 1 sample each treated for 60 and 120 s by S2 the thermoduric bacteria were not detectable by direct plating (Table 4.5). The enrichment results revealed that 3 samples treated for 180 s, 1 sample each treated for 60 and 120 s, and 2 samples treated for 150 s were positive for thermoduric bacteria (Table 4.5). Two samples each treated for 120 and 180 s by S3 were negative for thermoduric bacteria (Table 4.5), which were all found to contain thermoduric bacterial after enrichment (Table 4.5).

Statistical analysis revealed that all three cleaning systems were effective in reducing the populations of *E. coli* O157:H7, total aerobes, and thermoduric bacteria on pork skin samples artificially inoculated with ca. 7.39 or 5.01 $\log_{10}$ CFU/cm^2 (Table 4.1 and Table 4.3). With regard to the inactivation of *E. coli* O157:H7 and thermoduric bacteria, S1 performed better than ($P > 0.05$) S2 or S3 and S2 performed better than S3 when pork skin samples were inoculated with ca. 7.39 $\log_{10}$ CFU/cm^2 of *E. coli* O157:H7 (Table 4.2). The overall mean populations of *E. coli* O157:H7 inoculated with ca. 5.01 $\log_{10}$ CFU/cm^2 and treated by S1, S2, and S3 were 1.34, 1.69, and 2.44 $\log_{10}$ CFU/cm^2, respectively (Table 4.4). At this level of inoculation, S1 and S2 performed significantly better than S3 ($P > 0.05$) with respect to the inactivation of *E. coli* O157:H7 (Table 4.4). However, no significant difference ($P \leq 0.05$) was observed between the performance of S1 and S2 (Table 4.4). Furthermore, S1 performed significantly better ($P > 0.05$) than S2 and S3 (Table 4.4), while S2 performed better ($P > 0.05$) than S3 (Table 4.4) with regard to the inactivation of thermoduric bacteria.

DISCUSSION

It was noticed in the present study that the reduction of *E. coli* O157:H7 populations increased as the initial bacterial load decreased (Table 4.1 and Table 4.3). This observation suggests that the initial loads of bacterial populations on the surface of meats and animal carcasses are critical in determining the intensity of the steam treatment. Thus, in order to ensure the safety of meat and meat products, it is essential to have control over the populations of the pathogens as well as spoilage bacteria in food animals entering the processing plants.

In the current study, none of the three steam cleaning systems evaluated were able to reduce the total aerobic bacteria to the undetectable level (< 20 CFU/cm^2) at any treatment time (Table 4.1 and Table 4.3). This could be attributed to the presence of heat resistant mesophilic aerobic spore-formers in food animals as well as in the meat processing environment. Similar observation has been reported by Castillo *et al*. (1999), who demonstrated that treatment with steam and vacuum for 6 s reduced the total aerobes by 2.7 Log_{10} CFU/cm^2 from an initial level of ca. 6 Log_{10} CFU/cm^2.

The results of the present study revealed that the reductions in the populations of *E. coli* O157:H7, total aerobes, and thermoduric bacteria were directly correlated to the quality of the steam, which in general was determined by the technical parameters of each system. Steam system S1 was more effective than S2 and S3 in reducing the populations of *E. coli* O157:H7, total aerobes and thermoduric bacteria at ca. 7.39 log_{10} CFU/cm^2 inoculation level (Table 4.2). However, when *E. coli* O157:H7 inoculation level was ca. 5.01 log_{10} CFU/cm^2, no statistical differences were observed between S1 and S2 with regard to inactivation of *E. coli* O157:H7 and total aerobes (Table 4.4).

It was observed in the present study that the number of samples that became positive for *E. coli* O157:H7 after enrichment procedure did not always correlate to the level of inoculation and the length of steam treatment. For instance, when pork skins were inoculated with ca. 7.39 $\log_{10}$ CFU/cm^2 3 samples treated for 90 and 180 s by S1 were positive for *E. coli* O157:H7 whereas, 1 sample each treated for 120 and 150 s by S1 was positive for *E. coli* O157:H7 after enrichment (Table 4.5). This randomness in the number of positive samples could be explained by the presence of folds and wrinkles on the pork skin samples which could have prevented the exposure of bacteria to the steam. Additionally, the numbers of pathogen on pork skin samples became extremely low (< 2 X 10^1 CFU/cm^2) after steam treatment, which could also be the reason for the randomness. Similar results were observed by Muriana *et al.* (2002) while submersion heating 10^7 CFU/ml *L. monocytogenes* inoculated on packaged, ready-to-eat deli meat. A relatively lower level of inactivation was observed at 205^{o}F (96.1^{o}C) compared with the levels of inactivation at 195^{o}F (90.6^{o}C) or 200^{o}F (93.3^{o}C). The researchers attributed these inconsistencies to the imperfections of the product surfaces such as cuts, folds, deep grooves, wrinkles, or tears present on the surface of the deli meat.

Different research groups have reported varying degrees of reductions in *E. coli* populations using steam de-contamination regimes in their studies. Phebus *et al.* (1997) reported the reduction of 3.5 $\log_{10}$ CFU/cm^2 in the populations of *E. coli* O157:H7, when freshly slaughtered beef carcasses were treated with steam at 91 to 93°C for 15 s. Nutsch *et al.* (1997) reported that a steam treatment of 90.5 to 94°C for 6 to 8 s reduced the populations of *E. coli* on beef carcasses to undetectable levels from an initial level of 0.60 - 1.53 $\log_{10}$ CFU/cm^2. In another study, using a commercial steam pasteurization system; SPS-Frigoscandia, Nutsch *et al.* (1998) determined the efficacy of steam pasteurization at five anatomical locations on beef

carcasses and showed that a steam treatment temperature of 82.2°C and 6.5 s exposure time were effective in reducing the *E. coli* populations by 0.5 $\log_{10}$ CFU/cm^2.

It is however, not a practical approach to compare the results of microbial inactivation obtained from the aforementioned studies, with those of the present study, because most if not all the systems used by the other research groups are designed for continuous operation with more than one stage involved in a single treatment. These systems are designed for medium or large scale (400 heads per day) meat processing plants and uses different treatment protocols. The S1 and S3 systems in the present study use a relatively low pressure steam which is released onto the pork skins kept at atmospheric pressure. The steam condensing at atmospheric pressure is in a gaseous form, which facilitates rapid heat transfer (Kozempel *et al.* 2003). However, air at the surface of pork skin acts as an insulating medium (Kozempel *et al.* 2003), and as a result, a longer steam time is required for attaining similar surface temperature to that achieved by a large scale steam pasteurizer.

Use of the large commercial steam pasteurization systems in small and very small scale meat processing plants is not practical due to high cost of installation, maintenance and operation. Secondly, small meat processors may not need continuous operation systems since the throughput of such plants ranges from approximately 30 to 200 heads per week (USDA-FSIS 1998). The steam cleaning systems used in the present study could easily meet the requirements of small and very small meat processors as it can offer affordability, ease of operation, reusability, and effectiveness against pathogens such as *E. coli* O157:H7.

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TABLE 4.1. MEAN REDUCTION IN THE POPULATIONS (LOG_{10} CFU/CM^2) OF *E. COLI* O157:H7, TOTAL AEROBES, AND THERMODURIC BACTERIA RECOVERED FROM PORK SKIN SAMPLES, INOCULATED WITH CA. 7.39 LOG_{10} CFU/CM^2 OF *E. COLI* O157:H7, AFTER TREATMENT BY STEAM OR STEAM/VACUUM FOR 0, 30, 60, 90, 120, 150, OR 180 SEC USING THE HOUSEHOLD CLEANING SYSTEM S1, S2, AND S3.

Cleaning system	S1			S2			S3		
Time (sec)	*E. coli* O157:H7	Total aerobes	Thermoduric bacteria	*E. coli* O157:H7	Total aerobes	Thermoduric bacteria	*E. coli* O157:H7	Total aerobes	Thermoduric bacteria
0	7.39 a*	7.69 a	7.06 a	7.39 a	7.69 a	7.06 a	7.39 a	7.69 a	7.06 a
30	5.16 b	5.39 b	5.22 b	5.35 b	5.89 b	5.57 b	5.11 b	5.93 b	5.75 b
60	0.00 c	3.36 c	0.00 c	3.21 c	5.58 b	4.36 c	4.34 bc	5.37 bc	4.29 bc
90	0.00 c	3.56 c	0.93 c	3.79 c	4.95 bc	4.24 c	2.64 bc	4.84 c	4.41 bc
120	0.00 c	3.04 c	0.00 c	3.10 c	4.32 c	3.45 c	2.78 bc	4.57 cd	3.94 bc
150	0.00 c	3.02 c	0.00c	2.86 c	4.48 c	3.75 c	2.08 c	3.61 de	2.46 cd
180	0.00 c	1.97 d	0.00 c	2.74 c	3.23 d	2.39 c	1.69 c	2.87 e	1.15 d

*Means in the same column with the same letter are not significantly different.

