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Influence of Market Setting and Time of Purchase on Counts of Aerobic Bacteria, Escherichia Coli, and Coliform and Prevalence of Salmonella and Listeria in Beef, Pork, and Chicken in Vietnam

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Influence of market setting and time of purchase on counts of aerobic bacteria, *Escherichia coli*, and coliform and prevalence of *Salmonella* and *Listeria* in beef, pork, and chicken in Vietnam

By

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Influence of market setting and time of purchase on counts of aerobic bacteria, *Escherichia coli*, and coliform and prevalence of *Salmonella* and *Listeria* in beef, pork, and chicken in Vietnam

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The objective of this study was to determine the influence of market type and sampling time on *Salmonella* and *Listeria* prevalence and microbiological quality of 540 beef, pork, and whole chicken samples collected in 6 supermarkets (SM), 6 indoor markets (IM), and 6 open markets (OM) at opening (T0) and 4 h after the opening (T4) in Vietnam. *Salmonella* and *Listeria* prevalence ranged from 30.4 to 71.0% and 56.6 to 99.9 %, respectively, in beef, pork, and chicken in Vietnam. Aerobic bacteria counts ranged from 10.5 to 11.6 log CFU/g, whereas, *E. coli* and coliform counts ranged from 7.2 to 11.4 log CFU/g in beef, pork, and chicken in Vietnam. *E. coli* counts were influenced by the interaction of market type and sampling time in beef and pork. Market characteristic data that were considered relevant to microbiological safety of fresh meat and poultry products were collected for individual samples

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CHAPTER I

LITERATURE REVIEW

Introduction

Each year, the world wastes approximately 1.3 billion tons of food produced for human consumption. The United States, a developed countries, loses approximately 165 billion dollars of foods a year, even with various food safety policies and interventions for meat and poultry products. Food loss poses an even greater challenge in the developing countries, such as Vietnam, because of the financial and technological constraints. Food security emerged as a priority after sudden spikes in the prices of food commodities in 2007 – 2008 (Feed the Future, 2014). Asia was the epicenter of this worldwide crisis, and severity of food shortage in this region was primarily caused by lack of good manufacturing practices (Feed the Future, 2014). Price increases exposed the vulnerability of the poorest segments of the population, who spend half of their income to buy food (Feed the Future, 2014). With losses of other commodities exceeding 30% and in some cases over 50% during post-harvest processing, consumers in Vietnam and other developing countries cannot afford to waste precious sources of protein such as meat and poultry. Therefore, the microbiological safety and quality of meat and poultry are extremely important in developing countries. *Salmonella, Listeria, and Escherichia coli* are three major foodborne pathogens that have been the center of food safety research in developed countries such as the U.S. All three of these pathogens can cause

severe illnesses with heavy financial burdens to societies and economic loss and societal chaos if large quantities of animal proteins are recalled. Therefore, the link between microbiological safety and quality of foods and food security is undeniable, especially, that of meat and poultry products. Microbiological research in developing countries is needed to address the safety and security of meat and poultry.

Food Safety and Security

Relationship between food safety and security

Food safety is not only about safe food, but safe consumption of food and is recognized as an integral part of food security (Unnevehr, 2015). The World Health Organization (WHO) has recognized food safety as part of the enabling environment for reducing hunger and malnutrition (WHO and FAO, 2014). However, the most recent focus has been towards production, processing, and distribution of foods that are secure from bioterrorism so that foods cannot be deliberately contaminated with an agent that makes people ill and causes death or economic chaos (Johnstone et al., 2015). Buzby (2001) and Antle (1999) found that food safety economics are complicated because it is difficult to measure the value of "food safety", which depends on perception of safe food by the consumer and producer (Verbeke et al., 2007). The presence of food hazards can also lead to food losses and reduced food availability for food insecure populations (Unnevehr, 2015). Identifying a hazardous organism and its associated foodborne illness is only part of the debate on policymaking, which involves science, politics, culture, and international consensus (Kinsey, 2005). It is important to devise regulations based on science; however, it also essential to reach consensus on scientific evidence and application. The International Commission on Microbiological Specification for Foods

(ICMSF) and Codex Alimentarius sets food safety standards. The ICMSF assesses risk and establishes protocols, and the Codex is the consensus-building arm of the United Nations that identifies international standards for food safety. These organizations are responsible for ensuring regulations are realistic and maintain consumers' trust. Monetary cost seems to be underestimated because foodborne illnesses are underreported by both consumers and doctors. Most consumers did not think foodborne illness as the cause. However, in 2013, *Escherichia coli* O157 caused 63,153 cases of foodborne illness, resulting in 217,418,690 dollars spent (Hoffmann et al., 2012; Batz et al., 2014). *Listeria monocytogenes* caused 1,591 cases which cost 2,834,444,202 dollars in hospitalizations, newborn disabilities, and deaths (Hoffmann et al., 2012; Batz et al., 2014). Lastly, *Salmonella* infection resulted in 1,027,561 total cases and 3,666,600,031 dollars total cost for illness (Hoffmann et al., 2012; Batz et al., 2014). *Salmonella* and *Listeria* cause 35 and 19% of foodborne illnesses in the U.S., respectively (Scallan et al., 2011). In addition, *Salmonella* caused over one million illnesses with 19,000 hospitalizations and 380 deaths (CDC, 2015a) and *Listeria* was associated with approximately 1600 illnesses with 260 deaths in 2014 (CDC, 2015a; CDC, 2015b). Ollinger et al, (2003) reported that a Pathogen Reduction/Hazard Analysis and Critical Control Point program in meat and poultry plants would cost approximately 1.1 % of total costs, adding approximately 1.2 cents to a pound of beef, 0.7 cents to a pound of pork, and 0.4 cents to a pound of poultry. The benefits ranged from 1.9 to 171.8 billion dollars annually, which is twice as much as the initial implementation cost to the industry. An analysis of adopting Hazard Analysis and Critical Control Point (HACCP) programs in meat and poultry slaughterhouses in the U.S. using a Social Accounting

Matrix (Golan et al., 2000) provided a comprehensive picture of how well an entire economy performs after investments in food safety. The authors' model showed that for every dollar saved by preventing a premature death from a foodborne illness, there was an economy-wide gain of $1.92. They also found that for every dollar of household income saved from medical expenses, the whole economy would gain $0.27. To implement a HACCP program, every dollar to be spent in initial investment to food safety would result in an economic gain to the industry and consumers.

Economic effect of foodborne diseases

Foodborne diseases result in suffering and even in the loss of lives. It is estimated that one in three people worldwide suffers annually from a foodborne disease and 1.8 million die from severe food- and water-borne diarrhea. Foodborne diseases cause heavy social and economic burdens on communities, especially, their health care systems and economic productivity (Othman, 2003). Lack of regulations in developing countries affects the international food trade. The imposition of bans on food export results in extreme economic losses for exporting countries.

In recent years, many developing countries have participated in food export. However, access to world trading markets is dependent on developing countries' ability to meet regulatory requirements of the importing countries (Gillson and Fouad, 2014). Developing countries must have long-term food safety solutions to remain competitive and to gain the trust and confidence of consumers. Developing countries can suffer financial losses and damage their reputation in the world markets if their products do not meet safety requirements. In 1999, there was an international spread of recycled fat used in animal feeds contaminated with dioxin from a single source in Belgium to every

continent within weeks. Belgium's reputation in the global food trading markets suffered dramatically for many years after the problem was solved (WHO, 1980). The U.S. and the European Union provide yearly reports regarding import detentions and refusals. Developing countries dominate these reports, with Vietnam, Thailand, and Indonesia appearing most frequently (Unnevehr, 2005).

Food safety has received increased attention as an important public health issue in developing countries (Unnevehr, 2015). Food safety risks contribute to the burden of illness in developing countries. For example, foodborne pathogens are an important cause of diarrheal disease, which is estimated to cause 2.2 million deaths every year (WHO and FAO, 2014). Global trading regulations enforce sanitation, cold chain control, and hygienic conditions (Unnevehr and Gregory, 2006). However, microbial pathogens can enter the food supply at any point during processing and transportation and spread in commingled supply sources. The World Trade Organization (WTO) addresses the "weakest link", usually a developing country, by focusing on sanitation infrastructure and implementation of HACCP systems (Trade Capacity Building Database, 2006). Improving food safety in international trade will require policy and technical interventions (Schillhorn van Veen, 2005), including increasing awareness of food standards for exporters, importers, and policymakers, promoting food safety habits, increasing skills and competence, improving food safety and sanitary infrastructure, encouraging developing countries to play a more active role in the international bodies such as WTO, International Office for Epizootics (OIE), the North American Free Trade Agreement (NA- FTA), and Mercosur, adapting HACCP systems, and avoiding overlapping regulations that may be cost-prohibitive in small countries. Most

importantly, developing countries need strong participation in the international standard setting organizations (Schillhorn van Veen, 2005). Moreover, the economic perspective on food safety provides an important foundation for policy design (Unnevehr, 2015). The justification for government intervention to address food safety must arise from information collected and observed at retail markets. The economic benefits of improved food safety results in an increase in productivity and decreased loss of life from foodborne illnesses. Recent estimates suggest that foodborne illness results in between 14 and 152 billion dollars in lost productivity and life in the U.S. (Hoffmann et al., 2012). It is clear that the most important linkage between food safety and food security is through the reduction of hazards and foodborne illness by understanding the foodborne risks for countries with various economic statuses.

Development of Food Safety Programs

Much of the early HACCP development was conducted in the U.S. (Ropkins and Beck, 2000). The Pillsbury Company first discovered weaknesses in the microbiological quality control systems of food production when attempting to fulfill contracts with the U.S. Army and National Aeronautics and Space Administration (NASA) in the 1960's. NASA did not want to risk astronauts becoming ill during a space mission, thereby requiring very stringent microbiological acceptance criteria, with 100% product testing to assure that a food product was safe to consume (Sperber, 2005). At that time, quality control systems only tested the safety of food products at the end of production, resulting in costly and inaccurate results.

After a serious *Salmonella* and *Clostridium botulinum* incident occurred post World War II, the U.S. government could not guarantee food safety with end-product

inspections. Significant proportions of a foodstuff had to be sub-sampled for analysis to be representative of the entire food production chain. New food safety testing procedures were expensive, time consuming, difficult to interpret, and destructive to product quality. Thus, the HACCP concept was developed by The Pillsbury Company, the U.S. Army, and NASA.

The original Pillsbury HACCP procedure contained three components: (1) the identification and assessment of all hazards associated with the final foodstuff, (2) the identification of the steps or stages within food production at which these hazards may be controlled, reduced, or eliminated: the critical control points (CCPs), (3) the implementation of monitoring procedures at those CCPs (FDA, 1973). Therefore, in 1973, the U.S. Food and Drug Administration (FDA) conducted a pilot program of random HACCP audits of manufacturing sites of low-acid canned foodstuffs to develop Good Manufacturing Practices (GMP). Although this approach was ahead of its time, the procedures developed were criticized for focusing attention on control points that were already monitored, as opposed to identifying operations that were effective CCPs. The initial lack of interest in HACCP programs has been attributed to this pilot program and the failure of other early attempts at implementation (Bernard, 1998; Ropkins and Beck, 2000). The food industry attention to HACCP principles generally remained insignificant until they were endorsed by the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) in the 1980's (Ropkins and Beck, 2000). HACCP was intended for use by individual food companies, such as food producers, manufacturers, distributers, and retailers, as a procedure for the development of unique

safety assurance procedures to meet their individual needs. Sources such as the International Commission on Microbiological Specifications for Foods (WHO, 1980), the NACMF (Buchanan, 1997), the Codex Alimentarius Commission (WHO, 1963), and the International Life Science Institute (International Life Sciences Institute, 1993) all recommended very similar implementations of the seven basic HACCP principles: (1) conduct hazard analysis, considering all ingredients, processing steps, handling procedures and other activities involved in a foodstuff's production, (2) identify CCPs, (3) define critical limits for ensuring the control of each CCP, (4) establish monitoring procedures to determine if critical limits have been exceeded and define procedures for maintaining control, (5) define corrective actions to be taken if control is lost, (6) establish effective documentation and record keeping procedures for developed HACCP procedure, and (7) establish verification procedures for routinely assessing the effectiveness of the HACCP procedure, once implemented.

The harvest of livestock and the subsequent processing of raw meat products from livestock must consistently produce safe meat products for public consumption. However, history has shown that bacterial pathogens will evade even the best efforts by the industry, government, and consumers (Huffman, 2002). When an animal is slaughtered, bacteria may contact carcasses throughout the process. External surfaces of carcasses are exposed to potential sources of contamination such as fecal materials, paunch contents, and the hide (Huffman, 2002). Additional sources of cross-contamination are processing tools, equipment, structural components of facility, human contact, and carcass-to-carcass contact. Most microorganisms that are transferred to carcass surfaces, although undesirable, are non-pathogenic (Huffman, 2002; Institute of

Food Technologist, 2002). However, they cause meat spoilage. These spoilage bacteria are *Pseudomonas, Acinetobacter/Moraxella, Aeromonas, Alteromonas putrefaciens, Lactobacillus*, and *Brochothrix thermosphacta*. Pathogenic bacteria are *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes, Campylobacter, Clostridium botulinum, Clostridium perfringens, Staphylococcus aureus, Aeromonas hydrophila,* and *Bacillus cereus* (Huffman, 2002). Although meat processors have put forward their best effort, contamination is unavoidable in a production environment. Therefore, recent research has focused on interventions for live animals. On-farm technologies such as feeding probiotics to reduce shedding of acid-resistant *E. coli* are being researched. Such research will not only decrease bacterial shedding in packing plants, but also decrease food and water contamination. Water has shown to be a primary reservoir of *E. coli* O157:H7 in pre-harvest environments (LeJeune et al., 2001; Huffman, 2002). *E. coli* O157:H7 can survive up to 245 days in the sediment of a simulated water trough (LeJeune et al., 2001).

Carcass decontamination step should be a part of a slaughter HACCP programs. Physical interventions play a vital role in the decontamination of carcasses since they do not leave chemical residues and do not affect meat quality attributes and nutritional composition (Chen et al., 2012). Physical interventions can be applied throughout all processing stages of meat production such as pre-slaughter (animal washing), slaughter (trimming and hot-water washing), processing (steam pasteurization, refrigeration, super-chilling), and post-packaging (irradiation and high-pressure processing; Chen et al., 2012). Steam pasteurization is a fast, cost-effective method, which is suitable for almost any meat processing plant. Steam pasteurization is a 3-step process, including water

removal, steam to face temperature of 85 – 90°C, and rapid chilling. Nutsch et al. (1997) have reported that steam pasteurization is capable of reducing total aerobic bacterial counts on carcasses by 1.5 logs from initial levels and virtually eliminating coliforms on carcasses. Although this technology is favorable in the meat industry, there are notable disadvantages if non-uniform temperature is applied. McCann et al. (2006) reported a cooked appearance after prolonged treatment, which could result in improper cooking of the food product.

Irradiation is one of the most efficient physical preservation techniques (Wilcock et al., 2004; Loretz et al., 2011; Mukhopadhyay and Ramaswamy, 2012; González-Fandos and Herrera, 2013). Irradiation exposes meat to an ionizing radiation source that targets water molecules, and produces hydroxyl radicals. It is a highly oxidizing agent and can form stable products with large molecules and compounds (González-Fandos and Herrera, 2013). This process is primarily used to control illness-causing microorganisms and the dose is strictly regulated by the USDA and FSIS. An irradiation dose of up to 4.5 kGy for refrigerated red meat, up to 7 kGy for frozen meat, and up to 3 kGy for poultry is permitted in the United States to control pathogens (Loretz et al., 2011; Chen et al., 2012; Baer et al., 2013). However, only "dried aromatic herbs, spices, and vegetable seasonings" is can be treated with irradiation within the EU. Furthermore, although irradiation is the one of the most effective antimicrobial interventions, consumer acceptability is the limiting factor (Chen et al., 2012).

Today, many food producers are using multi-hurdle technology, which combes various interventions that alone are insufficient at preventing growth of spoilage and pathogenic bacteria, but are very effective when used in combination (Leistner, 2000;

Beales, 2004; Thévenot et al., 2006; Havelaar et al., 2010; Buncic and Sofos, 2012; Mani-López et al., 2012). There are increasing demands from the meat industry for more advanced alternative technologies to meet safety requirements and meet consumer expectations.

Food safety development in developed countries

After the 1970's, HACCP plans were more widely implemented in the United States because of the FDA's active encouragement within the food manufacturing sector. The FDA considers HACCP highly comprehensive approach to food safety because it accounts for safety risks in the whole food supply chain. More comprehensive HACCP regulations were subsequently developed and introduced into U.S. law. The legal requirements for HACCP compliance in the U.S. food industry have changed the way by which HACCP was implemented. Previously implemented on a voluntary basis, under which individual companies identified their own safety requirements, HACCP systems are currently mandatory, requiring food companies to adhere to governmental regulations (Bernard, 1998). HACCP implementation in small to medium size companies has shown difficulties because they lack financial resources, knowledge, and access to expertise (Aruoma, 2006).

In the EU, individual countries have their own legal structures for food safety legislation, surveillance, and assurance (Ropkins and Beck, 2000). Some of these countries began developing HACCP independently of the EU. Consequently, the European Commission decided to develop a systematic approach to HACCP for adoption throughout the EU. As a result, an international training exchange program between the United Kingdom, France, Denmark, Spain, Sweden, Portugal, Belgium, The Netherlands,

Ireland, and Greece were established. The EU subsequently produced a series of directives for incorporation into the legal systems of all member states. Three, 'vertical', directives were developed for specific foodstuffs, meat, and milk products, which required commercial food producers to: (1) identify CCPs in their individual manufacturing procedures, (2) establish and implement methods for monitoring and checking such CCPs, (3) collect samples for analysis in an approved laboratory, (4) maintain a written record of these procedures and subsequent data with a view for submission to relevant authorities and their representative inspectors. Adoption of these directives by EU member states has varied widely because of differences in the level of compatibility of directives with individual production needs.

In other developed countries such as Australia and New Zealand, early interest in HACCP development was much greater and more focused on exportation. However, there was some degree of inconsistency between food safety assurance schemes developed for export compared to domestic food markets. By the mid-1990's, some HACCP-based systems were being employed in Australia, such as the Australian Quarantine and Inspection Service (AQIS), Codex Alimentarius (WHO, 1963), International Commission on Microbiological Specifications for Food (WHO, 1980), and the National Advisory Committee on Microbiological Criteria for Foods (Buchanan, 1997). In order to standardize food safety, the Australian and New Zealand Food Authority (ANZFA) endorsed the principles of HACCP. The ANZFA developed a template that an individual food company could modify to employ within their own operation, which is summarized as follows: (1) prerequisite program, (2) the identification of the HACCP procedure scope, and (3) HACCP development.

Developed countries such as the United States, the countries inEuropean Union, and Australia are continuously improving and researching new technologies and interventions for the food industry. Consumers in developed countries have a desire to be educated about their food system from farm to fork. Consumers in developed countries have an increased willingness to pay for products that are perceived healthier such as organic foods, GMO-free (Bourn and Prescott, 2002) although there is no evidence of difference in nutritional composition compared with conventionally produced foods. Many consumers are aware of possible food contamination at home through education programs on proper handling, cooking methods, and cooking temperatures, as well as home food safety interventions.

Development of food safety in developing countries

Application of HACCP programs in developing countries has been widely recommended (WHO, 1963; WHO, 1980; Schillhorn van Veen, 2005). A number of limitations and problems associated with HACCP implementation in developing countries are similar to those in small to medium businesses in the developed countries. Some are also related to cultural or language differences (Ropkins and Beck, 2000). The Association of Southeast Asian Nations (ASEAN) is involved in food safety for the region. The ASEAN Expert Group on Food Safety provides the oversight and coordination of food safety in Southeast Asia (Othman, 2003). Ten program areas have been identified for improvement such as legislation, laboratory facilities, monitoring and surveillance, implementations of food safety systems, food inspection and certification, education and training, information sharing, research and development, international participation, and consumer participation and empowerment. Throughout the region,

multiple countries have been appointed to govern individual programs (Othman, 2003). The experts, however, found difficulties in language barriers and cultural differences among countries when implementing and enforcing region-wide protocols. A recent study of the implementation of HACCP in Thailand identified a number of constraints including education and training, availability of native language HACCP documents, and availability of hazard information (Minami et al., 2010). In most developing countries, the food industries lack the necessary scientific information such as, national food poisoning statistics or national foodborne disease databases to develop reliable hazard assessments. Therefore, many food industries in Southeast Asia cannot establish parameters at critical control points for their HACCP system. Moreover, the commitment to food safety has not been fully integrated into the cultures of many food-producing establishments.

Rapid urbanization and rural reform in developing countries have changed food demand and supply (Schillhorn van Veen, 2005). The main issue for many developing countries continues to be food security, meaning affordability. Food affordability is associated with food safety, which is not comprehended by developing countries in Southeast Asia. There is not an awareness of consequences caused by contaminated food, which is detrimental to the nation's health status and economic development. There is a lack of urgency to investigate or research food safety, partially because there are not cost-effective methods to identify specific food safety problems and these countries do not have financial resources for extensive investigation (Othman, 2003). Laboratories set up by ASEAN compete against each other for limited resources, thus, discouraging collaboration between countries and agencies. Exposure to foodborne

pathogens is common but epidemics are rare; therefore, foodborne diseases have not been a high priority in public health.

Investment in food safety infrastructure (slaughterhouses, quarantine facilities, laboratories) and skills development is low; except when large epidemics occur or when countries were major exporters. Participation in global trade requires that all countries follow international guidelines and must consider major investments in food safety and production monitoring. In most cases, the improvement of inspection or chain control systems are only applied to specific export products from large companies, but not in domestic markets. Many food companies have developed their own quality and safety standards for their export operations within country (Schillhorn van Veen, 2005). The major challenge to developing countries is establishing food safety guidelines that are applicable to local cultures. Food safety and risk analysis are largely in the realm of the consumer. Consumers handle the risk by careful buying, proper food preparation, and an acquired tolerance to certain pathogens (Schillhorn van Veen, 2005).

Bacterial Pathogens in Meat and Poultry

Highly publicized outbreaks of foodborne diseases in the U.S. caused by pathogenic bacteria, such as the *E. coli* outbreak in 2014 in ground beef from the Wolverine Packing Company, *Salmonella* outbreak in 2015 in pork products from Kapowsin Meats and live poultry from backyard flocks, and the *Listeria* outbreak in Blue Bell Ice Cream, have brought meat safety and food security to the forefront of societal concerns (CDC, 2015a; CDC, 2015b). Such challenges will continue and in some cases may become intensified in the future. Major pathogens of concern that have caused recalls of fresh meat product recalls include *E. coli* O157:H7 and *Salmonella.* In 2012,

the Centers for Disease Control listed *Salmonella* infections as the number one cause of death in the U.S. (CDC, 2015c). Moreover, *L. monocytogenes* is the pathogen of concern in ready-to-eat meat and poultry products because of the refrigerated storage environment that allows growth of the organism (Malley et al., 2015).

Efforts to control pathogens associated with meat will continue as a major focus in the future. Important issues that contribute to pathogen control and meat safety are animal health and welfare, animal identification, traceability and recall activities, application of antimicrobial interventions, and novel processing technologies. There is also a need for development of improved and rapid pathogen detection methods for laboratory and field applications. Such advances will assist in identifying pathogen sources for interventions and verification of critical control points in HACCP programs. It is important to recognize that management of meat safety risks should be based on an integrated effort and approach that applies to all sectors, including producers, processors, distributors, packers, retailers, food service workers, and consumers. However, microbial testing should not be a routine method in HACCP monitoring or a final step in assuring product safety (J. N. Sofos, 2008). Moreover, most foodborne illnesses are caused by mishandling of foods by the consumers. Thus, consumer education and good management practices should be targeted to improve meat safety (J. N. Sofos, 2008; O'Bryan et al., 2014; Proietti et al., 2014).

Salmonella

Salmonella spp. infections lead to high morbidity rates not only in developing countries but also in industrialized countries. *Salmonella* spp. are a group of gram-negative, non-spore-forming, rod-shaped bacteria belonging to the *Enterobacteriacae*

family (Akyala and Alsam, 2015). The CDC recognizes the genus *Salmonella* to contain two species, *Salmonella enterica* and *Salmonella bongori* (Sánchez-Vargas et al., 2011). *Salmonella enterica* includes more than 2500 serotypes of six subspecies (Mayrhofer et al., 2004; Coburn et al., 2006) and is recognized as one of the most common causes of bacterial foodborne illness worldwide (Mayrhofer et al., 2004), especially in Southeast Asia (Ta et al., 2012). The majority of documented *Salmonella* illnesses in the U.S. are attributed to foodborne contamination (CDC, 2011). Scallan et al. (2011) reported that 11% of foodborne illness in the U.S. was attributed to *Salmonella* and that 35% of hospitalizations and 28% of deaths from foodborne pathogens involved *Salmonella*. Meat-producing livestock, including poultry, pigs, and cattle, can be carriers of *Salmonella* and can shed the pathogen through feces without any extrinsic symptoms, which leads to further spread in the production chain (Buncic and Sofos, 2012). Control of *Salmonella* in the production chain can reduce contamination in final products (Schmidt et al., 2012). Contamination of *Salmonella* at retail causes a great risk to consumers; therefore, retail vendors and consumers should be educated in food safety principles, proper meat cookery, personal hygiene, and sanitation of processing equipment (J. N. Sofos, 2008)

Infection of *Salmonella*

Salmonella can survive remarkably well by using its invasive techniques and its defense mechanisms. Infection begins with ingestion of contaminated food or water so that *Salmonella* reaches intestinal epithelium and triggers gastrointestinal disease. *Salmonella*, e.g., *S. typhimurium*, overcomes the acidity of the stomach by activating an acid tolerance response (ATR) that provides an inducible pH-homeostatic function to

maintain the intracellular pH at values greater than those of the extracellular environment (Foster and Hall, 1991; Fàbrega and Vila, 2013). After entering the small intestines, *Salmonella* must reach and pass through the intestinal mucus layer before encountering and adhering to intestinal epithelial cells. In mice, salmonellae appear to preferentially adhere to and enter the M-cells of the Peyer's patches (PPs) in the intestinal epithelium, although invasion of normally non-phagocytic enterocytes can also occur (Takeuchi, 1967; Jones et al., 1994; Fàbrega and Vila, 2013). Shortly after adhesion, the invasion process appears as a consequence of engaged host cells, signaling pathways leading to profound cytoskeletal rearrangements (Finlay et al., 1991; Francis et al., 1992; Fàbrega and Vila, 2013). These internal modifications disrupt the normal epithelial brush border and induce the subsequent formation of membrane ruffles that engulf adherent bacteria in large vesicles called *Salmonella*-Containing Vacuoles (SCVs; Finlay and Falkow, 1988; Francis et al., 1993; Garcia-del Portillo and Finlay, 1994; Fàbrega and Vila, 2013). *Salmonella* is an excellent intracellular pathogen, whose abilities to colonize the host are extremely versatile. Its genome includes several virulence systems, including genes required for motility and chemotaxis, adhesion, invasion, replication, and survival within host cells, as well as biofilm formation, which cover the whole pathogenic process from the intestinal stage to systemic dissemination. As a result, *Salmonella* evolves to a complex state of interactions with the human body, in which a large number of effectors trigger specific actions in the host signaling pathways. These inputs require the pathogen to balance intracellular changes so that it can internalize and survive within the host. Moreover, coordination in the incredibly large set of bacterial virulence properties plays a critical role, since the effectors can show antagonistic functions. Therefore, this bacterial

balancing mechanism must be synchronized to facilitate expression of the appropriate virulence properties at the correct times and locations. Specific and global regulators organize this orchestra and mirror the complicated interactions between the invading *Salmonella* and the host (Fàbrega and Vila, 2013).

Salmonella **in beef, pork, and chicken**

Puncture of the bowel and rumen during evisceration can lead to cross-contamination of *Salmonella* during processing (Galland, 1997). In addition, *Salmonella* is easily transferred to the carcass during hide removal (Galland, 1997). During slaughter, pathogens can be directly translocated onto the carcasses, thereby affecting the safety of the beef products (Dong et al., 2014). The current baseline study conducted by the USDA and FSIS, (2014) revealed that *Salmonella* prevalence in retail ground beef products in the U.S. was 1.6 %. In addition, Vipham et al, (2012) observed 0.66% baseline in whole muscle beef products, which is much lower than the 60% incidence level in Vietnam (Van et al., 2007a). Ground beef in the U.S. is made by grinding and mixing trimmings from various sources and has greater *Salmonella* incidence levels than whole muscle meat (Johnston, 2015).