TABLE 4.2. MEAN POPULATIONS (LOG_{10} CFU/CM^2) OF *E. COLI* O157:H7, TOTAL AEROBES, AND THERMODURIC BACTERIA RECOVERED FROM PORK SKIN SAMPLES, INOCULATED WITH 7 LOG_{10} CFU/CM^2 OF *E. COLI* O157:H7, AFTER TREATMENT WITH STEAM OR STEAM/VACUUM FOR 30-180 SEC USING THE THREE HOUSEHOLD CLEANING SYSTEMS S1, S2, AND S3.

Cleaning System	*E. coli* O157:H7	Total aerobes	Thermoduric bacteria
S1	1.79 a*	4.00 a	1.89 a
S2	4.06 b	5.16 b	4.40 b
S3	3.72 c	4.98 b	4.15 c

* Means in the same column with the same letter are not significantly different.

TABLE 4.3. MEAN REDUCTION IN THE POPULATIONS (LOG_{10} CFU/CM^2) OF *E. COLI* O157:H7, TOTAL AEROBES, AND THERMODURIC BACTERIA RECOVERED FROM PORK SKIN SAMPLES, INOCULATED WITH 5 LOG_{10} CFU/CM^2 OF *E. COLI* O157:H7, AFTER TREATMENT BY STEAM OR STEAM/VACUUM FOR 0, 30, 60, 90, 120, 150, OR 180 SEC USING THE HOUSEHOLD CLEANING SYSTEM S1, S2, AND S3.

Cleaning system	S1			S2			S3		
Time (sec)	*E. coli* O157:H7	Total aerobes	Thermoduric bacteria	*E. coli* O157:H7	Total aerobes	Thermoduric bacteria	*E. coli* O157:H7	Total aerobes	Thermoduric bacteria
0	5.01 a	5.40 a	5.61 a	5.01 a	5.40 a	5.61 a	5.01 a	5.40 a	5.61 a
30	2.25 b	3.52 b	2.00 b	2.80 b	3.84 b	2.50 b	3.31 b	3.90 b	3.49 b
60	0.92 b	2.57 b	0.93 bc	1.88 bc	2.93 bc	1.79 bc	2.86 bc	3.67 bc	2.79 bc
90	0.90 b	2.51 b	0.67 c	1.32 bcd	3.03 bc	1.97 bc	2. 18 bc	3.16 bcd	2.36 bc
120	0.34 b	2.35 b	0.73 c	0.82 cd	2.59 c	1.64 bc	1.87 c	3.19 bcd	1.32 c
150	0.00 b	2.15 b	0.78 c	0.00 d	2.41 c	1.28 bc	1.50 cd	2.80 cd	1.63 c
180	0.00 b	2.12 b	0.82 c	0.00 d	2.24 c	0.82 c	0.34 d	2.57 d	1.41 c

*Means within columns (mean bacterial reductions) with same letters are not significantly different.

TABLE 4.4. MEAN POPULATIONS (LOG_{10} CFU/CM^2) OF *E. COLI* O157:H7, TOTAL AEROBES, AND THERMODURIC BACTERIA RECOVERED FROM PORK SKIN SAMPLES, INOCULATED WITH 5 LOG_{10} CFU/CM^2 OF *E. COLI* O157:H7, AFTER TREATMENT WITH STEAM OR STEAM/VACUUM FOR 30-180 SEC USING THE THREE HOUSEHOLD CLEANING SYSTEMS S1, S2, AND S3.

Cleaning system	*E. coli* O157:H7	Total aerobes	Thermoduric bacteria
S1	1.34 a*	2.95 a	1.65 a
S2	1.69 a	3.21 ab	2.23 b
S3	2.44 b	3.53 b	2.66 c

*Means in the same column with the same letter are not significantly different.

TABLE 4.5. THE NUMBERS OF SAMPLES ENRICHED AND THE NUMBERS OF SAMPLES POSITIVE FOR *E. COLI* O157:H7, TOTAL AEROBES, AND THERMODURIC BACTERIA AFTER THE ENRICHMENT.

Inoculation level of *E. coli* O157:H7	10^7 CFU/cm^2						10^5 CFU/cm^2					
Steam time (s)	30	60	90	120	150	180	30	60	90	120	150	180
E. coli O157:H7												
S1	-[P cP]	4[a](4[b])	4(3)	4(1)	4(1)	4(3)	1(1)	1(1)	1(1)	3(2)	4(3)	4(1)
S2	-	-	-	-	-	-	-	-	2(1)	2(1)	4(4)	4(2)
S3	-	-	-	-	-	2(2)	-	-	-	1(1)	-	3(2)
Total aerobes												
S1	-	-	-	-	-	-	-	-	-	-	-	-
S2	-	-	-	-	-	-	-	-	-	-	-	1(1)
S3	-	-	-	-	-	-	-	-	-	-	-	-
Thermoduric bacteria												
S1	-	4(3)	2(0)	4(1)	4(1)	4(2)	1(1)	3(3)	2(2)	3(3)	1(1)	-
S2	-	-	-	-	-	-	-	1(1)	-	1(1)	2(2)	3(3)
S3	-	-	-	-	-	2(2)	-	-	-	2(2)	-	2(2)

[a:] The numbers of samples enriched;
[b:] The numbers of samples positive for the respective microorganisms after the enrichment;
[c:] All four samples were positive by direct plating, and none of the samples was subjected to the enrichment procedure

CHAPTER 5

Use of a Commercial Household Steam Cleaning System to Decontaminate Beef and Hog Carcasses Processed by Four Small or Very Small Meat Processing Plants in Georgia[1]

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ABSTRACT

Small and very small meat processing facilities in the United States are in need of a pathogen reduction technology that would be both effective and economical. The present study evaluated the effectiveness of a commercial household steam cleaner in reducing naturally-occurring bacterial populations on freshly-slaughtered beef and hog carcasses in four small or very small meat processing plants. Three anatomical sites on the right half of each carcass were exposed to a 60 s steam treatment while the corresponding left half of the carcass remained untreated. In total, 72 beef and 72 hog carcasses were sampled before, immediately after, and 24 h after the steam treatment. The mean populations of total aerobes, coliforms and *Enterobacteriaceae* recovered from three anatomical sites of the beef carcasses were 1.88, 1.89, and 1.36 log CFU/cm^2, respectively, before the steam treatment, 1.00, 0.71, and 0.52 log CFU/cm^2, respectively, immediately after the steam treatment, and 1.10, 0.95, and 0.50 log CFU/cm^2, respectively, 24 h after the steam treatment . On hog carcasses, the mean populations of total aerobes, coliform, and *Enterobacteriaceae* recovered from the three anatomical sites were 2.50, 2.41, and 1.88 log CFU/cm^2, respectively before the steam treatment, 0.50, 0.94, and 0.21 log CFU/cm^2, respectively immediately after the steam treatment, and 0.91, 1.56, and 0.44 log CFU/cm^2, respectively 24 h after the steam treatment. The steam treatment significantly reduced ($P < 0.05$) the levels of total aerobes, coliforms, and *Enterobacteriaceae* at all three anatomical locations on both types of carcasses. The order of mean bacterial populations recovered from the beef and hog carcasses before steam treatment was midline > neck > rump, and belly > jowl > ham, respectively, except for the total coliform counts at the midline and neck areas on the beef carcasses. Of the 144 carcasses evaluated, 5 were positive for *Salmonella* before steam treatment (3.47%) but all carcasses tested negative for *Salmonella* after the

treatment. Results indicate that the household steam cleaner can effectively reduce overall bacterial populations on freshly-slaughtered beef and hog carcasses in small and very small meat processing facilities.

Keywords: *Salmonella*, indicator and spoilage microorganisms, steam treatment, household steam cleaning systems, carcasses

The Centers for Disease Control and Prevention (CDC) estimates that there are approximately 76 million cases of foodborne illnesses, resulting in 300,000 hospitalizations and 5,000 deaths each year in the United States *(13)*. This high rate of morbidity and mortality has a substantial impact on public health as well as the national economy *(5)*. According to an estimate by the Economic Research Service of the United States Department of Agriculture (ERS-USDA), the economic losses associated with product recalls, disease treatment, and loss of productivity were approximately $6.9 billion in the year 2000 *(23)*.