Pork products that are sold in markets in developing countries are from various farms with varying pathogenic status and transported as whole carcasses to markets, thereby creating numerous opportunities for cross-contamination of *Salmonella*. Researchers have suggested that retail displays are the weakest links in the commercial cold chain (James and Bailey, 1990), adding to the concern that *Salmonella* may proliferate to dangerous numbers because of temperature abuse in display cases (Lo Fo Wong et al., 2002). However, compared to beef production, pork production has less

pathogen prevalence. In a USDA study in commercial slaughter facilities, 91% of pre-scald, 19.1% of pre-evisceration, and 3.7% of post-chill carcasses were contaminated with *Salmonella* (Schmidt et al., 2012). The reduction in prevalence as carcasses proceed through processing stages indicates that appropriate critical control points during slaughter will reduce *Salmonella* incidence (Schmidt et al., 2012; Baer et al., 2013). However, developing countries, such as Vietnam, lack these interventions.

Salmonella is isolated from raw poultry with greater prevalence than other meats (CDC, 2007) because of its survival in the intestinal tract of birds. Currently, many developing countries including Vietnam do not have a complete foodborne disease surveillance system to estimate the annual incidence of human salmonellosis (Ta et al., 2012). Poultry processing includes bleeding, scalding, defeathering, evisceration, washing, and chilling. The main differences in a poultry processing between developed countries and developing countries are that the poultry industries in developed countries employ rapid chilling and decontamination treatments (Belluco et al., 2016). To control *Salmonella* contamination at the retail level, the entire production process has to be evaluated to establish critical control points and the need for interventions such as antimicrobial application.

Listeria

Listeria epidemiology and listeriosis

Listeria spp. are gram-positive, non-spore forming, faculatively anaerobic rods, which grow between -0.4 to 50°C (Walker and Stringer, 1987; Junttila et al., 1988; Farber and Peterkin, 1991). The taxonomy of the genus *Listeria* includes the species *L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. ivanovii, L. gray, and L.*

murray (Farber and Peterkin, 1991; Low and Donachie, 1997). Only *L. monocytogenes* and *L. ivanovii* are pathogenic with *L. ivanovii* being strictly an animal pathogen (Cossart, 2007). *L. monocytogenes* is recognized as a foodborne pathogen that can be unknowingly present in the gastrointestinal tract of healthy humans (Cossart, 2007). The incubation time can be as long as three months, and the disease occurs mainly in immune-compromised individuals such as newborn babies, elderly, or pregnant women with suppression of their T-cell-mediated immunity (Farber and Peterkin, 1991; Cossart, 2007). However, the infection may also occur in people with no known predisposing factors (Swaminathan and Gerner-smidt, 2007). In the 1960s, Mackaness and colleagues were the first to report a mouse model in which *L. monocytogenes* resisted intracellular killing in macrophages. Although a primary infection by *Listeria* induced a protective cellular immune response, antibodies played no critical role in recovery from infection and protection against a secondary infection (Mackaness et al, 1962; Cossart et al, 2007). This response was rapid and sterilizing. Since these pioneering studies, *Listeria* has become one of the very few intracellular organisms used to study mechanisms underlying the induction and establishment of T-cell responses (Cossart, 2007; Zenewicz and Shen, 2007).

Human listeriosis can be caused by multiple serovars of *L. monocytogenes*; however, geographic differences in the global distribution of serotypes do exist (Farber and Peterkin, 1991). The incidence of listeriosis varies between 0.1 and 11.3 per 1,000,000 people in various countries (Swaminathan and Gerner-smidt, 2007). Most reported cases are life-threatening with either maternofetal listeriosis/neonatal listeriosis, blood stream infection, or meningoencephalitis (Swaminathan and Gerner-smidt, 2007).

Listeriosis has an average case-fatality rate of 20 to 30% despite adequate antimicrobial treatment (Swaminathan and Gerner-smidt, 2007). Maternofetal or neonatal listeriosis occur within the first week of life (early-onset neonatal listeriosis) and the fetus is thought to acquire the infection in utero through transplacental migration of organisms from the bloodstream of the mother. The mother usually experiences non-specific flu-like symptoms, whereas the fetus develops a systemic infection because its immune system is not sufficiently developed, which leads to fetal distress, death, or premature birth of a severely ill infant. The risk of death caused by listeriosis in an infant is inversely related to gestational age. If maternofetal listeriosis is diagnosed early, antimicrobial treatment applied to the mother will prevent the disease in the infant (Silver, 1998; Swaminathan and Gerner-smidt, 2007). However, this course of treatment rarely occurs because the diagnosis is usually missed because of non-specific nature of the symptoms. Listeriosis that occur in an infant more than a week after birth is called late-onset neonatal listeriosis. The route of transmission in this condition may be transplacental, as in early-onset disease, orally acquired during passage through a contaminated birth canal, or through contact with an external source (Silver, 1998; Swaminathan and Gerner-smidt, 2007). Other immunocompromising health conditions, such as HIV/AIDS, have been identified to increase the risk of listeriosis. The major defense of the body against listeriosis is cell-mediated immunity; therefore, people with T-cell dysfunction seem to be particularly prone to contracting the disease (Swaminathan and Gerner-smidt, 2007).

***Listeria* in beef, pork, and chicken**

Data on *Listeria* prevalence in beef and beef packing plants are minimal (Guerini et al., 2007). However, in hides of cattle, the pre-evisceration stage of beef carcasses, and retail raw ground beef, the prevalence of *Listeria* was reported at 77, 14.5, and 2.5% during sampling in U.S. facilities (Samadpour et al., 2006; Guerini et al., 2007). However, Guerini et al, (2007), also reported that post-intervention contamination on cattle hides were almost undetectable. A similar study in Malaysian wet markets on retail meat reported a *Listeria* incidence level of 25 to 50%. Moreover, a high incidence is not unusual because in Canada, a developed country, *L. monocytogenes* was found in 52% of raw ground beef (Bohaychuk et al., 2006). Fecal contamination during the slaughter process, vendor hygiene, and unsafe food processing, packaging, and handling could lead to an increase of *Listeria* in raw meat (Rahimi et al., 2012; Ismaiel et al., 2014; Stea et al., 2015). These data indicate that *Listeria* prevalence can be sporadic and although less detected, it is an emerging pathogen in fresh meat.

Columbian and Japanese researchers observed a 33.9 and 35.7% prevalence, respectively, in raw pork products (Ochiai et al., 2010; Gamboa-Marín et al., 2012). In contrast, developed countries such as, the U. S., Finland, Bulgaria, Greece, and Canada show much lower contamination ranging from 0.15 – 24% (Wesley and Ashton, 1991; Samelis and Metaxopoulos, 1999; Bohaychuk et al., 2006; Karkolev, 2009; Hellstrom et al., 2010). The implementation of HACCP regulations throughout the production chain could be attributed to the decreased prevalence in these countries (Gamboa-Marín et al., 2012) The variation in data can be shown in other studies conducted in developed and developing countries, such as Australia, France, Ireland, Japan, Serbia, and the U.K.,

which ranged from 30.0 to 90.0% prevalence, respectively (Ibrahim and Mac Rae, 1991; Ryu et al., 1992; MacGowan et al., 1994; Sheridan, 1998; Thévenot et al., 2006; Dimic et al., 2010). The variation in prevalence between developed countries that have regulations in place confirms that there is a problem throughout the production chain that needs to be identified and corrected. *Listeria* prevalence in raw, whole muscle, pork is very dangerous because many products are further processed to deli products, where *Listeria* can survive and flourish at refrigeration temperature.

Listeria has been isolated from raw poultry in many countries (Miettinen et al., 2001). However, prevalence is greatly varied. Pini and Gilbert (1988) observed 60% prevalence of *Listeria* in raw chickens in the U. K., whereas, Bailey et al. (1989) only reported 23% in the U.S. Moreover, Loncarevic et al., (1994) reported 0 to 64% prevalence of *Listeria* in raw broiler meat. The widespread occurrence of *Listeria* spp. in the environment can result in the contamination of poultry carcasses in processing facilities (Chiarini et al., 2009). *Listeria* can survive in the environment of food processing plants for extended time such as the floor drains (Lunden et al., 2003; Loura et al., 2005; Berrang et al., 2013). Studies have indicated that the improper cleaning and disinfecting of processing equipment in poultry facilities can lead to cross-contamination (Loura et al., 2005; Adeyanju and Ishola, 2014) during cutting and further processing (Uytttendaele et al., 1999). These authors documented an increase in *Listeria* incidence from 41.3% in whole chicken carcasses to 46.7% in parts and 61.0% in retail products. Furthermore, additional handling of carcasses after chilling can increase possibility of contamination (Genigeorgis et al., 1989). Poultry products are recommended to be cooked to 74°C (FSIS, 2014) and are assumed of low risk for *Listeria.* However,

opportunities for cross-contamination to occur in other foods in consumers' food preparation areas must be considered (Loura et al., 2005; Voidarou et al., 2011). Similar to *Salmonella*, implementing HACCP plans, promoting vendor hygiene, and educating processors and vendors on the importance of food safety are essential to reduce the incidence of *Listeria*.

Escherichia coli

***Escherichia coli* pathogenicity**

Escherichia coli (*E. coli)* O157:H7 is a member of the Enterhaemorrhagic group of *E.coli* (EHEC) and was first implicated as an infectious disease in the early 1980s (Riley et al., 1983). The symptoms of infection include bloody diarrhea and severe abdominal pain. Hemolytic uremic syndrome (HUS), a cause of acute renal failure, may be a complication of the illness, and neurological problems in the form of thrombotic thrombocytopenic purpura (TTP) may occur (Duffy et al., 2006). Immuno-compromised patients, including young children and the elderly, are at particular risk of developing HUS. The time from exposure to onset of symptoms ranges from 1 to 14 days (Coia, 1998). However, with complications the illness may last many months and lead to permanent damage or even death. Despite clinicians' and microbiologists' familiarity with *Escherichia coli*, there is a general underappreciation of the enormous differences among different groups of *E. coli*, and the clinical implications of these differences. From a genetic and clinical perspective, *E. coli* that is biologically important to humans can be broadly categorized as (1) commensal *E. coli* (i.e. harmless intestinal colonizers), (2) intestinal pathogenic E. coli (i.e. enteric or diarrheagenic strains), and (3) extra-intestinal pathogenic *E. coli* (ExPEC; Johnson and Russo, 2005). Pathogenicity is related

to the ability of the organism to adhere to and colonize the human large intestinal epithelial tissue, forming attachment and lesions, and the production of verocytotoxins (Duffy et al., 2006). All six categories of diarrheagenic *E. coli* carry at least one virulence-related property upon a plasmid. *E. coli* follows a four-step infection: (1) colonization of a mucosal site, (2) evasion of host defenses, (3) multiplication, and (4) host damage (Salayers and Whitt, 2002). Once dismissed as a harmless inhabitant of the intestinal tract, *E. coli* is now recognized as a pathogenic species with remarkable versatility in its ability to cause disease in humans and animals.

***Escherichia coli* in beef, pork, and chicken**

E. coli O157:H7 first emerged as a food borne pathogen in the mid-1980s (Duffy et al., 2006). *E. coli* has been linked to many cases of food poisoning across the world (Duffy et al., 2006). Sources and reservoirs of *E. coli* O157 including beef, lamb, lettuce, sprouts, fruit juices, vegetables, raw milk, and water have been implicated as vehicles of transmission (Bell et al., 1998 ; Hilborn et al., 2000; Cowden et al., 2001; Duffy et al., 2006). Direct contact with a human carrier (O'Donnell et al., 2002), animals carrying the organism, or fecally contaminated mud (Crampin et al., 1999) are recognized as sources of infection through cross-contamination (Duffy et al., 2006). Improper handling of unpackaged meat or leakage from wrapped packages may also lead to cross-contamination. During distribution, storage, and retail display, failure to maintain temperatures (4°C) may allow for the growth of the *E. coli*. Studies on beef and beef products in retail establishments in various countries have shown that *E. coli* O157:H7 was present in 0.43 to 5.22% of beef products. Jones et al. (2014) reported that vacuum-packaged beef in Canada had 1.1 to 2.5 log CFU/100 cm^2 of *E. coli* and that beef from

retail establishments had a maximum of 3.1 log CFU/100 cm^2 of coliforms. It is important to note that most beef packing plants in the U.S. and other developed countries employ antimicrobial interventions (Pohlman et al., 2002) during lairage and carcass dressing (Buncic and Sofos, 2012). Although carcass decontamination interventions are important for microbiological safety and quality of beef (Huffman, 2002), these interventions, with the exception of washing, are unavailable in developing countries. Moreover, most small beef processing facilities in developing countries, including Vietnam, are not required to follow HACCP plans or adhere to any regulations for microbial decontamination.

The composition of the bacterial flora in pork in retail outlets is caused by initial bacterial contamination and bacterial colonization occurring during slaughter, processing, and distribution (Berends et al., 1998). Approximately 6.7 and 7.2 log CFU/g of *E. coli* in minced pork in butcher's shops and supermarkets in Greece, respectively (Andritsos et al., 2012). Moreover, in Nigeria, a developing country, 5.6 log CFU/g for *E. coli* were found in pork retail markets, other than supermarkets, that can be attributed to the increased bacterial count during the slaughter process or from water contamination (Adesiji et al., 2011). *E. coli* is not harbored in the intestines of pork and the interventions at critical control points such as, scalding and removal of hair, are very effective at decreasing contamination. However, *E. coli* is a common adulterant in pork products in developed countries, which could be caused by the lack of interventions during processing and hygiene of workers.

E. coli counts on poultry carcasses have been increasing and routinely linked with inadequate or unhygienic processing, improper handling, and insufficient storage

conditions (Whyte et al., 2004; Williams et al., 2015). Good management practices should be used during slaughter and processing to minimize bacterial contamination. Carcass cleanliness is very important to identify critical control points and correctly manage the production process (Belluco et al., 2016). Poultry production includes bleeding, scalding, defeathering, evisceration, washing, and chilling. Chilling and decontamination treatments are also important for decontamination in poultry processing (Belluco et al., 2016). Allen et al, (2000) observed a reduction in *E. coli* when water chilling was used. However, water chilling can also be a primary vehicle for foodborne pathogens (Demirok et al., 2013). Extensive bird-to-bird contact during water chilling can result in cross-contamination (Bilgili et al., 2002).

Conclusion

Developed countries have established laws, regulations, and various interventions to combat the recurring and persistent pathogens that can cause foodborne illnesses. The antimicrobial interventions and novel technologies are extensively researched and widely available to the meat industry in developed countries to reduce bacteria prevalence and counts. However, developing countries have not established microbiological safety and quality baselines. Moreover, the industries in developing countries do not have financial and technological capabilities to meet the modern requirements for microbiological safety and quality. A comprehensive baseline study of pathogen prevalence and microbial loads on meat and poultry products is needed. Also, development of regulatory guidelines for food safety that are applicable to local meat merchandising cultures is necessary in developing countries. Lastly, food safety education programs should be implemented for all stakeholders involved in the meat industries. Food safety is a fundamental step

towards food security. Therefore, great efforts, especially in research, must be made by developing countries, such as Vietnam. **Our overall objective was to generate baseline data of bacterial counts and prevalence of pathogens in beef, pork, and chicken in Vietnam**.

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CHAPTER II

INFLUENCE OF MARKET SETTINGS AND TIME OF PURCHASE ON COUNTS OF AEROBIC BACTERIA, *ESCHERICHIA COLI*, AND COLIFORM, AND PREVALENCE OF *SALMONELLA* AND *LISTERIA* IN BEEF IN VIETNAM.

Abstract

The objective of this study was to determine the influence of market type and sampling time on *Salmonella* and *Listeria* prevalence and microbiological quality of 180 beef samples collected in 6 supermarkets (SM), 6 indoor markets (IM), and 6 open markets (OM) at opening (T0) and 4 h after the opening (T4) in Vietnam. *Salmonella* prevalence was greater than 50% and was influenced by both market type ($P = 0.082$) and sampling time ($P = 0.019$). *Listeria* prevalence was greater than 90% and did not differ among markets and sampling times ($P > 0.773$). Beef samples had more than 11, 7, and 9 logs of aerobic bacteria, *E. coli*, and coliforms, respectively. In SM, *E. coli* was greater at T0, whereas it was greater at T4 in IM ($P_{market\ type \times sampling\ time} = 0.029$). Covered meat displays were used by 63.3, 33.0, and 0.0% of SM, IM, and OM vendors at T0 and by 100.0, 0.0, and 13.0% of SM, IM, and OM vendors at T4, respectively. Only at T4 when 100.0% of SM vendors used refrigeration. Gloves and hairnets were used only by SM vendors at T4. Hot water was used only by 16.7% SM vendors at T4. In addition, only 29.2, 2.5, and 8.3% of SM, IM, and OM vendors, respectively, used cold water for

cleaning purposes. These results highlighted substantial bacterial contamination in beef at retail in Vietnam, which requires immediate intervention and education so that the public health can be protected.

Keywords: Beef*, Salmonella, Listeria, Escherichia coli*, coliforms, retail, developing countries, safety, quality, Vietnam

Introduction

Despite global efforts to combat foodborne pathogens, the societal consequences of foodborne illnesses is only available to industrialized countries (Chaves et al., 2015). In developing countries, this information gap has hindered epidemiological investigations and limited approaches towards public health interventions that could minimize the number of cases of foodborne illness (Kaferstein, 2003; Chaves et al., 2015). Poor hygienic conditions of vendors, lack of clean water, and poorly designed and regulated packing plants in developing countries subject meats to a greater risk of contamination. Many markets and vendors in developing countries do not use refrigeration and expose fresh meat and poultry products to pathogenic contamination by practicing unsafe food processing, packaging, handling, and cooking. All of these factors pose serious challenges to food security (Kinsey, 2005).

Beef has great nutritive value with balanced composition of essential nutrients (Maharjan et al., 2006; Mcneill, 2007; USDA, 2014a). The Nutrition Collaborative Research Support Program (NCRSP) reported positive associations between meat intake and physical growth, cognitive function, school performance, physical activity, and social behaviors (Mcneill, 2007). Unfortunately, because of its nutritional composition, beef is a suitable medium for the growth of various microorganisms and a reservoir through which

foodborne illnesses may spread (K. Milios et al., 2014). Although the interior of beef carcasses is considered to be free of bacteria, cross-contamination may occur on the carcass surface during hide removal (Rivera-betancourt et al., 2006) and evisceration or through contact with equipment, humans, and other carcasses (Huffman, 2002; Maharjan et al., 2006). Moreover, whenever beef primals or subprimals are cut, additional surfaces are exposed and beef becomes more susceptible to contamination (Maharjan et al., 2006).

Animals are one of the major sources of the major foodborne bacterial pathogens, *E. coli* and *Salmonella* (Rivera-betancourt et al., 2006). In addition, *Listeria* is also a confirmed pathogen in beef carcasses (Korsak et al., 1998; Rivera-betancourt et al., 2006). Recently, *Listeria monocytogenes* has been identified as a foodborne pathogen with an increased lethality in raw beef products (Rivera-betancourt et al., 2006*)*. These pathogens are associated with the hide, the intestinal tract of healthy animals, and the environment (Rebhun, 1987; Galland, 1997; Brown et al., 2000; Elder et al., 2000; Bell, 2002; Rivera-betancourt et al., 2006; J N Sofos, 2008). *Salmonella* and *Listeria* cause 35 and 19% of foodborne illnesses in the U.S., respectively (Scallan et al., 2011). In addition, *Salmonella* caused over one million illnesses with 19000 hospitalizations and 380 deaths (CDC, 2015a) and *Listeria* was associated with approximately 1600 illnesses with 260 deaths in 2014 (CDC, 2015a; CDC, 2015b).

In developed countries, many studies have focused on the prevalence of *Salmonella*, *Listeria*, and *E. coli* at the beef production stage (Capita et al., 2004; Hussein and Sakuma, 2005; Arthur et al., 2010; Meyer et al., 2011; Schneider et al., 2011; Martínez-Chávez et al., 2015). The focus of *Listeria* contamination has been associated with ready-to-eat meat products because *Listeria monocytogenes* is a zero-tolerance

adulterant in these products (FSIS, 2014). However, evidence indicates that it is possible for *Listeria* contamination to occur in fresh beef, although the risk is relatively low at feedlots (Mohammed et al., 2010). The meat industry in developed countries minimizes the amount of processing at retail stores because most retail subprimals and cuts are provided by the packing plants or large purveyors. Therefore, there have been fewer studies pertaining to bacterial pathogens in the retail setting (Vipham et al., 2012; Martínez-Chávez et al., 2015) In addition, many studies have explored the use of indicator organisms such as *E. coli* to predict the potential presence of a pathogen on carcasses (Brown et al., 2000; Brown et al., 2002; K. Milios et al., 2014).

Meat is among the most nutritious foods in developing countries, especially for young children (Muir et al., 2010). Meat consumption increases with improved standards of living (J N Sofos, 2008); therefore, meat safety is increasingly important in developing countries. Foodborne illnesses mostly occur during processing and retail fabrication or because of inadequate cooking (McMeekin, 2007). Consumers in developing countries are accustomed to traditional fresh meat markets because of their loyalty to familiar vendors, perceived availability of fresher meat, and competitive prices through bargaining. Traditional markets pose serious safety risks to consumers because of the lack of refrigeration and exposure of meats to the open atmosphere (Trappey and Lai, 1997). Supermarkets store meat products in refrigerated display cases but still face safety challenges because they primarily sell meats from similar sources (Chamhuri and Batt, 2013). In developing countries such as Nepal, Vietnam, and China, most studies have focuses on the contamination of one microorganism on meat products (Maharjan et al., 2006; Van et al., 2007b; Yang et al., 2010). Similar to developed countries, multi-

pathogen data in the retail setting are lacking because it is difficult to account for many sampling variations and biases and to pinpoint the sources of contamination. However, it is important that a comprehensive retail study be conducted to establish a baseline of contamination so that further mapping and risk mitigation strategies can be elucidated. In developing countries, Vietnam in particular, and even in the developed countries, the influence of market setting, time of purchase, and meat merchandising has never been evaluated. Therefore, it was the objective of this study to investigate the prevalence of *Salmonella* and *Listeria*, microbiological quality, and vendors' practices in various beef markets at two sampling times in three regions of Vietnam.

Materials and Methods

Sample Collection

Ho Chi Minh City, Da Nang, Ha Noi, and their surrounding areas were selected to achieve adequate representation of regional variation in meat merchandising in Vietnam. The three types of markets, supermarkets (SM), indoor markets (IM), and open markets (OM), were classified by their infrastructure (Table 1). Within each market type, two of the most popular grocery markets were selected in each region, resulting in six markets per region. Domestically produced beef were purchased at two sampling times at each market after careful exploration of the distribution and purchase patterns of each market type. The opening time (T0) was the opening of individual markets, which varied from 5 A.M. (most open markets) to 8 A.M. (most supermarkets), and the closing time (T4) was 4 h after opening. Five 200-g beef *Longissimus* muscle samples were purchased aseptically and separately from various vendors in each market at each sampling time, resulting in 180 samples. Vendors were randomly selected for sampling. If a market had

less than five vendors, at least one vendor was sampled repeatedly in a rotating order so that samples from the same vendors were purchased separately and from different beef strip loins. There was no vendor randomization in the SM because each SM was the sole meat vendor. However, beef samples in the SM were purchased individually from different beef strip loins and by different purchasers. The randomization at T4 was performed in the same manner as at T0. The samples were placed separately in sterile Whirl-Pak® bags (Nasco, Fort Atkinson, WI) and the bags were sealed immediately after the meat surface temperature was recorded by a Fisher Scientific™ Traceable™ Infrared Thermometer Gun (Fisher Scientific, Waltham, MA). Samples were stored in an Igloo Super Tough Sportsman ice chest (Igloo, Katy, TX) with frozen ice packs.

Sample Preparation

Meat samples were transported in the ice chests back to a local university in each region. Samples were weighed and shaken for 60 s in 90 mL of Buffered Peptone Water broth (BPW; 25.5 g/L; 3M, St. Paul, MN), which was added to the Whirl-Pak® bags (Nasco, Fort Atkinson, WI; Vipham et al., 2012). Two sterile 15-mL polypropylene tubes (Greiner Bio-One, Monroe, NC) of BPW rinsate were collected and stored on ice for transportation to Ho Chi Minh City University of Technology for further analyses.

Microbiological Analysis

Except for sterile sampling bags, all apparatuses and solutions were autoclaved before microbiological analyses. Blank enrichment, isolation, and incubation of all solutions including sterile water were performed for all microbiological analyses.

Salmonella spp. were identified by using the Official Method of Analysis 2014.01 (AOAC International, 2014) with modifications for 3M™ Petrifilm™ Salmonella Express System (3M, St. Paul, MN). The previously collected BPW rinsate was shaken for 60 s and 2.5 mL of the rinsate was combined with 22.5 mL of Salmonella Enrichment Broth (3M, St. Paul, MN) in a sterile Whirl-Pak® bag (Nasco, Fort Atkinson, WI). The solution was incubated at 45°C for 24 h. After incubation, 1 mL of the solution was transferred into a 15-mL sterile polypropylene tube (Greiner Bio-One, Monroe, NC) containing 10 mL of Rappaport-Vassiliadis R10 Broth (RVR10; 3M, St. Paul, MN), which was then incubated at 41.5°C for 24 h. A single streak of 10 µL of RVR10 solution was made onto a hydrated 3M™ Petrifilm™ of the Salmonella Express System. The Petrifilm™ was incubated at 41.5°C for 24 h. *Salmonella* colonies were identified by a red color with yellow halo (3M, 2015a). Presumptive positive colonies were isolated, inoculated in Tryptic Soy Agar (3M, St. Paul, MN) slants, and stored under refrigeration.

Listeria spp. were detected according to the Official Method of Analysis 911.02 (AOAC International, 2002) using ALOA® medium (BioMerieux, St. Louis, MO) with modifications to the enrichment process. After being shaken for 60 s, 2.5 mL of BPW rinsate was combined with 22.5 mL of Demi-Fraser Listeria Enrichment Broth (3M, St. Paul, MN) in a sterile Whirl-Pak® bag (Nasco, Fort Atkinson, WI). The solution was incubated at 30°C for 24 h. A volume of 0.1 mL of the solution was subsequently spread onto an ALOA® agar petri dish. The dish was inverted and incubated at 37°C for 24 h. *Listeria* colonies were identified by a blue to green color with or without halo.

Presumptive positive colonies were isolated, inoculated in Tryptic Soy Agar (3M, St. Paul, MN) slants, and stored under refrigeration.

Aerobic Plate Count (APC), *E. coli ,* and coliforms analyses were performed according to the Official Method of Analysis 990.12 (APC; AOAC International, 2012) and 998.08 (*E. coli* and coliforms; AOAC International, 2008) with 3M™ Petrifilm™ Aerobic Count Plates and 3M™ Petrifilm™ *E. coli*/coliforms Plates instructions, respectively (3M, 2015b; 3M, 2015c). Original BPW rinsate (15 µL) was serially diluted (1:100) to a volume of 1.5 mL with sterile BPW broth in two 2-mL sterile polypropylene microcentrifuge tubes for either APC or *E. coli*/coliforms. One mL of each dilution was spread onto an APC Petrifilm™ or an *E. coli*/Coliform Petrifilm™. The Petrifilms™ were incubated with clear side up in a stack of 10 at 35°C for 24 h. Colony forming units (CFU) were counted according to the 3M interpretation guides (3M, 2015b; 3M, 2015c).

Market Characteristics

An observational data form was developed to collect data that were considered relevant to microbiological safety of fresh meat products. Outdoor temperature (ºC), relative humidity (%), meat surface temperature (ºC), type of retail display (display case, suspended by hook, or open counter), use of refrigeration, gloves and hairnets, cleaning of knife before cutting meat, and use of water for cleaning purposes (hot water or fresh cold water) were recorded for individual samples.

Calculation and Statistical Analysis

The prevalence of *Salmonella* and *Listeria* was reported as percentage of positive samples estimated by the statistical model. Aerobic Plate Count (APC), *E. coli,* and coliforms were reported as log CFU/g, calculated from CFU as follows:

$$\log \text{CFU/g} = \log \left(\frac{N}{V} \times DF \times V_0 \times \frac{1}{m} \right) \quad (1)$$

with N, V, DF, V_0, and m being number of colony forming units on a Petrifilm™, volume of a dilution spread onto a Petrifilm™ (1 mL), dilution factor, original volume of BPW rinsate (90 mL), and sample weight (g), respectively. Market characteristic data were recorded for each sample and reported as crude percentage without statistical analysis.