The microbial contamination of animal carcasses that occurs during animal slaughtering is an undesirable but unavoidable repercussion of meat processing *(6)*. Although animal carcasses may become contaminated with bacteria from the processing environment, the primary source of such contamination is the animal itself *(6)*. The animal may harbor bacterial pathogens such as *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* which are all known to pose a threat to consumer health *(3, 10, 19)*. The contamination of meat and poultry products with these pathogenic bacteria results in as many as 5,000,000 illnesses and 4,000 deaths each year nationally *(26)*. In order to reduce the health risks and economic losses associated with the presence of bacterial pathogens on animal carcasses, the USDA has imposed the establishment of the Hazard Analysis Critical Control Point (HACCP) system as well as Sanitation Standard Operating Procedures (SSOPs) in meat and poultry processing plants of all sizes in the United States *(24, 25)*. As a result, extensive research has been conducted on developing pathogen reduction technologies in order to improve the microbiological safety of slaughtered animal carcasses *(21, 22)*. Amongst these technologies, steam pasteurization (*18*) and steam vacuuming (*2, 4, 7, 8, 9, 11, 17, 18*) have been recognized as two of the most effective methods for decreasing bacterial populations on animal carcasses. Using Frigoscandia, a commercial steam

pasteurization apparatus, Nutsch et al. demonstrated that steam pasteurization could reduce the mean aerobic plate counts from 2.19 to 0.84 log CFU/cm^2 on beef carcasses *(15)*. Although Frigoscandia is effective in reducing overall bacterial populations on the surfaces of animal carcasses, its high cost of installation and operation would limit its use to medium and large meat processing facilities *(22)*. The establishment of the system in a commercial meat processing plant would require the installation of utilities such as a steam generator, chilled water, and a regeneration plant, which at this time, is not an economical proposition for small meat processing facilities with an average production throughput of 30 to 200 head per week *(25)*. Thus, a carcass decontamination technology suitable for small and very small meat processing operations is in demand. A system which can offer ease of operation and installation, reusability, effectiveness against pathogens, and economic affordability would meet the needs of both small and very small meat processors. The objective of the present study was to determine the effectiveness and feasibility of using a commercial household steam cleaner for reducing natural microflora on the surfaces of beef and hog carcasses commercially slaughtered by small and very small meat processing facilities.

MATERIALS AND METHODS

Slaughter facilities. Four small or very small meat processing plants in Georgia were selected in the present study to examine the efficacy of a commercial household steam cleaner in reducing the levels of bacterial contamination on naturally contaminated beef and hog carcasses. Plant A slaughtered hogs twice a week with an operating capacity of 50 to 60 hogs per day. This plant also slaughtered steers and heifers once a week with a production throughput of 20 to 24 head per day, which was often followed by hog slaughtering on the same day. Plant B slaughtered only steers and heifers once or twice a month with a production throughput of 12 to

20 head per day. Plant C was a very small plant and operated once a week with a production throughput of 24 to 40 hogs per week. It also custom slaughtered steers and heifers, with the slaughter schedule depending upon the season and availability of animals. Plant D operated twice a week and mostly slaughtered steers, heifers, and calves with a production throughput of 75 to 120 animals per day. Slaughtering procedures employed by the four plants followed the standard industry practices and included one or more decontamination intervention technologies. Plant A sprayed the carcasses with chlorinated tap water, while Plant B used 2.0 to 2.6% of lactic acid spray after the final carcass wash. Plant C and Plant D both used acetic acid spray as a decontamination intervention after the final carcass wash. Plant D however used the acetic acid spray at two points of slaughtering, with the first one being applied immediately after evisceration and the second after the final carcass wash.

Steam treatment. A commercial household steam cleaner, Steam Fast SF 275 (Top Innovation Inc., Riverside, MO), with a steam capacity of 1,500 W was used in the present study. The steam cleaner consisted of a water tank (capacity: 48 oz. max.), a steam chamber, and a hosepipe with a nozzle at the end. The steam flow from the nozzle could be controlled manually by a switch on the handle. The steam treatment was applied after the final carcass wash but before spraying any organic acid solutions on the carcasses. The steam was applied to three anatomical locations on the carcasses, specifically the rump, midline, and neck areas of the beef carcasses, as well as the ham, belly, and jowl areas of the hog carcasses. The designated anatomical locations of the carcasses (100 cm^2) were steamed for a total of 60 s with the nozzle being held approximately 6-7 cm away from the surface of the carcasses. The average atmospheric temperature inside steam chamber was 180 to 185^oF (82 to 85^oC), whereas the surface temperature of animal carcasses was approximately 75^oC after the 60 s steam treatment.

Experimental design. Samples (n = 1,296) were collected by sponge swabbing randomly selected beef (n = 72) and hog (n = 72) carcasses before steam treatment, immediately after steam treatment but before the application of acid or chlorine wash, and 24 h after the application of steam treatment. The three anatomical locations on the right side of each carcass were treated with steam and samples collected were designated as after treatment specimens. The left side of the respective carcass was not treated with steam and the samples collected from the three anatomical sites were designated as untreated controls. Both sides of each carcass were marked for identification before placement in a chilling room. On the following day, the treated areas at the three anatomical sites on the right side of the respective carcass were sponge swabbed again in order to obtain specimens at 24 h after the steam treatment.

Sample collection. The sponge method used to collect samples from the carcasses was similar to that outlined by the USDA *(24)*. Immediately before sampling, sterile sponges (Nelson Jameson Inc; Marshfield, WI) were hydrated with 25 ml of Butterfield's phosphate buffer (pH 7.2). The residual moisture was expelled from the sponge inside the sampling bag, and the sponge was then removed from the bag. Sterile gloves were worn by the personnel handling the sterile sponges. Using a sterile vinyl template (Nelson Jameson Inc), the 100 cm^2 sampling area at each carcass site on beef or hog carcasses was delineated and sponge swabbed for 10 vertical passes (up-and-down being considered as 1 pass) and 10 horizontal passes (side-to-side being considered as 1 pass) with a pressure equivalent to that which would be used to remove dried blood from the carcass. The sponge was then returned to the original sampling bag containing the Butterfield's phosphate buffer. One sponge was used per anatomical location. The sterile gloves and templates used for the sponge swabbing were changed between the sampling of each carcass site in order to maintain aseptic sampling and avoid cross

contamination. After the excess air was expelled, the sponge bags were folded down and placed in a cardboard box with icepacks. The samples were then placed into a cooler of 4ºC and were immediately transported (< 2 to 3 h) to our laboratory at the Georgia Experiment Station, Griffin, GA for microbiological analysis.

Microbiological analysis. Following arrival at the laboratory, the sponge samples were held at 4ºC until analysis on the same day. The content of each sampling bag was stomached for 1 min at normal speed using a Stomacher 400 lab blender (Seward Ltd., London, UK). The liquid content in the sampling bag was released by manual squeezing in order to maximize the recovery of microorganisms. Serial dilutions were prepared using sterilized Butterfield's buffer, and 50 µl of appropriate dilutions was spirally plated in duplicate on tryptic soy agar (TSA) plates for the enumeration of total aerobic bacteria, on MacConkey (MAC) agar plates for the enumeration of total coliform, and on violet red bile glucose agar (VRBGA) plates for the enumeration of *Enterobacteriaceae* using the Autoplate® 4000 automated spiral plating system (Spiral Biotech, Bethesda, MD, USA). The TSA plates were incubated at 35°C for 48 h and the MAC and VRBGA plates were incubated at 37°C for 24 h. The colonies on each plate were enumerated using a **Q***COUNT*™ automatic colony counter (**Q***COUNT*™ software, Spiral Biotech). All microbiological media used in the study were purchased from Difco (Sparks, MD, USA). The detection limit of the enumeration procedure was approximately 20 CFU/cm^2.

Enrichment. All samples collected were screened for the presence of *Salmonella,* along with the enumeration of indicator and spoilage microorganisms. The procedures followed for the isolation of *Salmonella* were similar to those outlined by the USDA *(17)* and United States Food and Drug Administration *(1)*. Five milliliters of the liquid content from the sample bag described above were transferred to 20 ml of lactose broth for pre-enrichment. The lactose broth was

incubated at 37ºC for 22-24 h, and at the end of the incubation period, 0.1 ml of the pre-enriched culture in lactose broth was transferred to 10 ml of modified Rappaport-Vassiliadis (mRV) broth and 0.5 ml was transferred to 10 ml of Tetrathionate broth (TT). The inoculated mRV and TT broth was incubated for 24 h at 42 and 35°C, respectively. One loopful of each selective enrichment culture was inoculated on xylose lysine tergitol™ 4 (XLT4) agar plates. All plates were incubated at 37°C for 24 h. Presumptive *Salmonella* colonies from the XLT4 agar plates were transferred to triple sugar iron (TSI) and lysine iron (LIA) agar slants and were incubated for 24 h at 37°C. The isolates giving typical reactions on the TSI and LIA agar slants were further confirmed using the *Salmonella* polyvalent O (poly A-1, Vi) and polyvalent H serum, both were purchased form Difco.

Statistical analysis. All microbiological data were transformed into log CFU/cm^2 before comparison of means. Analysis of data was accomplished using the Fisher's least significant difference of means of bacterial populations calculated with the General Linear Model (GLM) procedure of SAS based on a 95% confidence level *(20)*. The data were analyzed as a completely randomized design within split plot structure, with anatomical location (three levels), sampling time (three levels), and type of animal (two levels).

RESULTS AND DISCUSSION

The mean populations of total aerobes recovered from the beef carcasses before, immediately after, and 24 h after the steam treatment are shown in Table 1. Approximately 74.5 or 50.0% of the 216 samples collected from the 72 beef carcasses tested positive for total aerobic bacteria before and immediately after the steam treatment, respectively (Table 5.2). The rump area of the untreated beef carcasses had the lowest mean total aerobic count, 1.83 log CFU/cm^2, followed by a count of 1.87 log CFU/cm^2 on the neck area. The midline area had the highest

mean aerobic count of 1.94 log CFU/cm^2 (Table 5.1). All these counts were however, statistically equivalent ($P > 0.05$). Immediately after the steam treatment, the mean total aerobic counts at each anatomical location were significantly lower ($P \leq 0.05$) than the same counts from the untreated control carcasses (Table 5.1). The rump area had the maximum reduction of 1.04 log CFU/cm^2, and the midline and neck area had a reduction of 0.81 and 0.79 log CFU/cm^2, respectively. The mean populations of total aerobes recovered immediately after versus 24 h after the steam treatment at any of the sampling locations of the beef carcasses were not significantly different ($P > 0.05$) (Table 5.1).

Approximately 75.5 or 46.3% of the 216 samples collected from the 72 beef carcasses tested positive for coliforms before or immediately after the steam treatment (Table 5.2). The rump area of the untreated carcasses had the lowest mean total coliform population of 1.53 log CFU/cm^2 while the midline area had an intermediate total coliform population of 2.02 log CFU/cm^2 ($P < 0.05$) (Table 5.1). The neck area was the most heavily contaminated with a mean coliform population of 2.11 log CFU/cm^2. Immediately after the steam treatment, the mean coliform populations at the rump, midline, and neck area were 0.38, 0.84, and 0.92 log CFU/cm^2, respectively. At each anatomical location, a significant reduction ($P \leq 0.05$) was observed between the coliform populations recovered before and immediately after the steam treatment. The mean coliform counts recovered from the rump, midline, and neck area after the 24 h chilled storage were 0.69, 1.11, and 1.05 log CFU/cm^2, respectively. These counts were higher than the mean coliform populations recovered immediately after the steam treatment at all sampled sites. However, no significant differences ($P > 0.05$) were observed between the mean populations of total coliforms recovered immediately after and 24 h after the steam treatment at any of the three anatomical locations on the beef carcasses.

Without the application of steam, 61.6% of the 216 samples taken from the 72 beef carcasses tested positive for *Enterobacteriaceae* (Table 5.2). The steam treatment effectively reduced the number of *Enterobacteriaceae* positive samples to 33.8% (Table 5.2). The *Enterobacteriaceae* populations were 1.27, 1.35, and 1.47 log CFU/cm^2, respectively at the rump, neck, and midline areas of the untreated beef carcasses. After the steam treatment the mean *Enterobacteriaceae* populations at the rump, neck, and midline areas were 0.20, 0.65, and 0.71 log CFU/cm^2, respectively.

Approximately 89.9 or 40.7% of the 216 samples collected from the 72 hog carcasses tested positive for total aerobic bacteria before and immediately after the steam treatment, respectively (Table 5.2). The mean total aerobic counts recovered from the untreated control carcasses were 2.91 log CFU/cm^2 at the belly area, 2.52 log CFU/cm^2 at the jowl area, and 2.06 log CFU/cm^2 at the ham area ($P < 0.05$) (Table 5.1). The total aerobic counts recovered immediately after the steam treatment were reduced ($P \leq 0.05$) by 2.32 log CFU/cm^2 at the belly, 1.85 log CFU/cm^2 at the jowl, and 1.82 log CFU/cm^2 at the ham area. No significant differences ($P > 0.05$) were observed between the mean populations of total aerobes recovered immediately after and 24 h after the steam treatment at any of the sampling locations on the hog carcasses (Table 5.1).

Without the steam treatment, 80.6% of the 216 samples collected from the 72 hog carcasses tested positive for coliforms; however only 45.4% of these samples were positive for coliforms immediately after the steam treatment (Table 5.2). The mean total coliform populations recovered from the untreated hog carcasses were 2.94 log CFU/cm^2 at the belly area, 2.34 log CFU/cm^2 at the jowl area, and 1.95 log CFU/cm^2 at the ham area (Table 5.1). Immediately after the steam treatment, the mean populations of total coliforms were reduced (*P*

≤ 0.05) by 1.13 log CFU/cm^2 at the ham area and 1.64 log CFU/cm^2 at both the belly and jowl area. The mean total coliforms recovered after the 24 h chilling period were 1.45 and 2.18 log CFU/cm^2 at the ham and belly area, respectively which were significantly higher ($P \leq 0.05$) than the coliform counts recovered immediately after the steam treatment from the respective locations. The populations of coliforms recovered at 24 h after the steam treatment however, remained statistically lower than those observed before the treatment ($P \leq 0.05$). No differences ($P > 0.05$) were observed between the mean coliform counts recovered immediately after and 24 h after the steam treatment at the jowl area.

The results for *Enterobacteriaceae* populations were similar to those of total aerobes recovered from the beef carcasses (Table 5.1). Before the steam treatment, 78.7% of the 216 samples collected from the 72 hog carcasses tested positive for *Enterobacteriaceae* and 19.9% of these samples tested positive for *Enterobacteriaceae* immediately after the steam treatment (Table 5.2). The mean *Enterobacteriaceae* populations recovered from the untreated control carcasses were 2.19 log CFU/cm^2 at the belly area, followed by 2.12 log CFU/cm^2 at the jowl area, and 1.34 log CFU/cm^2 at the ham area. With the steam treatment, the *Enterobacteriaceae* were reduced ($P \leq 0.05$) by 1.91 log CFU/cm^2 at the belly, 1.86 log CFU/cm^2 at the jowl, and 1.26 log CFU/cm^2 at the ham area. The steam treatment significantly reduced ($P \leq 0.05$) the mean *Enterobacteriaceae* populations at each anatomical location. However, no differences ($P > 0.05$) were observed between the mean *Enterobacteriaceae* populations recovered immediately after and at 24 h after the steam treatment.

A total of 1,296 samples collected from the 72 beef and 72 hog carcasses were analyzed for the presence of *Salmonella*. Without the steam treatment, 1 out of the 216 samples (0.46%) collected from the beef carcasses and 4 out of the 216 samples (1.85%) collected from the hog

carcasses were found to harbor *Salmonella*. However, the samples collected from the same carcass immediately after and at 24 h after the steam treatment tested negative for *Salmonella*.

The results of this study indicated that the numbers of samples positive for total aerobes, coliforms, and *Enterobacteriaceae* were effectively reduced after the 60 s steam treatment using the household steam cleaner (Table 5.2). Similar results have been obtained by Nutsch et al. (*15*). Before an 8 s steam treatment using the Frigoscandia steam pasteurizer, 16.4% of the samples were positive for generic *E. coli* (0.6-1.5 log CFU/cm^2), 37.9% for coliforms (0.6-2.3 log CFU/cm^2), and 46.4% for *Enterobacteriaceae* (0.6-2.3 log CFU/cm^2). The steam treatment successfully reduced the prevalence of *E. coli*, coliforms, and *Enterobacteriaceae* to 0, 1.4, and 2.9%, respectively *(15)*.