The prevalence of *Salmonella* and *Listeria* were analyzed as a 3 × 2 factorial arrangement in a randomized complete block design with region as block, market type (SM, IM, and OM) and sampling time (T0 and T4) as two factors, and a specific market at a specific sampling time as experimental unit (n = 6 per factorial combination). For APC, *E. coli*, and coliforms, the experimental unit was beef sample (n = 30 per factorial combination). The effects of market type and sampling time on pathogenic prevalence (%) and bacterial count (log CFU/g) were statistically analyzed by SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). Analysis of variance for binomially distributed data (prevalence) was performed through logistic regression, whereas that for normally distributed data (log CFU/g) was conducted through linear regression. A generalized linear mixed model was used for both analyses in the GLIMMIX procedure of SAS, with market type, sampling time, and their interaction being the fixed effects and region being the random effect. Means were separated by the protected t-test, using the LSMEANS

statement with the PDIFF option in the GLIMMIX procedure. Statistical significance was determined at $P \leq 0.10$.

Results and Discussion

Microbiological Quality

Beef in all markets had more than 11 log CFU/g of APC (Table 3). Many of the APC Petrifilm™ were too numerous to count (TNTC) at 10^{-6} dilution, because they contained a pink color in the entire growth area (3M, 2015b). These TNTC Petrifilms™ were estimated at 10^8 CFU. However, there were differences between the OM and SM ($P = 0.030$; Figure 1) and the two sampling times ($P = 0.054$; Figure 2). *E. coli* counts were greater than 7 log CFU/g and there was no market type or sampling time effect ($P = 0.380$ and 0.837, respectively; Table 3). However, the market type × sampling time interaction was different ($P = 0.029$). The IM had a 1.2-log increase ($P = 0.052$; Figure 3), whereas the SM had a 1.1-log decrease in *E. coli* from T0 to T4 ($P = 0.074$; Figure 3). *Coliforms*, excluding *E. coli*, was greater in the IM (10.29 log CFU/g) and OM (10.38 log CFU/g) than in the SM (9.43 log CFU/g; Figure 1; $P = 0.016$ and 0.009, respectively). Similarly, many *E. coli*/coliforms Petrifilms™ were TNTC for either *E. coli* or coliforms and were indicated by a purple (*E. coli)* or pink (coliforms) color in the entire growth area at the 10^{-6} dilution (3M, 2015c). These levels of contamination were much greater than those reported in most studies in the U.S. Arthur et al. (2004) reported 7.8 log of APC and 6.2 log of *Enterobacteriaceae* on the hide and only 1.4 log of APC and 0.4 log of *Enterobacteriaceae* on chilled beef carcasses. Jones et al. (2014) reported that vacuum-packaged beef in Canada had 1.1 to 2.5 log CFU/100 cm^2 of *E. coli* and that beef from retail establishments had a maximum of 3.1 log CFU/100 cm^2 of coliforms. It is

important to note that most beef packing plants in the U.S. and other developed countries employed various interventions (Pohlman et al., 2002) during lairage and carcass dressing (Buncic and Sofos, 2012). Lactic acid (Castillo et al., 2001), acetic acid, and chlorine sprays have been used as carcass decontamination treatments to decrease *Salmonella* counts by 1.3 to 5.1, 2.0 to 4.8, and 0.6 to 1.3 log CFU/cm^2 (Buncic and Sofos, 2012). Various studies have indicated that up to 4-log reduction can be achieved through carcass chilling (Buncic and Sofos, 2012). Although carcass decontamination interventions are important for microbiological safety and quality of beef (Huffman, 2002), these interventions, with the exception of washing, are unavailable in Vietnam. Moreover, most domestically produced beef in Vietnam is processed in small to very small processing facilities, where interventions are unavailable and microbiological evaluation is neither required nor regulated.

Indicator bacteria are widely used as a measure of hygienic conditions and microbiological quality of foods (Jordan et al., 2007). Indicator organisms such as aerobic bacteria, *E. coli,* and coliforms can be enumerated and quantified more inexpensively and easily than other bacterial pathogens (Jordan et al., 2007). *E. coli* and total coliform counts have been used in packing plants as indicator organisms (K. Milios et al., 2014). Arthur et al. (2004) reported correlations between APC, *Enterobacteriaceae*, and *E. coli* O157 loads on pre- and post-evisceration carcasses. Therefore, there are benefits of monitoring indicator organisms to evaluate the effectiveness of interventions or risk mitigation strategies. Moreover, indicator organisms are commonly indicative of specific pathogenic species. For example, Ghafir et al., (2008) reported both that *E. coli* and APC counts on beef carcasses were correlated

and that *E. coli* counts were greater on beef carcasses that were the origin of *Salmonella* contaminated beef samples. These authors suggested that *E. coli* count was a reliable index of *Salmonella* incidence in beef. *E. coli,* coliforms and *Enterobacteriaceae*, and APC are indicators of fecal contamination, environmental contamination, and overall hygienic conditions. Although the measures may be correlated, each can be indicative of different bacterial pathogens, which infers that multiple indicators should be used (Milios et al., 2014). A decrease in the population of indicators is generally assumed to correspond to a similar decrease in the population of pathogens (Brown et al., 2000), although there are no clear correlation between indicator organisms and the contamination of specific pathogens. It is generally accepted that pathogens occur less frequently and with lower counts than indicators (Milios et al., 2014).

Prevalence of *Salmonella*

Salmonella prevalence for each market at a specific sampling time was reported in Table 3. The average prevalence of *Salmonella* in SM, IM, and OM was 66.0, 71.0, and 50.0%, respectively (Figure 4). Across two sampling times, SM and IM had greater *Salmonella* incidence than OM ($P = 0.098$, $P = 0.037$; Figure 4). No difference was found between IM and SM ($P = 0.587$; Table 3; Figure 4). Across three market types, the *Salmonella* prevalence in beef was greater at T4 than at T0 (71.7 and 52.6%, respectively; $P = 0.019$; Figure 5). Puncture of the bowel and rumen during evisceration can lead to cross-contamination during processing (Galland, 1997). In addition, *Salmonella* is easily transferred to the carcass during hide removal (Galland, 1997). During the slaughter process, pathogens can be directly translocated onto the carcasses, thereby affecting the safety of the beef products (Dong et al., 2014). The *Salmonella*

prevalence in beef could be attributed to the tropical climate with increased temperature and humidity (greater than 26.3°C and 68.5%, respectively; Table 2) than most regions of the U.S., which might allow more growth of *Salmonella* on carcasses and increase the likelihood of cross-contamination onto the final retail products (Van et al., 2007b). A similar study screened retail meat products collected from various regions in China and observed greater *Salmonella* prevalence (44%) than the U.S. (6 to 35%; Yang et al., 2010). However, several factors must be considered when comparing *Salmonella* prevalence among countries (Yang et al., 2010), including origin, type of meat samples (ground or whole muscle), sampling seasons, plant sanitation, and collection methods. Baseline studies revealed that *Salmonella* prevalence in retail whole muscle beef products in the U.S. was at 0.66% (Vipham et al., 2012), which is much less than that in Vietnam. Ground beef in the U.S. is made by grinding and mixing trimmings from various sources and has similar *Salmonella* incidence levels to the whole muscle meat. The FSIS tested 2983 raw ground beef samples under the MT43 project (Risk-based Sampling for Raw Ground Beef) during the first quarter of 2015 and 0.9% (27 samples) were positive for *Salmonella* (USDA, 2015a). Although the *Salmonella* prevalence in beef in Vietnam was substantial in the current study, similar incidence (62%) was previously reported for raw beef in Ho Chi Minh City of Vietnam (Van et al., 2007b). It is important to note that the current study confirmed *Salmonella* prevalence in beef on a much larger scale throughout Vietnam in various market settings, including supermarkets. A Spearman rank correlation between *E. coli* count and *Salmonella* prevalence in this study was not different ($P = 0.628$). As mentioned previously, Gill and Baker (1998) suggested that such a correlation between count on carcasses and incidence

in meats existed. However, correlation on the same retail samples has not been reported. This may be because contamination on retail meats comes from various sources, including random cross-contamination.

Prevalence of *Listeria*

Market type and sampling time did not affect *Listeria* prevalence in beef across all three regions of Vietnam ($P > 0.773$; Table 3). The prevalence of *Listeria* was determined at 90.0, 100.0, and 93.6% for SM, IM, and OM, respectively (Figure 4) and at 92.5 and 99.9% for T0 and T4, respectively (Figure 5). *Listeria*, especially *L. monocytogenes*, is predominantly a safety concern for ready-to-eat meat products. The latest incidence prompted Shirk's Meat in New York to recall approximately 2478 pounds of ready-to-eat pork and beef products that might have been contaminated with *L. monocytogenes* (USDA, 2015b). The data on *Listeria* in beef and beef packing plants are minimal (Guerini et al., 2007). Rivera-Betancourt et al. (2004) reported a maximum of 14.6% *Listeria* prevalence in pre-evisceration beef carcasses at two geographically distant commercial beef packing plants in the U.S., which was decreased to 0.0 to 1.1% post-intervention. Approximately 3.5% (18 of 512 samples) incidence of *L. monocytogenes* was reported for retail raw ground beef in the state of Washington (Samadpour et al., 2006). Guerini et al. (2007) reported a consistently high prevalence (up to 77 to 92%) of *Listeria* on the hide of cows and bulls, but also reported that post-intervention contamination was almost undetectable, with the exception of a 19% incidence at one packing plant. Ibrahim (1991) conducted a similar study in Malaysian wet markets and reported a *Listeria* incidence level of 25 to 50%. Such an incidence is not unusual because even in Canada, a developed country, *L. monocytogenes* was found

in 52% of raw ground beef (Bohaychuk et al., 2006). Similarly, Yucel et al. (2005) and Buncic (1991) observed 86.4 and 69.0% *Listeria* contamination in raw minced meat collected from supermarkets and local butcher shops in Turkey and Yugoslavia, respectively. The increased incidence of *Listeria* in raw meat could be attributed to fecal contamination during the slaughter process, vendor hygiene, or unsafe food processing, packaging, and handling (Rahimi et al., 2012; Ismaiel et al., 2014; Stea et al., 2015). These data indicate that *Listeria* prevalence can be sporadic and although having been less detected in beef, it is an emerging pathogen in fresh meat.

In whole muscle meat, the majority of contamination occur on the surface until further processing such as mincing or slicing creates additional surface area that are susceptible to cross-contamination (K.T. Milios et al., 2014). However, in whole muscle raw meat purchased at retail stores, relatively high degree of *Listeria* contamination were observed in Japan (56.6%; Ryu et al., 1992) and Australia (24.0%; Ibrahim and Mac Rae, 1991) although they are less than the incidence level in this study. More recently, *L. monocytogenes* were undetectable in raw beef in South Korea (Park et al., 2015). In general, *Listeria* is capable of surviving on meat surfaces regardless of extrinsic factors. Freezing, surface dehydration, and simulated spray chilling do not appear to affect to the survival of *Listeria* (Farber and Peterkin, 1991). Growth of *Listeria*, however, appears greatly dependent on the temperature and the pH of the meat, the muscle tissue type, and the type and amount of background microflora (Farber and Peterkin, 1991). *Listeria* grows between -0.4 to 45°C with 37°C being the optimum temperature (Low and Donachie, 1997). Surface temperature of beef samples in this study were 19.2, 25.9, and 25.5°C in the SM, IM, and OM, respectively. The environmental temperature was 26.3

to 29.0°C. Guerini et al. (2007) reported that *Listeria* prevalence was greater on the hide during cooler weather in their investigation into cull cows and bulls; however, temperature-dependent phenomenon could not be evaluated in this study, because temperature variation was minimal.

Market Characteristics

Characteristics of markets and vendors as they related to the safety of beef in Vietnam were summarized in Table 2. Cover meat displays, a physical barrier between consumer and non-refrigerated meat, were used at T0 and T4 by 63.3 and 100.0% of the SM across all three regions of Vietnam, respectively. Similarly, refrigeration was used at T0 by 50.0% of the SM for storage to replenish the displayed products throughout the day. At T4, 100.0% of the SM used refrigeration for storage of products to be sold the next day. In comparison, only 33.3 and 16.7% of IM vendors used cover displays at T0 and T4, respectively. In addition, 36.7% of IM vendors used refrigeration at T0 and no vendor used refrigeration at T4. No OM vendor used refrigeration at either sampling times. At T0 and T4, 76.7 and 70.0% of OM vendors, respectively, used open meat displays without any physical barrier between consumer and products. Appropriate use of gloves and hairnets were lacking in IM, at both T0 (16.7 and 33.3%, respectively) and T4 (0.0 and 0.0%, respectively). Similarly, OM vendors used neither at both sampling times. However, in the SM, gloves and hairnets were used predominately at T4 (50.0 and 83.0%, respectively), compared with 16.7% and 33.3% at T0. The Centers for Disease Control estimates that 20% of foodborne illnesses are the result of cross-contamination from workers to food products (Michaels, 2015). The author also reported that bare hand contact with meat surfaces in the U.S. resulted in 182 of 308 foodborne illness outbreaks

(59%) because bare hand contact directly caused contamination. Proper hand washing decreased the possibility of pathogens being transmitted onto foods (Guzewich and Ross, 1999; Montville et al., 2002; Michaels et al., 2004). The authors reported a 30 to 40% decrease in foodborne illnesses when hand washing programs were implemented (Michaels et al., 2013). Hot water was only used by 16.7% of the SM vendors for cleaning purposes at T4. No vendors used hot water at T0. In addition, 16.7% of the SM vendors used fresh water at T0 and 41.7% used fresh water at T4. Furthermore, only 16.7% of IM vendors used fresh water at T0 to clean the retail area and 1.7% of OM vendors indicated that cold water was used for cleaning purposes at T4. Although water was available in all markets, at the time of surveying, no SM, IM, or OM vendor indicated that knives were cleaned before cutting meat. These practices could be related to the high degree of bacterial contamination found in the current study. *Salmonella* and *E. coli* counts can be reduced if beef carcasses are treated decontaminated by hot water washing, lactic acid spray, and carcass trimming (Castillo et al., 1998). Developing countries with limited resources can apply these physical interventions to reduce bacterial contamination levels.

Vendors in the IM and OM provide reasonably priced and conveniently available meat products for the lower income population. However, most foods sold in these markets create major food safety and quality concerns because meat products are being prepared and distributed under poor hygienic conditions, with limited access to safe water and sanitary services (WHO, 2002). There is an increased health risk to consumers because of the lack of knowledge about food safety measures and incentives for vendors to comply with food safety guidelines and regulations (Choudhury et al., 2011).

Chamhuri and Bratt (2013) reported that consumers in Malaysia still preferred to shop at traditional markets, i.e., open and street vendor markets, than supermarkets even though they were informed that meat from supermarkets were safer (Chamhuri and Batt, 2013). Some reports claims that traditional markets will soon be displaced and lose their customers to more modern retailers that offer greater quality and safer products (Trappey and Lai, 1997; Goldman et al., 1999; Giovannucci and Reardon, 2000). However, consumers in developing countries have not abandoned traditional markets when purchasing fresh meat because of the loyalty to a vendor, the perception of the availability of "fresher" meat, and competitive prices through bargaining. Even though traditional markets do not provide a clean and hygienic environment, they do provide a personal relationship that is lacking at other more modern market types. Emphasis on the importance of hygiene and food safety is needed in all markets because unsafe behaviors were not limited to traditional market types. Furthermore, it is important to intensify the efforts in educating food-handlers and consumers in food safety principles, proper cooking of foods of animal origins, personal hygiene, and sanitation of processing equipment (Sofos, 2008).

Conclusion

This study documented the levels of contamination of *Salmonella, Listeria,* and *E. coli*, three of the most important pathogens, in beef products. The occurrence of *Salmonella* and *Listeria* on beef products was much more frequent than reported in the literature. In addition, there were greater than 7 logs of indicator organisms such as APC, *E. coli*, and coliforms, which can be dangerous for the consumers if beef is not properly cooked. The high incidence and bacterial loads could be partially attributed to absence of

good manufacturing practices at markets and possibly at various points of production, such as lack of refrigeration, cleanliness, water usage, and proper attire. Therefore, more research is needed in this area to map the prevalence of pathogens from live animals to retail display so that risk mitigation strategies can be devised. Moreover, regulations and the control of hazards of beef processing in Vietnam are lacking. These data justify the establishment of food safety regulations and training in Vietnam.

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Tables and Figures

Table 1 Characteristics used to classify supermarkets (SM), indoor markets (IM), and open markets (OM) across three regions of Vietnam.

Market Characteristics	Market Type		
	SM	IM	OM
Multiple vendors		√	√
Air-conditioning	√		
Refrigeration	√		
Walls	√	√	
Roof	√	√	
Clean water availability	√	√	√

√ Existing characteristics

Table 2 Observational and environmental data collected during the purchase of beef from supermarkets (SM), indoor markets (IM), and open markets (OM) at the market opening (T0) and 4 h after the opening (T4) across three regions of Vietnam (Ho Chi Minh City, Da Nang, and Ha Noi).

Market Characteristics	SM		IM		OM	
	T0	T4	T0	T4	T0	T4
Outdoor temperature, °C	26.7 ± 1.1	29.3 ± 1.7	25.2 ± 0.5	27.5 ± 1.6	27.2 ± 1.6	30.8 ± 1.8
Humidity, %	69.3 ± 6.0	67.2 ± 6.7	82.7 ± 4.1	72.9 ± 5.5	73.5 ± 5.8	63.5 ± 5.6
Meat surface temperature, °C	20.2 ± 2.0	18.3 ± 1.4	25.8 ± 0.7	25.3 ± 0.9	25.8 ± 1.0	26.2 ± 0.8
Cover display, %	63.3 ± 18.2	100.0 ± 0.0	33.0 ± 21.1	0.0 ± 0.0	0.0 ± 0.0	13.0 ± 0.1
Hang display, %	3.3 ± 3.3	0.0 ± 0.0	30.0 ± 19.2	16.7 ± 16.7	23.3 ± 16.7	16.7 ± 10.9
Open display, %	33.3 ± 16.9	0.0 ± 0.0	36.7 ± 20.3	66.7 ± 21.1	76.7 ± 16.7	70.0 ± 13.4
Refrigeration, %	50.0 ± 16.9	100.0 ± 0.0	36.7 ± 20.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Gloves, %	16.7 ± 22.4	50.0 ± 22.4	16.7 ± 16.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Hairnet, %	33.3 ± 21.1	83.0 ± 16.7	33.3 ± 21.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cleaned knife before cutting, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Hot water, %	0.0 ± 0.0	16.7 ± 16.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fresh water, %	16.7 ± 0.1	41.7 ± 8.3	16.7 ± 10.5	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 1.7

*Values were reported as means ± standard error of the means.

Table 3 Bacterial counts and the prevalence of *Salmonella* and *Listeria* in beef procured from supermarkets (SM), indoor markets (IM), open markets (OM) at the market opening (T0) and 4 h after the opening (T4) across three regions of Vietnam (Ho Chi Minh City, Da Nang, and Ha Noi).

Microbiological Measurement*	SM		IM		OM		*P* market type	*P* time	*P* interaction
	T0	T4	T0	T4	T0	T4			
APC[1], log CFU/g	11.6 ± 0.1^{bY}	11.6 ± 0.1^{bX}	11.6 ± 0.1^{abX}	11.6 ± 0.1^{bX}	11.6 ± 0.1^{aX}	11.7 ± 0.1^{aX}	0.060	0.034	0.398
E. coli[2], log CFU/g	8.3 ± 4.1^{aX}	7.1 ± 3.9^{aY}	6.6 ± 4.8^{bY}	7.8 ± 3.6^{aX}	7.3 ± 3.2^{abX}	7.0 ± 3.8^{aX}	0.380	0.837	0.029
Coliform[3], log CFU/g	9.1 ± 2.0^{bX}	9.7 ± 1.9^{bX}	10.2 ± 2.0^{aX}	10.4 ± 1.9^{aX}	10.3 ± 2.8^{abX}	10.5 ± 2.7^{aX}	0.005	0.196	0.790
Salmonella[4] prevalence, %	61.2 ± 10.3^{aX}	70.4 ± 9.3^{aX}	53.4 ± 10.3^{aY}	83.8 ± 7.2^{aX}	43.2 ± 10.3^{aX}	56.8 ± 10.3^{abX}	0.082	0.019	0.380
Listeria[5] prevalence, %	89.6 ± 6.1^{aX}	90.4 ± 5.7^{aX}	93.6 ± 4.6^{aX}	100.0 ± 0.0^{aX}	93.6 ± 4.6^{aX}	93.6 ± 4.6^{aX}	0.773	0.975	0.998

[1] Aerobic Plate Count, enumerated using 3M™ Petrifilm™ Aerobic Plate Count (3M, St. Paul, MN)
[2] *Escherichia coli*, enumerated using 3M™ Petrifilm™ E. coli/Coliform Count Plates (3M, St. Paul, MN)
[3] Coliform, enumerated using 3M™ Petrifilm™ E. coli/Coliform Count Plates (3M, St. Paul, MN)
[4] *Salmonella,* detected using 3M™ Petrifilm™ *Salmonella* Express System (3M, St. Paul, MN)
[5] *Listeria,* detected using ALOA® media (BioMerieux, St. Louis, MI)
^{xy} within market type, means without common letters differ, $P \leq 0.1$.
^{ab} within sampling time, means without common letters differ, $P \leq 0.1$.
*Values were reported as estimated least squares means ± standard error of the means

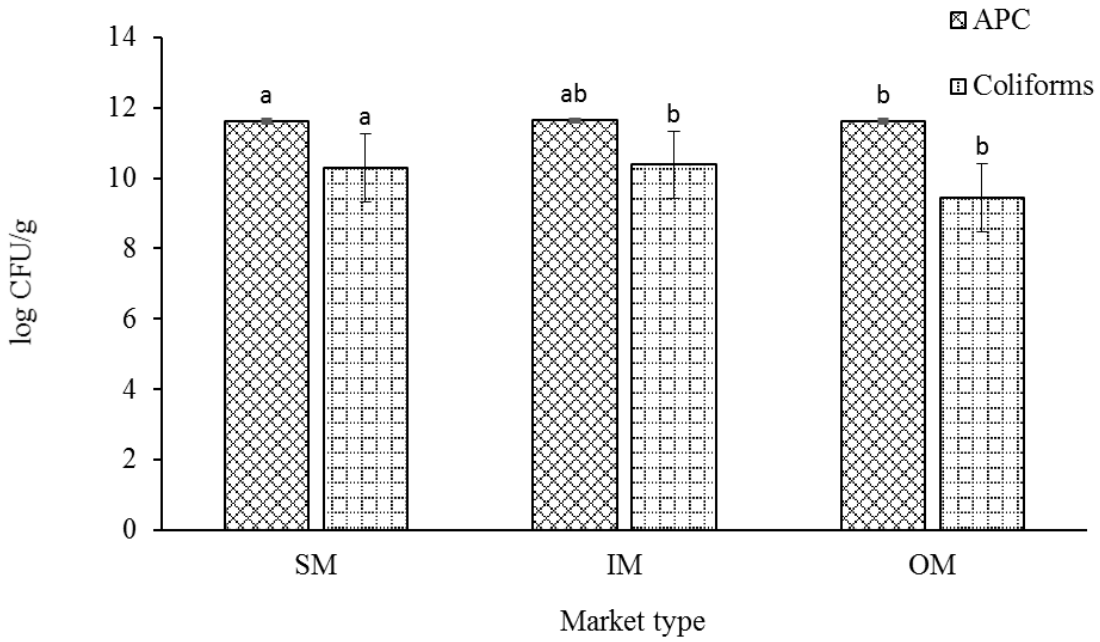

Figure 1 Aerobic Plate Count (APC) and Coliform counts (log CFU/g) of beef purchased from supermarkets (SM), indoor markets (IM), and open markets (OM) in Ho Chi Minh City, Da Nang, and Ha Noi of Vietnam, averaged across two sampling times

Within a category of bacterial count, means without common letters differ, (*Pmarket type* = 0.060 and 0.005, respectively)

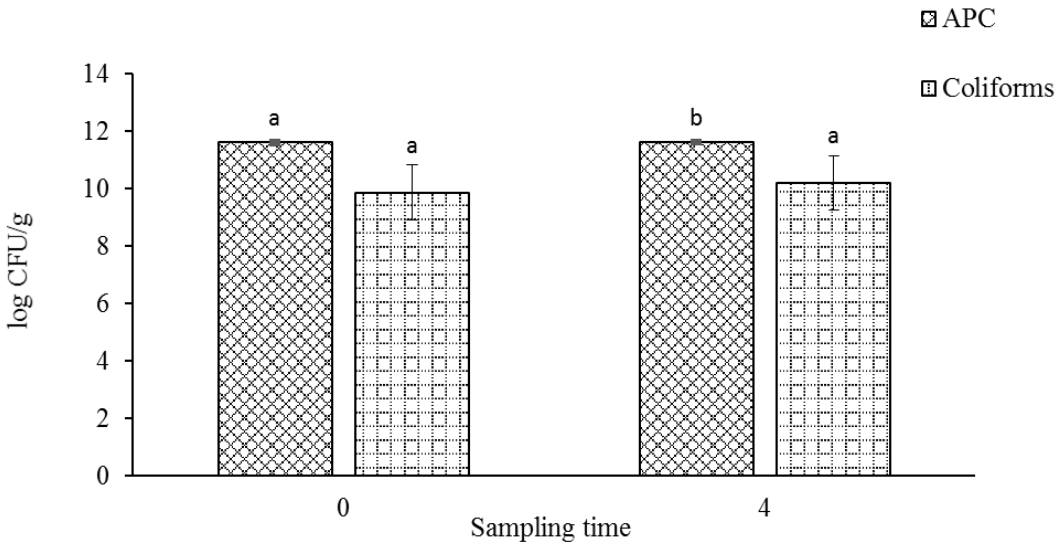

Figure 2 Aerobic Plate Count (APC) and coliform counts of beef purchased at two sampling times (opening - T0 and 4 h after opening - T4) in Ho Chi Minh City, Da Nang, and Ha Noi of, averaged across supermarkets, indoor markets, and open markets

Within a category of bacterial count, means without common letters differ, ($P_{sampling\ time}$ = 0.034, and 0.196, respectively).

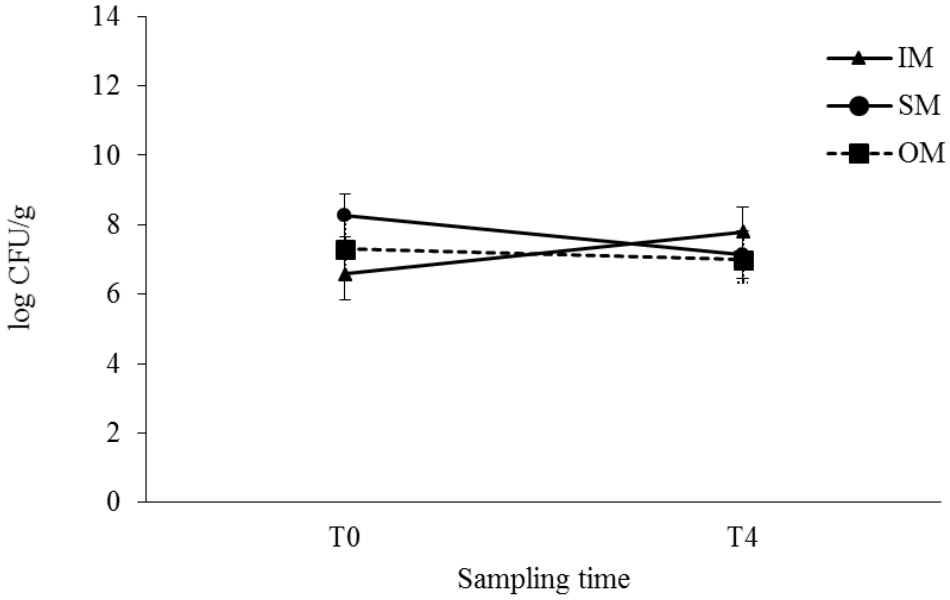

Figure 3 *E. coli* counts at opening (T0) and 4 h after opening (T4) in supermarkets (SM; P = 0.074), indoor markets (IM; P = 0.052), and open markets (OM; P = 0.623), varied by market type × sampling time interaction ($P_{market\ type\ x\ sampling\ time}$ = 0.029).