The populations of total aerobes, total coliforms, and *Enterobacteriaceae* recovered from the three anatomical sites of the treated and untreated carcasses were significantly different ($P \leq 0.05$) (Table 5.1). No statistical differences ($P > 0.05$) were, however observed for the populations of total aerobes, total coliforms, and *Enterobacteriaceae* recovered immediately after versus 24 h after the steam treatment at the three anatomical sites of the beef carcasses and jowl area of the hog carcasses (Table 5.1). For both the ham and belly areas of the hog carcasses, the total coliform counts recovered 24 h after steam treatment were significantly higher ($P \leq 0.05$) than those recovered immediately after steam treatment from the respective locations (Table 5.1). These results suggest that post treatment handling and storage could contribute to the re-contamination of treated animal carcasses. In spite of our instructions to plant personnel to keep their hands off the treated sites on the animal carcasses, it was noticed that personnel in Plant C touched the treated sites of the hog carcasses while transferring them to the chilling room. Additionally, Plant C had a relatively smaller chilling room; thus, the treated

hog carcasses in the chilling room had to be placed in a manner that the untreated areas of the hog carcasses were sometimes in contact with the treated areas of adjacent hog carcass. This may have also contributed to post treatment contamination. Furthermore, the temperatures in the chilling rooms of Plant A and Plant C were between 7 and 10^{o}C during our visits at 24 h after the steam treatment. Storage of beef and hog carcasses at 10^{o}C is inappropriate since it provides favorable conditions for psychrotrophs as well as pathogens to grow *(14)*. Koutsoumanis et al. showed that the growth rate of pathogens such as *L. monocytogenes* on fresh beef stored aerobically was greater at 10^{o}C than at 4^{o}C after fresh beef samples were decontaminated using single or sequential treatments of hot water and lactic acid *(12)*.

The higher microbial loads at the belly or jowl areas of the hog carcasses and neck and midline areas of the beef carcasses prior to steam treatment could be attributed to standard industrial slaughter practices *(15)*. The carcasses are hung upside down and are split vertically, exposing the belly and jowl regions of hog carcasses and neck and midline areas of beef carcasses to internal organs such as the intestines of slaughtered animals providing ample opportunity for these areas to be contaminated by bacteria.

Although some similarities exist between the findings of the present study and the studies of others relevant to steam pasteurization *(15, 16)*, a comparison of the results should proceed with caution because of the differences in the technical as well as operational parameters of the steam pasteurization systems used in the respective study. The steam cleaning system used in the present study could easily meet the requirements of small and very small meat processing facilities as it can offer an ease of operation, reusability, effectiveness against pathogens, and affordability. This cleaning system is inexpensive ($150-$200), portable, and does not require any additional external utilities such as a steam generator or chilled water. The results of the

current study indicate that the 60 s steam treatment by the household steam cleaner is effective in reducing the populations of total aerobes, total coliforms, and *Enterobacteriaceae* at the three anatomical locations on both the beef and hog carcasses. The household steam cleaning systems could be used as a critical control measure in small and very small meat processing plants. However, many factors in the meat processing environment could contribute to the re-contamination of processed animal carcasses. An effective steam treatment must be therefore, accompanied by SSOPs, good manufacturing practices, and regulatory supervision during post treatment handling and storage in order to reduce the levels of bacterial contamination on animal carcasses.

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Table 5.1. Mean populations (log CFU/cm^2) of total aerobes, total coliforms, and *Enterobacteriaceae* recovered from the three anatomical locations of the beef (n =72) and hog carcasses (n =72), before and immediately after the 60 s steam treatment using a commercial household cleaning system and after 24 h of cold storage.

	BEEF				HOG			
	RUMP	MIDLINE	NECK	MEAN	HAM	BELLY	JOWL	MEAN
Total aerobes								
Control [a]	1.83 a [d]A [e]	1.94 aA	1.87 aA	1.88	2.06 aX	2.91 aY	2.52 aZ	2.50
After [b]	0.79 bA	1.15 bA	1.06 bA	1.00	0.24 bX	0.59 bY	0.67 bY	0.50
24 h After [c]	0.91 bA	1.30 bA	1.09 bA	1.10	0.80 bX	0.97 bX	0.97 bX	0.91
Total coliforms								
Control	1.53 dA	2.02 dAB	2.11 dB	1.89	1.95 dX	2.94 dY	2.34 dX	2.41
After	0.38 eA	0.84 eB	0.92 eB	0.71	0.82 fX	1.30 fY	0.70 eX	0.94
24 h after	0.69 eA	1.11 eA	1.05 eA	0.95	1.45 eX	2.18 eY	1.06 eX	1.56
Enterobacteriaece								
Control	1.27 gA	1.47 gA	1.35 gA	1.36	1.34 gX	2.19 gY	2.12 gX	1.88
After	0.20 hA	0.71 hA	0.65 hA	0.52	0.08 hX	0.28 hY	0.26 hY	0.21
24 h after	0.44 hA	0.61 hA	0.44 hA	0.50	0.32 hX	0.53 hX	0.46 hX	0.44

[a]: Control: not treated with steam
[b]:After: immediately after the steam treatment
[c]:24 h after: 24 h after the steam treatment and cold storage
[d]:Within a given bacterial population, means in the same column followed by the same lower case letter are not significantly different.
[e]:Within a given animal carcass, means in the same row followed by the same upper case letter are not significantly different.

Table 5.2. Number of samples tested positive for total aerobes, total coliforms, and *Enterobacteriaceae* on beef and hog carcasses.

	Beef Carcasses (n = 216) [d]			Hogs Carcasses (n = 216)		
	Total aerobes	Total coliforms	*Enterobacteriaceae*	Total aerobes	Total coliforms	*Enterobacteriaceae*
Control [a]	161 (74.5%) [e]	163 (75.5%)	133 (61.6%)	194 (89.9%)	174 (80.6%)	170 (78.7%)
After [b]	108 (50.0%)	100 (46.3%)	73 (33.8%)	88 (40.7%)	98 (45.4%)	43 (19.9%)
24 h after [c]	107 (49.5%)	95 (44.0%)	55 (25.5%)	106 (49.0%)	144 (66.7%)	81 (37.5%)

[a]: Control: not treated with steam
[b]: After: immediately after the steam treatment
[c]: 24 h after: 24 h after the steam treatment
[d]: Number of samples enumerated for each count type within each carcass type
[e]: Number of samples tested positive for total aerobes, total coliform or *Enterobacteriaceae* (detection limit: 20 CFU/cm^2).

CHAPTER 6

Microbiological Quality of Beef and Pork Carcasses Processed by Four Small and Very Small Meat Processing Plants in Georgia[1]

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ABSTRACT

Aims: This study evaluated the microbiological quality of beef and pork carcasses processed by four small and very small meat processing facilities in Georgia.

Methods and Results: A sterile sponge was used to sample the rump, midline, and neck area (100 cm^2) of 72 beef carcasses along with the ham, belly, and jowl area (100 cm^2) of 72 pork carcasses collected from four different slaughtering facilities in Georgia (A, B, C, and D) over a period of two months. All samples were collected at the end of the slaughter line but prior to the application of any antimicrobial interventions. Each collected sample was analyzed for the populations of total aerobes, total coliforms, and *Enterobacteriaceae*, as well as the incidence of *Salmonella*. No significant differences ($P > 0.05$) were found in the overall mean populations of aerobic bacteria or *Enterobacteriaceae* recovered from the different locations on the beef carcasses. The total coliforms however, were higher ($P \leq 0.05$) on the neck area (2.11 Log_{10} CFU cm^{-2}) than on the rump area (1.53 Log_{10} CFU cm^{-2}) and were intermediate at the midline area (2.02 Log_{10} CFU cm^{-2}). In the pork carcasses, total aerobes, total coliforms, and *Enterobacteriaece* recovered from the belly area (2.91, 2.94, and 2.19 Log_{10} CFU cm^{-2} respectively) were higher ($P \leq 0.05$) than respective populations recovered from the ham and jowl areas. The total aerobes on the jowl area (2.52 Log_{10} CFU cm^{-2}) were higher ($P \leq 0.05$) than those on the ham area (2.06 Log_{10} CFU cm^{-2}). Out of the 72 beef carcasses evaluated, only 1 was positive for *Salmonella* (1.38% incidence rate) whereas, of the 72 pork carcasses evaluated, 4 were positive for *Salmonella* (5.5% incidence rate).

Conclusions: The overall mean populations of total aerobes and total coliforms on the beef carcasses processed by Plant B and Plant D, and on the hog carcasses processed by Plant A are either arithmetically similar to or lower than the national baseline values collected by the

USDA. The overall mean populations of total aerobes and total coliforms on beef carcasses processed by Plant A were however, arithmetically higher than the national baseline value. Furthermore, the total coliform populations on the hog carcasses processed by Plant C were almost double the national baseline value for market hogs.