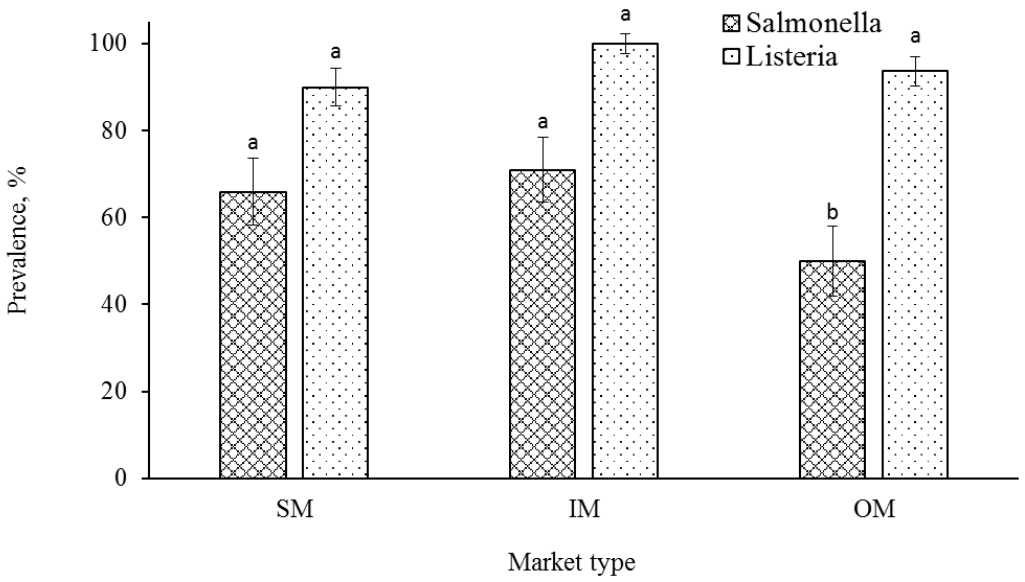

Figure 4 *Salmonella* and *Listeria* prevalence in beef purchased from supermarkets (SM), indoor markets (IM), and open markets (OM)) in Ho Chi Minh City, Da Nang, and Ha Noi of Vietnam, averaged across two sampling times

Within a pathogen category, means without common letters differ, ($P_{market\ type}$ = 0.082 and 0.773, respectively).

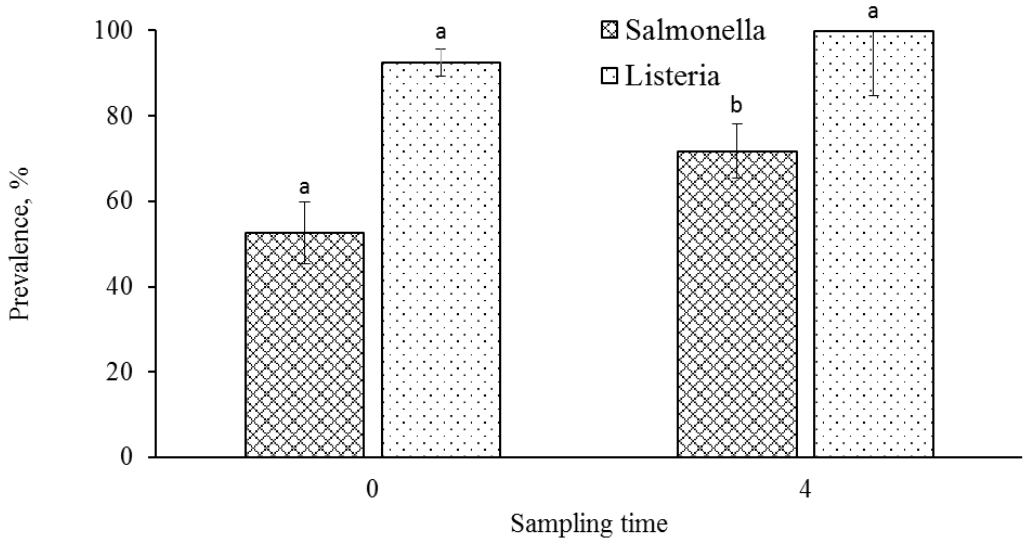

Figure 5 *Salmonella* and *Listeria* prevalence in beef purchased at opening (T0) and 4 h after opening (T4) in Ho Chi Minh City, Da Nang, and Ha Noi of Vietnam, averaged across supermarkets, indoor markets, and open markets.

Within a pathogen category, means without common letters differ, ($P_{sampling\ time}$ = 0.019 and 0.975, respectively).

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CHAPTER III

INFLUENCE OF MARKET SETTINGS AND TIME OF PURCHASE ON COUNTS OF AEROBIC BACTERIA, *ESCHERICHIA COLI*, AND COLIFORM AND PREVALENCE OF *SALMONELLA* AND *LISTERIA* IN PORK IN VIETNAM.

Abstract

The objective of this study was to determine the influence of market type and sampling time on *Salmonella* and *Listeria* prevalence in and microbiological quality of 180 pork samples collected in 6 supermarkets (SM), 6 indoor markets (IM), and 6 open markets (OM) at opening (T0) and 4 h after the opening (T4) in Vietnam. *Salmonella* and *Listeria* prevalence were greater than 42 and 64%, respectively. *Salmonella* prevalence was influenced by market type ($P = 0.049$), but not sampling time ($P = 070$). On average, pork from these markets had greater than 11, 7, and 10 logs of aerobic bacteria, *E. coli*, and coliforms, respectively. *E. coli* counts of pork at IM and OM were increased at T4 by 2.9 and 1.5 logs ($P < 0.001$ and $P = 0.045$, respectively), whereas they were similar in SM at both sampling times ($P = 0.925$). Cover meat displays were used by 50.0, 33.3, and 0.0% of SM, IM, and OM vendors at T0 and by 83.3, 0.0, and 0.0% of SM, IM, and OM vendors at T4, respectively. Refrigeration was used by 50.0 and 100.0% of SM vendors at T0 and T4, respectively and only by 53.3% of IM at T0 for storage. No OM pork vendor used refrigeration, gloves, or hairnets. No SM, IM, or OM

pork vendor used hot water. Cold water was used at T0 by 16.7, 25.5, and 0.0% of SM, IM, and OM vendors and by 45.0, 8.3, and 1.7% of SM, IM, and OM vendors at T4. Pork at retail establishments in Vietnam had substantial bacterial counts and occurrence of *Salmonella and Listeria* in addition to widespread improper handling practices, which highlights an immediate need of mandatory interventions and educational programs to protect public health.

Keywords: Pork, *Salmonella, Listeria, Escherichia coli,* coliforms, retail, developing countries, safety, quality, Vietnam.

Introduction

Pork is the most consumed meat in the world (FAO, 2014) and is a source of foodborne diseases (Baer et al., 2013). In the U.S., pork consumption has remained steady over the past 20 years (Baer et al., 2013). However, in Asian countries, pork has always been a major source of animal proteins, and it continues to increase with economic development (USDA, 2013). Because of the popularity of pork products in developing countries, microbiological safety and quality of pork supply are essential.

Small-scale operations with less than 20 pigs constitute 70% of pig production in Vietnam (Huynh et al., 2007). There are also few large-scale swine farms that can accommodate 18000 pigs, accounting for 15 to 20% of pig production (La et al., 2002; Northoff, 2006). Swine farms in Vietnam serves multiple purposes because Vietnamese producers use an integrated system, combining animal species with crops and fish, in which manure production may become more important and more profitable than pork (Huynh et al., 2007) Because of small-scale production, a major challenge in pork production in Vietnam is the lack of knowledge in zoonotic disease control (Foley et al.,

2008). Zoonotic diseases, such as *Salmonellosis*, can be spread by poor hygienic practices and improper waste disposal (Northoff, 2006). *Salmonella* resides in the intestinal tract of pigs and shedding of the bacteria is the major route for *Salmonella* infection (Baer et al., 2013). Similarly, *Listeria monocytogenes* can also persist in wet feeds and moist areas of farms (Baer et al., 2013). When pigs are slaughtered, carcass contamination can occur through infected live animals or cross-contamination from environment (Li et al., 2016), processing equipment, and other carcasses (Van Damme et al., 2015). However, prevalence of *Salmonella* and *Listeria* can be decreased by physical interventions such as removal of lymph nodes, hot water wash, acid sprays, carcass rinse, and carcass chilling (Schmidt et al., 2012). These interventions are commonly used in developed countries. However, developing countries lack information and capabilities to develop systematic approach towards processing interventions and epidemiological investigations to minimize the impact of foodborne illnesses (Kaferstein, 2003; Chaves et al., 2015). In addition, lack of good manufacturing practices of meat by market vendors, and poorly designed and regulated packing plants in developing countries increase risk of contamination. Many meat vendors in developing countries do not refrigerate fresh meat and poultry products, allowing pathogenic bacteria such as *Salmonella* and *Listeria* to grow. Unsafe foods cause serious food security challenges (Kinsey, 2005).

Meat is among the most nutritious foods in developing countries, especially for young children (Muir et al., 2010). Moreover, pork is the most important source of animal proteins in Vietnamese households (Tisdell, 2009). Per capita consumption of all meats has been increasing with increased incomes; however, pork still remains most consumed in Vietnam (USDA, 2013). Therefore, the safety of pork is increasingly

important in developing countries. The government of Vietnam believe that large-scale pig production can improve safety and quality of pork supply (Tisdell, 2009). However, cultural factors make traditional markets and small-scale pig processing plants valuable to consumers in developing countries because they are loyal to familiar vendors, perceive meat and poultry there as being fresher and cheaper. Traditional meat markets expose products to open atmosphere without refrigeration and supermarkets, although being capable of refrigeration and cover display, still face safety challenges because they primarily sell meats from similar sources (Chamhuri and Batt, 2013). Studies in developing countries such as Nepal, Vietnam, and China, have focuses on the contamination of one microorganism on meat products (Maharjan et al., 2006; Van et al., 2007b; Yang et al., 2010). Multi-pathogen data in the retail setting are lacking. Therefore, prevalence of important pathogenic bacteria such as *Salmonella* and *Listeria* and aerobic microbial loads in meat and poultry products, specifically pork, are important to establish a baseline of contamination so that further investigations into contamination sources and interventions can be devised. Market setting and time of purchase are important meat merchandising factors; therefore, the objective of this study was to investigate the effects of market type and sampling time on *Salmonella* and *Listeria* prevalence in and microbiological quality of fresh pork in Vietnam.

Materials and Methods

Sample Collection and Preparation

Sampling plan was similar to the one described in chapter II. Ho Chi Minh City, Da Nang, Ha Noi, and their surrounding areas were selected to represent regional variation in meat merchandising in Vietnam. Supermarkets (SM), indoor markets (IM),

and open markets (OM) were described in chapter II and in Table 4. Two markets per market type within in each region were geographically selected to procure domestically produced pork at two sampling times, the opening of individual markets (T0) and 4 h after the opening (T4). Five 200-g pork *Longissimus* muscle samples were collected separately and aseptically from various vendors at each sampling time, resulting in 180 samples. Vendors were randomized as described in chapter II. Samples were placed separately in sterile Whirl-Pak bags® (Nasco, Fort Atkinson, WI) and the bags were sealed immediately after meat surface temperature was recorded by a Fisher Scientific™ Traceable™ Infrared Thermometer Gun (Fisher Scientific, Waltham, MA). Samples were stored in an Igloo Super Tough Sportsman ice chest (Igloo, Katy, TX) with frozen ice packs and transported to a local university in each region. Samples were weighed and shaken for 60 s in 90 mL of Buffered Peptone Water broth (BPW; 25.5 g/L; 3M, St. Paul, MN), which was added to Whirl-Pak® bags (Nasco, Fort Atkinson, WI; Vipham et al., 2012). Two sterile 15-mL polypropylene tubes (Greiner Bio-One, Monroe, NC) of BPW rinsate were transported on ice to Ho Chi Minh City University of Technology for further analyses.

Microbiological Analysis

Salmonella was analyzed as described in chapter II. Briefly, 2.5 mL of BPW rinsate was combined with 22.5 mL of *Salmonella* Enrichment Broth (3M, St. Paul, MN) in a sterile Whirl-Pak® bag (Nasco, Fort Atkinson, WI) and incubated at 45°C for 24 h. One mL of the incubated solution was combined with 10 mL of Rappaport-Vassiliadis R10 Broth (RVR10; 3M, St. Paul, MN) in a 15-mL polypropylene tube (Greiner Bio-One, Monroe, NC) and incubated at 41.5°C for 24 h. Ten µL of the incubated RVR10

solution was streaked onto a hydrated 3M™ Petrifilm™ of the Salmonella Express System. The Petrifilm™ was incubated at 41.5°C for 24 h. Presumptive positive *Salmonella* spp. colonies were identified by a red color with yellow halo (3M, 2015a).

Listeria was detected as described in chapter II. Similarly, a volume of 2.5 mL of BPW rinsate was combined with 22.5 mL of Demi-Fraser Listeria Enrichment Broth (3M, St. Paul, MN) in a sterile Whirl-Pak® bag (Nasco, Fort Atkinson, WI) and incubated at 30°C for 24 h. A volume of 0.1 mL of the incubated solution was spread onto an ALOA® agar petri dish and incubated at 37°C for 24 h. Presumptive positive *Listeria* spp. colonies were identified by a blue to green color with or without halo.

Analyses of aerobic bacteria (Aerobic Plate Count, APC), *E. coli,* and coliforms analyses were performed as described in chapter II. Fifteen µL of BPW rinsate was serially diluted (1:100) by combining with 1485 µL of sterile BPW broth. One mL of each dilution was spread onto an APC Petrifilm™ or an *E. coli*/Coliform Petrifilm™. The Petrifilm™ was incubated at 35°C for 24 h. Colony forming units (CFU) were counted according to the 3M interpretation guides (3M, 2015c; 3M, 2015d).

Market Characteristics

Market and environmental data were collected by using a form containing outdoor temperature (ºC), relative humidity (%), meat surface temperature (ºC), type of retail display, availability of refrigeration, use of gloves and hairnets, knife cleaning, and water availability. Data were recorded for individual samples.

Calculation and Statistical Analysis

Salmonella and *Listeria* prevalence was reported as percentage of positive samples estimated by the statistical model. Counts of aerobic bacteria, *E. coli,* and coliforms were reported as log CFU/g, calculated from CFU as follows:

$$\log \text{CFU/g} = \log \left(\frac{N}{V} \times DF \times V_0 \times \frac{1}{m} \right) \quad (2)$$

with N, V, DF, V_0, and m being number of colony forming units on a Petrifilm™, volume of a dilution spread onto a Petrifilm™ (1 mL), dilution factor, original volume of BPW rinsate (90 mL), and sample weight (g), respectively. Market characteristic data were recorded for each sample and reported as crude percentage without statistical analysis.