Significance and Impact of the Study: The findings of this study address a need to identify appropriate critical control points in, and effective interventions for small and very small meat processing plants in order to further improve the microbial quality of meats.

Keywords: *Salmonella*, total aerobes, *Enterobacteriaceae*, total coliforms, small and very small meat processing facilities, beef carcasses and hog carcasses.

INTRODUCTION

It is estimated by the Centers for Disease Control and Prevention (CDC) that there are approximately 76 million cases of foodborne illnesses each year in the United States (Mead *et al.* 1999). During the period of 1993 and 1997, bacterial pathogens were responsible for 75% of the outbreaks and 86% of the total number of cases of foodborne illnesses in the United States (CDC 2000). Among these, beef was identified as the source of transmission in a total of 66 out of the 2,747 reported cases of foodborne outbreaks (CDC 2000). To decrease the extent of microbial contamination in meat products and the incidence of foodborne diseases, the USDA imposed new meat and poultry inspection regulations in 1996 (FSIS 1996b). The new regulations required meat and poultry processing plants of all sizes to operate under Sanitation Standard Operating Procedures (SSOP) and the Hazard Analysis Critical Control Point (HACCP) program (FSIS 1996b).

The implementation of HACCP in small and very small meat processing plants took place in January 2000. Since then, all 3,159 federal and approximately 2,300 state-inspected small meat processing plants in the United States have been operating under HACCP along with microbiological testing of carcasses in order to meet the performance standards for *Salmonella* (FSIS 2000). The microbiological performance standards were set based on the nationwide microbiological baseline data collected by the USDA Food Safety and Inspection Service (FSIS) during the period of October 1992 to March 1996 (FSIS 1993, 1994, 1996a). These data, however, solely reflected the situation in federally inspected plants during the period of testing, and did not reflect the situation in small and very small meat processing facilities (FSIS 1993, 1994, 1996a). According to the FSIS, small and very small plants represent approximately 25%

of total meat production volume in the United Sates (FSIS 1999). In 1996, Gill et al. surveyed the hygienic conditions of raw beef materials that were destined for ground beef production and concluded that the hygienic quality of the finished product was heavily influenced by the quality of the raw materials, and that generally, raw materials from the carcasses of culled or market cow and bull had relatively lower levels of contamination than did raw materials from the carcasses of feed lot cows (Gill *et al.* 1996). In light of these findings, it was found necessary to determine the microbial quality of beef and hog carcasses processed by small and very small meat processors, for both food safety advancements and regulatory purposes.

The study reported here, evaluated the populations of total aerobic bacteria, total coliforms, and *Enterobactriaceae,* as well as the incidence of *Salmonella* on 72 beef and 72 hog carcasses processed by four small or very small meat processing facilities in Georgia.

MATERIALS AND METHODS

Slaughter facilities

Four small or very small meat processing plants in Georgia were selected for the present study. Plant A slaughters hogs twice a week with an operating capacity of 50 to 60 hogs per day. This plant also slaughters steers and heifers once a week with a production throughput of 20 to 24 heads per day, which is often followed by hog slaughtering on the same day. Plant B slaughters only steers and heifers once or twice a month as per requirement with a production throughput of 12 to 20 heads per day. Plant C is a very small plant and operates once a week with a production throughput of 24 to 40 hogs per week. It also slaughters steers and heifers, with the slaughter schedule depending upon the season and availability of animals. Plant D operates twice a week and predominately slaughters steers, heifers, and calves with a production throughput of 75 to

120 animals per day. Slaughtering procedures employed by the four plants followed the standard industry practices (Nutsch *et al.* 1997) and included one or more decontamination intervention technologies. Plant A sprays the carcasses with chlorinated tap water, while Plant B uses 2.0 to 2.6% of lactic acid spray after the final carcass wash. Plant C and Plant D both use acetic acid spray as a decontamination intervention after the final carcass wash. Plant D, however, uses the acetic acid spray at two points of slaughtering, the first one being applied immediately after evisceration and the second after the final carcass wash.

Experimental design

Samples (n = 432) were collected by sponge swabbing randomly-selected beef (n = 72) and hog (n = 72) carcasses immediately after the final carcass wash but before application of any antimicrobial interventions. The three anatomical locations on the beef carcasses were neck, midline, and rump and the three sites on the hog carcasses were jowl, belly, and ham.

Sample collection

The sponge method used to collect samples from the carcasses was similar to that outlined by the FSIS (FSIS, 1996b). Immediately before sampling, sterile sponges (Nelson Jameson Inc; Marshfield, WI) were hydrated with 25 ml of Butterfield's phosphate buffer (pH 7.2). The residual moisture was expelled from the sponge inside the sampling bag, and the sponge was then removed from the bag. Sterile gloves were worn by all personnel handling the sterile sponges. Using a sterile vinyl template (Nelson Jameson Inc; Marshfield, WI), a 100 cm^2 sampling area at each carcass site on the beef or hog carcasses was delineated and sponge swabbed using 10 vertical passes (up-and-down being considered as 1 pass) and 10 horizontal passes (side-to-side being considered as 1 pass) with a pressure equivalent to that which would be used to remove dried blood from the carcass. The sponge was then returned to the original

sampling bag containing the Butterfield's phosphate buffer. One sponge was used per anatomical location. The sterile gloves and templates used for sponge swabbing were changed between sampling of each carcass site in order to maintain aseptic sampling and avoid cross contamination. After the excess air was expelled, the sponge bags were folded down and placed in a cardboard box with icepacks. The samples were then placed into a cooler at 4ºC and were immediately transported (< 2 to 3 h) to our laboratory at the Georgia Experiment Station in Griffin, GA for microbiological analysis.

Microbiological analysis

Following arrival at the laboratory, the sponge samples were held at 4ºC until analysis. The content of each sampling bag was stomached for 1 min at normal speed using a Stomacher 400 lab blender (Seward Ltd., London, UK). The liquid content in the sampling bag was released by manual squeezing in order to maximize the recovery of microorganisms. Serial dilutions were prepared using sterilized Butterfield's buffer, and a 50 µl of appropriate dilutions was spirally plated in duplicate on tryptic soy agar (TSA) plates for the enumeration of total aerobic bacteria, on MacConkey (MAC) agar plates for the enumeration of total coliform, and on violet red bile glucose agar (VRBGA) plates for the enumeration of *Enterobacteriaceae* using the Autoplate® 4000 automated spiral plating system (Spiral Biotech, Bethesda, MD, USA). The TSA plates were incubated at 35^{o}C for 48 h and the MAC and VRBGA plates were incubated at 37^{o}C for 24 h. The colonies on each plate were enumerated using a **Q***COUNT*™ automatic colony counter (**Q***COUNT*™ software, Spiral Biotech, Bethesda, MD, USA). All microbiological media used in the study were purchased from DIFCO Laboratories (Sparks, MD, USA).

Enrichment

All samples collected were screened for the presence of *Salmonella* along with the enumeration of indicator and spoilage microorganisms. The procedures followed for isolation of *Salmonella* were similar to those outlined by the FSIS (FSIS 1996b) and Bacteriological Analytical Manual (Andrews et al., 1995). Five milliliters of the liquid content from the sample bag described above was transferred to 20 ml of lactose broth for pre-enrichment. The lactose broth was incubated at 37ºC for 22-24 h, and at the end of the incubation period, 0.1 ml of the pre-enriched culture in lactose broth was transferred to 10 ml of Rappaport-Vassiliadis (RV) broth and 0.5 ml was transferred to 10 ml of Tetrathionate broth (TT). The inoculated RV and TT broth was incubated for 24 h at 42 and 35^{o}C, respectively. One loopful of each selective enrichment culture was inoculated on xylose lysine tergitol 4 (XLT4) agar plates. All plates were incubated at 37^{o}C for 24 h. Presumptive *Salmonella* colonies from the XLT4 agar plates were transferred to triple sugar iron (TSI) and lysine iron (LIA) agar slants and were incubated for 24 h at 37^{o}C. The isolates giving typical reactions on the TSI and LIA agar slants were further confirmed using the *Salmonella* polyvalent O (poly A-1, Vi;) and polyvalent H serum, both were purchased from DIFCO Laboratories.

Statistical analysis

All microbiological data were transformed into Log_{10} CFU cm^{-2} before comparison of means. Analysis of data was accomplished using the Fisher's least significant difference of means of bacterial populations calculated with the General Linear Model (GLM) procedure of SAS based on a 95% confidence level (SAS 2000). The data were analyzed as a completely randomized design within split plot structure, with anatomical location (three levels), sampling time (three levels), and type of animal (two levels).

RESULTS

Beef carcasses

Table 1 shows the overall mean populations (Log_{10} CFU cm^{-2}) of total aerobes, total coliforms, and *Enterobacteriaceae* recovered from the three anatomical locations of the naturally contaminated beef and hog carcasses sampled in this study. On beef carcasses, the total coliforms recovered from the neck area were 2.11 Log_{10} CFU cm^{-2}, which were significantly higher ($P \leq 0.05$) than those recovered from the rump area (1.53 Log_{10} CFU cm^{-2}) (Table 1). No significant ($P > 0.05$) differences were noticed in the populations of total aerobes and *Enterobacteriaceae* recovered from the three anatomical locations on the beef carcasses, however the populations of total aerobes and *Enterobacteriacea*e in the midline area was higher than those at the neck area, and the same counts at the neck area were higher than those at the rump area (Table 1).

The populations of total aerobes recovered from beef carcasses at Plant A were significantly higher ($P \leq 0.05$) than those recovered from Plant B or Plant D (Table 2). The mean of total aerobes recovered from Plant A was 3.21 Log_{10} CFU cm^{-2}, followed by Plant B (0.76 Log_{10} CFU cm^{-2}) and Plant D (0.43 Log_{10} CFU cm^{-2}) (Table2). No significant difference ($P > 0.05$) was noticed between the populations of total aerobes recovered from the beef carcasses from Plant B and Plant D (Table 2).

The mean populations of total coliforms recovered from beef carcasses at Plant A were 2.59 Log_{10} CFU cm^{-2}, followed by Plant D (1.63 Log_{10} CFU cm^{-2}) and Plant B (0.29 Log_{10} CFU cm^{-2}) (Table2). The coliform populations recovered from Plant A were significantly higher ($P \leq 0.05$) than those recovered from Plant B or Plant D and those recovered from Plant B were lower ($P \leq 0.05$) than Plant D (Table 2). In total, 82% of the 108 samples collected from Plant A, 28%

of the 36 samples collected from Plant B, and 89% of the 72 samples collected from Plant D tested positive for coliforms (Table 3).

The populations of *Enterobacteriaceae* were similar to those of the total aerobes recovered from the beef carcasses. The populations of *Enterobacteriaceae* recovered from the beef carcasses at Plant A were significantly higher ($P \leq 0.05$) than those recovered from Plant B or Plant D (Table 2). The mean of *Enterobacteriaceae* recovered from Plant A was 2.48 Log_{10} CFU cm^{-2}, followed by Plant D (0.29 Log_{10} CFU cm^{-2}) and Plant B (0.15 Log_{10} CFU cm^{-2}) (Table2). No significant difference ($P > 0.05$) was noticed between the populations of total aerobes recovered from the beef carcasses of Plant B and Plant D (Table 2). Overall, 88% of the 108 samples collected from Plant A, 25% of the 36 samples collected from Plant B, and 42% of the 72 samples collected from Plant D tested positive for *Enterobacteriaceae* (Table 3).

Hog carcasses

On hog carcasses, the populations of total aerobes, total coliforms, and *Enterobacteriaceae* recovered from the belly area were significantly higher ($P \leq 0.05$) than those recovered from the jowl and ham areas, and the populations of the same groups of microorganisms at the jowl area were higher than those at the ham area (Table 1). The mean population of total aerobes recovered from the jowl region was 2.52 Log_{10} CFU cm^{-2}, which was significantly higher ($P \leq 0.05$) than those recovered from the jowl area (2.52 Log_{10} CFU cm^{-2}) and the ham area (2.06 Log_{10} CFU cm^{-2}) (Table 1). The differences between the populations of total coliforms and *Enterobacteriaceae* recovered from the ham and jowl regions were statistically insignificant ($P > 0.05$) (Table 1).

The mean populations of total aerobes, total coliforms, and *Enterobacteriaceae* recovered from the hog carcasses processed by Plant C were 2.71, 3.47, and 2.47 Log_{10} CFU cm^{-2}, and the

same populations recovered from the hog carcasses processed by Plant A were 2.28, 1.36, and 1.29 Log_{10} CFU cm^{-2}, respectively (Table 2). The mean populations of total aerobes, total coliforms, and *Enterobacteriaceae* recovered from the hog carcasses processed by Plant C were significantly higher ($P \leq 0.05$) than those processed by Plant A (Table 2). Overall, 61% of the 108 samples taken from Plant A and 100% of the 108 samples taken from Plant C tested positive for coliforms, additionally, 62% of the 108 samples taken from Plant A and 94% of the 108 samples taken from Plant C tested positive for *Enterobacteriaceae* (Table 3).

Samonella positive samples

A total of 432 samples collected from the 72 beef and 72 hog carcasses were analyzed for the presence of *Salmonella*. One sample out of the 216 samples (0.46%) collected from beef carcasses and 4 samples out of the 216 samples (1.85%) collected from hog carcasses were found to harbor *Salmonella*.

DISCUSSION

The nationwide microbiological baseline data collected by the USDA FSIS from steers and heifers indicated that mean populations of total aerobic bacteria and total coliforms were 2.68 and 1.55 Log_{10} CFU cm^{-2}, respectively (FSIS 1993). These national baseline values are arithmetically lower than the respective mean values derived from the beef carcasses collected at Plant A (Table 2). However, the mean populations of total aerobes and total coliforms derived from Plant B and Plant D are either arithmetically similar to or lower than the national baseline values (Table 2). The nationwide microbiological baseline data collected from market hogs indicated that mean populations of total aerobic bacteria and total coliforms were 3.69 and 1.88 Log_{10} CFU cm^{-2}, respectively (FSIS 1996a). The total aerobes recovered from both Plant A and Plant C were in compliance with the national baseline (Table 1). However, the total coliforms

recovered from Plant C were almost double the national baseline value for market hogs, indicating a poor sanitation condition in this plant (Table 2).

Small and very small meat processing facilities slaughter fewer animals at any given time, and in most of the cases, the same personnel perform different cuts on the same carcass. Due to a lack of space, all aspects of the slaughter process might be done in one small area, which may provide for an increased risk of fecal contamination. At a high capacity slaughterhouse, distance between the clean and unclean parts of the abattoir are longer than those typical to a small or very small meat processing facility. Among the plants involved in the present study, only Plant B had separate clean and unclean areas along with limited movement of workers between the unclean and clean areas, which appear to reduce the levels of contamination on carcasses processed by this plant (Table 2). This has also been observed in a Swedish study (Hansson 2001), in which microbial samples were collected from the beef and pork carcasses processed by a total of 16 high or low capacity slaughtering plants. Out of the 16 plants, four were high capacity and four were low capacity beef processing plants. Similarly, from the rest of eight plants, four were high capacity and four were low capacity pork processing plants. The study found that the mean population of total aerobes recovered from the four low capacity beef processing plants was 3.44 Log_{10} CFU/cm^2 which was significantly higher than the mean aerobic bacteria population recovered from the four high capacity beef processing plants (2.59 Log_{10} CFU/cm^2). However, no significant differences were observed in the mean populations of total aerobes recovered from the pork carcasses from high capacity (3.44 Log_{10} CFU/cm^2) and low capacity plants (3.34 Log_{10} CFU/cm^2).

Within the four small beef processing plants involved in this study, there were significant variations ($P \leq 0.05$) with regards to the populations of aerobic bacteria and total coliforms

(Tables 2 and 3). These variations could be attributed to the different evisceration techniques employed by each plant and variations in the precautions taken by plant personnel during and after the evisceration step. Based on our observations and the findings of other studies, it is essential that animal hide does not come in contact with an already dehided carcass in order to avoid carcass surface contamination during removal of the skin from the carcass (Hansson, 2001). Animal hide has been shown to be the potential source for the majority of the mesophilic or psychrotrophic bacteria found on cattle carcasses immediately after slaughter and dressing process (Newton *et al.* 1978). Both dirty and wet hides from cattle may serve as a source of contamination in de-hided carcasses (Snijders *et al.* 1984). At Plant A, a stainless steel trolley was used to support the carcass during the evisceration process, which allowed for the contact of the de-hided carcass surface with the stainless steel surface already contaminated with dirty hide. Three of the four plants involved in this study (Plants B, C, and D) applied an organic acid rinse to carcasses immediately before they entered the coolers. However, Plant D applied the organic acid rinse at two different times of carcass dressing; first, immediately after evisceration and second, before transferring to the cooler. Thus, all the samples collected from Plant D were collected after the first organic acid rinse, which may explain the significantly lower populations of total aerobes and total coliforms in the samples collected from Plant D (Table 2). Applying organic acids, such as lactic, acetic, or citric acid, to carcass surfaces has been shown to reduce bacterial populations (Quartey-Papafio *et al.* 1980; Acuff *et al.* 1987; Cutter and Siragusa 1994; Kochevar *et al.* 1997). Hardin *et al.* (1995) reported that an application of an organic acid rinsing treatment following the washing of beef carcasses was more effective in reducing the level of microbial contamination than washing only or trimming. In a study by Bell et al. (1997), lean beef and adipose tissue contaminated with a fecal inoculum containing *E. coli* ATCC 25922

was sprayed with 1% acetic acid (pH 2.92) at a temperature of 25°C (77°F) in a pilot scale model carcass washer. The spray was initially applied for 15 s, the beef tissue was then held for 90 s before the spray was repeated for another 15 s. The acetic acid treatment resulted in reductions of 2.47 Log_{10} CFU cm^{-2} and 3.12 Log_{10} CFU cm^{-2} in populations of *E. coli* ATCC 25922 on the adipose and lean tissue, respectively.

The higher microbial load at the belly or jowl area of the beef or hog carcasses could be attributed to standard industrial slaughter practices (Nutsch *et al.* 1997). In a conventional dressing system carcasses are hung by their hind legs then split vertically. This technique exposes the majority of the carcass surface to internal organs such as the intestine of the animal, providing ample opportunity for enteric bacteria to contaminate the carcass surface (Duffy *et al*. 2001). Studies with lamb carcass dressings have shown that an inverted dressing system (lamb carcasses are hung by their front legs during pelt removal) result in carcasses with lower levels of bacterial contamination compared to the conventional dressing system. In the conventional carcass dressing system, the pelt-opening cuts are made in the hindquarter region which is an area associated with high levels of contamination, while in the inverted dressing system, the pelt-opening cuts are made in the forequarter region of the carcass, keeping contaminants around the leg and anus from spreading across the carcass (Duffy *et al*. 2001).

During the carcass dressing process, the perianal area and rectal cavity are usually contaminated by microorganisms (Grau 1979). Using a plastic bag to seal off the rectum immediately after it had been exposed, the level of bacterial contamination on the carcasses could be considerably reduced. Nesbakken *et al.* (1994) demonstrated that the plastic bag technique prevents the dissemination of *Yersinia enterocolitica* and other pathogens that are

spread through fecal contamination. However, none of the processing plants evaluated in this study have implemented the plastic bag technique as a preventive measurement.

Singeing or flaming is one of the effective techniques in achieving the greatest reduction of bacterial load on the hog carcass skin (Snijders *et al.* 1984; Huis In't Veld *et al.* 1994). Hog carcasses processed by Plant C had more bristles left on the hog carcass rind compared with the hog carcasses from Plant A. This may be the primary reason for the greatest populations of total coliforms and *Enterobacteriaceae* recovered from the samples collected from Plant C. Furthermore, Plant C was a very small slaughtering plant employing only one worker on the floor to perform all operations of the hog slaughtering, ultimately resulting in inefficient dressing of the carcass as well as a higher microbial load (Table 2).

CONCLUSIONS

The study shows that the levels of microbial contamination on carcasses processed by some of the four small and very small meat processing facilities involved in this study are relatively higher than the national baseline data collected by the USDA. There are significant variations among the four plants with regards to the populations of total aerobes, total coliforms, and *Enterobacteriaceae* on their beef and hog carcasses. Therefore, it is important for these plants to adopt better and more effective intervention procedures in order to further improve the microbial quality of carcasses. Although many attempts have been made to identify simple yet effective techniques for prevention of microbial contamination of carcasses at slaughterhouses, only a few small meat processing plants are taking advantage of such technology, indicating a communication gap among small meat processors, research institutions, and regulatory agencies.

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Table 6.1. Overall mean populations (Log_{10} CFU cm^{-2}) of total aerobes, total coliforms, and *Enterobacteriaceae* recovered from three anatomical locations of naturally contaminated beef and hog carcasses before final carcass wash. (n = 72)

	BEEF			HOG		
	RUMP	**MIDLINE**	**NECK**	**HAM**	**BELLY**	**JOWL**
Total aerobes	1.83 a	1.94 a	1.87 a	2.06 g	2.91 e	2.52 f
Total coliforms	1.53 b	2.02 ab	2.11 a	1.95 f	2.94 e	2.34 f
Enterobacteriaece	1.27 a	1.47 a	1.35 a	1.34 f	2.19 e	2.12 f

* Means in the same row with the same letter are not significantly different.

Table 6.2. Overall mean populations (Log_{10} CFU cm^{-2}) of total aerobes, total coliforms, and *Enterobacteriaceae* recovered from naturally contaminated beef and hog carcasses processed by four small and very small slaughtering facilities.

	Beef			**Hogs**	
	Plant A	**Plant B**	**Plant D**	**Plant A**	**Plant C**
Total aerobes	3.21e	0.76f	0.43f	2.28x	2.71y
Total coliforms	2.59e	0.29g	1.63f	1.36x	3.47y
Enterobacteriaceae	2.48e	0.15f	0.29f	1.29x	2.47y

* Means in the same row with the same letter are not significantly different.

Table 6.3. Number of samples tested positive for total aerobes, total coliforms, and *Enterobacteriaceae* on beef and hog carcasses.

	Beef						**Hogs**			
	Plant A		**Plant B**		**Plant D**		**Plant A**		**Plant C**	
	No. of samples collected	No. of samples tested positive	No. of samples collected	No. of samples tested positive	No. of samples collected	No. of samples tested positive	No. of samples collected	No. of samples tested positive	No. of samples collected	No. of samples tested positive
Total aerobes	108[a]	104[b](96%)	36	22(61%)	72	34(47%)	108	96(89%)	108	98(91%)
Total coliforms	108	89(82%)	36	10(28%)	72	64(89%)	108	66(61%)	108	108(100%)
Enterobacteriaceae	108	95(88%)	36	9(25%)	72	30(42%)	108	67(62%)	108	102(94%)

[a]: Number of samples enumerated for each count type within each carcass type
[b]: Number of samples tested positive for total aerobes, total coliforms, or *Enterobacteriaceae* (detection limit: 20 CFU ml^{-1}).

CHAPTER 7

CONCLUSIONS

The following conclusions are drawn from the studies described in chapters 3, 4, 5, and 6:

1. The application of steam using three household steam cleaners, S1, S2, and S3 significantly ($P \leq 0.05$) reduced the population of artificially inoculated *L. monocytogenes* and *E. coli* O157:H7 as well as naturally contaminated total aerobic and thermoduric bacteria on the surface of pork skin. The performance of S1 was significantly better than S2 or S3 ($P \leq 0.05$) at both levels of inoculation with regard to inactivation of *L. monocytogenes* and total aerobes. For the inactivation of *E. coli* O157:H7, the performance of S1 was better ($P \leq 0.05$) than the performance of S2 or S3 when *E. coli* O157:H7 inoculation level was ca. 10^7 CFU/cm^2 however, no statistical differences ($P > 0.05$) were noticed between the performance of S1 and S2 when *E. coli* O157:H7 inoculation level was ca. 10^5 CFU/cm^2. Significant differences in microbial reductions were observed between different inoculation levels, treatment times, and types of cleaning systems used in the study ($P \leq 0.05$).
2. The 60 s steam treatment using the steam cleaner S1 significantly reduced ($P \leq 0.05$) the levels of naturally contaminated total aerobes, coliforms, and *Enterobacteriaceae* at rump, midline, and neck regions of beef carcasses as well as ham, belly, and jowl regions of hog carcasses processed by four small or very small meat processors in Georgia. The overall mean populations of total aerobes and total coliforms on the beef carcasses processed by Plant B and Plant D, and on the hog carcasses processed by Plant A are either arithmetically similar to or lower than the national baseline values collected by the

3. USDA. The overall mean populations of total aerobes and total coliforms on beef carcasses processed by Plant A were however, arithmetically higher than the national baseline value. Furthermore, the total coliform populations on the hog carcasses processed by Plant C were almost double the national baseline value for market hogs.
4. Commercial household steam cleaning systems could be used as a critical control measure in small and very small meat processing plants. However, an effective steam treatment must be accompanied by Sanitation Standard Operating Procedures (SSOPs), Good Manufacturing Practices (GMPs) and regulatory supervision.