Prevalence of *Salmonella* and *Listeria* were analyzed as a 3 × 2 factorial arrangement in a randomized complete block design with region as block, market type (SM, IM, and OM) and sampling time (T0 and T4) as two factors, and a specific market at a specific sampling time as experimental unit, using logistic regression. Bacterial counts were analyzed as the same design using linear regression; however, experimental unit was pork sample and the statistical model was linear regression. Statistical analyses were performed by using a generalized linear mixed model of SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA) in the GLIMMIX procedure. Market type, sampling time, and their interaction were the fixed effects, whereas region was the random effect. Means were separated by the protected t-test in the PDIFF option of the LSMEANS statement. Statistical significance was determined at $P \leq 0.10$.

Results and Discussion

Microbiological Quality

Bacterial count for each market at a specific sampling time was reported in Table 6. There was no overall market effect on bacterial counts. Pork purchased in these markets had greater than 11.4, 7.4, and 10.4 log CFU/g of aerobic bacteria, *E. coli*, and coliforms, respectively (Table 6 and Figure 6). Similar to previous study on beef, many of the APC Petrifilms™ were too numerous to count (TNTC) at 10^{-6} dilution (3M, 2015b) and estimated at 10^{8} CFU. *E. coli* counts were 7.4 and 8.6 logs at T0 for IM and OM, respectively; however, they were increased to 10.3 and 10.1 logs at T4 ($P < 0.001$ and P = 0.04, respectively; Table 6). *E. coli* counts remained the same on pork purchased from SM ($P = 0.92$). Although no sampling time effect was found for APC, coliform counts were greater at T4 (10.9 logs) than T0 (8.4 logs; $P = 0.08$). Major bacterial genera on post-slaughter meat surface are *Pseudomonas* spp., *Acinetobacter* spp., *Aeromonas* spp., *Brochothrix thermosphacta*, lactic acid bacteria such as *Lactobacillus* and Enterobacteriaceae (Duffy et al., 2008). Although meat is an excellent environment for microbial growth, the levels of bacterial counts in these pork products were greater than those normally observed in the U.S. and other developed countries, at approximately 3 to 4 logs on carcasses without trimming and interventions. Meat products with 7 to 8 logs of APC are considered spoiled (Duffy et al., 2008). Pork products was even classified as either spoiled or unacceptable quality with 4.5 to 6.0 logs of total bacteria counts (Zhao et al., 2015; Ma et al., 2014) The composition of the bacterial flora on pork in retail outlets is the end result of the initial bacterial contamination and the colonization occurring during slaughter, processing, and distribution (Van Damme et al., 2015). Pork

in Vietnam, although having much greater bacterial loads, did not show sign of spoilage. It is understood that bacterial profile, i.e. counts and species, depends on the initial contamination and environmental conditions (Duffy et al., 2008). In developed countries, most fresh meat products initially have less than 3 logs of total aerobic bacteria. Therefore, organoleptic quality is decreased drastically as bacterial counts reach 7 to 8 logs. However, in developing countries such as Vietnam, it is possible that meat products have much greater initial bacterial counts caused by contamination during distribution and in markets. Similar *E. coli* counts for minced pork were reported in Greece, a developed country (UN, 2012), at 6.7 and 7.2 log CFU/g in both butcher's shops and supermarkets, respectively (Andritsos et al., 2012). In Nigeria, a developing country with similar meat merchandising venues, 5.6 log CFU/g for *E. coli* were documented in pork retail establishments. These authors attributed the increased bacterial counts to contamination during slaughter processing and water contamination because the markets were close to a stream where fecal materials were to be disposed (Adesiji et al., 2011). In the current study, contaminations during processing, transportation, and hygienic conditions at the markets could contribute to the increased bacterial counts.

Prevalence of *Salmonella*

Salmonella prevalence for each market at a specific sampling time was reported in Table 6. *Salmonella* prevalence was 71.1, 65.9, and 48.1% in SM, IM, and OM, respectively (Figure 9). Market type influenced *Salmonella* prevalence in pork (P = 0.049) with OM being less than both IM and SM (P = 0.069 and P = 0.021, respectively), whereas IM and SM *Salmonella* prevalence was similar (P = 0.559). . However, there was no effect of sampling time on *Salmonella* incidence (P = 0.700; Figure 10). Vendors

in the IM and OM received pork carcasses that might be different in microbial profile and production settings. These carcasses were cut at the markets, thereby creating opportunities for cross-contamination of pathogenic microorganisms, such as *Salmonella*. Researchers agree that retail display is possibly the weakest link in a commercial cold chain (James and Bailey, 1990). Therefore, if meat products are not refrigerated, *Salmonella* may proliferate to a dangerous number of cells that can be carried over during display (Lo Fo Wong et al., 2002). A study conducted in Ha Noi in Vietnam discovered that more than 50% of pigs brought to packing plants carried *Salmonella* spp (Le Bas et al., 2006). These authors concluded that farm practices, including transportation and lairage conditions were favorable for *Salmonella* spp. shedding among pigs (Le Bas et al., 2006). They also revealed that water was greatly contaminated with *Salmonella* (62%), and was used for carcass rinsing after evisceration. Similar studies on pork carcasses, environmental surfaces in slaughter facilities, and retail markets conducted in Hue, Bac Ninh, Ha Noi, and Ha Tay in Vietnam found 30% or greater of retail pork (Thai et al., 2012), 15.5% of carcasses, and 16.7% of tank water to be contaminated with *Salmonella* (Takeshi et al., 2009). Although these authors (2012) reported similar results, *Salmonella* incidence in their studies was still less than that in the current study. In addition, the current study had a more comprehensive sampling plan across three regions of Vietnam. In similarly narrow studies, Phan et al. (2005) and Van et al. (2007) also reported 69.9 and 64.0% prevalence of *Salmonella* in pork in Mekong Delta region and Ho Chi Minh City, respectively, which was comparable to the incidence levels in the current study. Developed countries such as Austria, Ireland, the U.K., and the U.S. have found much lower prevalence of *Salmonella* in retail markets, at 1.8, 9.9, 1.9, and 2.6%, respectively

(Mayrhofer et al., 2004). However, a study in commercial pork slaughter facilities in the U.S., 91% of pre-scald, 19.1% of pre-evisceration, and 3.7% of post-chill carcasses were contaminated with *Salmonella* (Schmidt et al., 2012). The decrease in *Salmonella* prevalence as carcasses moved through processing stages indicated that appropriate critical control points during slaughter will decrease *Salmonella* incidence (Schmidt et al., 2012; Baer et al., 2013). However, Duggan et al. (2010) reported that a *Salmonella* incidence level of up to 69% on pork carcasses was the result of a contaminated slaughter environment. Differences between developing countries such as Vietnam and developed countries could be the contamination at various critical control points in the pork production chain. When pork products are contaminated, cross-contaminate can progress unless carcasses or cuts are decontaminated (Berends et al., 1998), possibly through interventions such as carcass sprays of organic acids (Castillo et al., 1998), which decreases pH to suppress bacterial growth (Baer et al., 2013). Hot water wash is as effective as organic acid spray (Baer et al., 2013), which can a applicable method for developing countries. Even with postharvest interventions, developed countries still face challenges in minimizing *Salmonella* prevalence in retail establishments, although it is not a the levels found in the current study. Sixty-four attendees in Hamilton County, Ohio were determined to suffer salmonellosis during a private event after consuming pulled pork (CDC, 2010). Most recently in 2015, the FSIS issued public health alert for pork from Kapowin Meats of Graham, WA because of possible *Salmonella* contamination, which was associated with whole pig used for pig roast (Johnston, 2015). Retail pork in Demark were found to have a *Salmonella* incidence at 3 to 8%, with butcher shops being positive twice as much as supermarkets (Hansen et al., 2010). The

authors indicated that this difference could be from hygienic conditions and cross-contamination caused by variation in handling procedures among retail venues (Hansen et al., 2010). However, in the current study, the prevalence of Salmonella in the IM and OM, similar merchandising model to butcher shops, was either similar or lower than that in the SM. The observation during the current study revealed that SM vendors in Vietnam behaved similarly to vendors at other market types, who did not adhere to good management practices such as cleaning knife, using hot water, or wearing gloves and hairnets.

Prevalence of *Listeria*

Listeria prevalence for each market at a specific sampling time was reported in Table 6. Market type did not affect *Listeria* prevalence in pork across all three regions of Vietnam ($P = 0.162$; Figure 9) with average of 77.7, 87.9, and 73.4% in SM, IM, and OM, respectively. Moreover, similar to the case of *Salmonella*, *Listeria* prevalence was not affected by sampling time ($P = 0.817$, Figure 10), an average of 79 to 81%. These levels of incidence in retail venues in Vietnam were much greater than those reported in various studies. Columbian researchers observed a 33.9% prevalence (Gamboa-Marín et al., 2012) in pork carcasses, which agreed with a study conducted in Tokyo with 35.7% positive samples in pork carcasses (Ochiai et al., 2010). In contrast, research in the U.S., Finland, Bulgaria, Greece, and Canada showed much lower *Listeria* contamination in pork products, ranging from 0.15 – 24% (Wesley and Ashton, 1991; Samelis and Metaxopoulos, 1999; Bohaychuk et al., 2006; Karkolev, 2009; Hellstrom et al., 2010). The decreased prevalence in these countries was the result of HACCP regulations implemented throughout the supply chain (Gamboa-Marín et al., 2012). Without proper

practices at critical control points, researchers in Ethiopia, a developing country, reported at a *Listeria* incidence level of 69.8% supermarkets (Molla et al., 2004), which was comparable to that in the SM in the current study (77.7%). Boerlin and Piffaretti (1991) found less *Listeria monocytogenes* on live pigs than in pork after slaughter and fabrication. van den Elzen and Snijders (1993) indicated that chilling and cold environment of cutting room could facilitate *Listeria* contamination because *Listeria* is psychrotrophic. Moreover, delicacies such as lungs, heart, diaphragm, kidneys, and liver are frequently consumed in Asian culinary cultures. In the current study, all markets in Vietnam had viscera on display and in contact with pork whole muscle products. These authors hypothesized that *Listeria* spread through contact with the viscera during processing (Autio et al., 2000). This can partially explain the high degree of *Listeria* contamination in pork in Vietnam's meat markets. Furthermore, chilling and cutting increased the contamination of *Listeria* in pork (Nesbakken et al., 1996), and van den Elzen and Snijders (1993) found that *Listeria* prevalence in the cutting areas was as high as 71 to 100%. These findings suggest that post-slaughter processing can increase bacterial contamination in meat, and that refrigeration may not enough to suppress *Listeria* growth. The current study only assessed retail establishment as source of contamination. However, with the current high *Listeria* incidence, it was suspected that processing facilities, transportation, and water could be potential sources of contamination. Postharvest interventions combined with antimicrobials decreased *Listeria* in pork products (Chen, 2005). Chlorine as well as thermal treatment can remove biofilm on processing equipment to reduce cross-contamination (Sánchez-Escalante et al., 2001) because *Listeria*, although more thermotolerant than other

pathogens, is inactivated when heated above 70°C (Thévenot et al., 2006). These technologies can be applied in a multi-hurdle approach to eliminate *Listeria* contamination in Vietnam's meat markets. However, it is important to recognize that *Listeria* has unique characteristics that help the bacteria adapt to environmental stress and become greatly resistant to pre- and post-harvest interventions (Thévenot et al., 2006).

Market Characteristics

Characteristics of markets and pork vendors were summarized in Table 5. Physical barrier between meat products and consumers were used only in SM and IM. At T0, 50 and 33.3 % of SM and IM vendors, respectively, used cover displays; however, 83.3% of SM vendors but no IM vendor used cover displays at T4. This variation in meat display was observed across various supermarkets and indoor markets in the current study. No OM vendor covered pork during sampling time. Unlike previously reported beef products (chapter II), pork loin was suspended from hooks in many markets in Vietnam. The hook suspension method was used to attract customers in IM and OM. Vendors in SM and IM used refrigeration, whereas OM vendors did not. The SM and IM vendors stored pork products under refrigeration at T0 to restock their meat displays. Unlike SM pork vendors, who always used refrigeration (100.0%), IM vendors did not use the refrigeration at T4 (0.0%) because refrigeration was only used for restocking purposes. Supermarkets stored pork products that were not purchased in the refrigerator to be sold the next day. Gloves and hairnets were not frequently used either at T0 by SM and IM vendors, at 16.7 and 6.7%, or at T4, at 50.0 and 16.7%, respectively. Gloves and hairnets were not worn by any OM pork vendors across three regions of Vietnam. Neither did pork vendors in SM, IM, or OM clean their knives before cutting meat nor

did they have access to hot water. However, clean water was available to 16.7, 25.0, and 0.0% of SM, IM, and OM vendors at T0, respectively. At T4, 45.0, 8.3, and 1.7% of SM, IM, and OM vendors, respectively had access to clean water. Lacking clean water could be detrimental to the safety pork in Vietnam. However, the vendors did not use much water for cleaning because pork was sold quickly across all markets. It is the animal protein in Vietnam (Tisdell, 2009). Vendors in IM and OM provided more reasonably priced pork products for Vietnamese population. It was initially thought that SM vendors would provide safer pork products. However, pork sold across all market type created major food safety concerns because of poor hygienic conditions and lack of good manufacturing practices by most vendors. In IM and OM, limited access to safe water and sanitary services increases safety risks of meat products (WHO, 2002). Although *Salmonella* and *Listeria* risks can be eliminated with proper cooking temperature, increased food safety knowledge and incentives for both consumers and vendors are needed to ensure compliance with food safety guidelines and regulations (Choudhury et al., 2011).

Conclusion

The current study investigated *Salmonella, Listeria,* and *E. coli* in pork, contributing to the baseline of bacterial counts and prevalence in retail establishments in Vietnam. The incidence of *Salmonella* and *Listeria* in pork products was greatly increased compared with previously reported data. Bacterial counts were also between 7.4 and 11.6 logs for indicator organisms such as aerobic bacteria, *E. coli,* and coliforms. This indicates the danger of pork products in Vietnam, which was similarly reported for beef products (chapter II), if they are not properly cooked because pork is the most

commonly consumed animal protein in Vietnam. *Listeria* prevalence is of particular concern because of the consistently high incidence across all markets instead of sporadic presence seen in developed countries. *Listeria* is much more difficult to eliminate in processing environment and must be an important factor to be considered when developing interventions in Vietnam. The high incidence and bacterial loads could be partially attributed to lack of good manufacturing practices at markets; however, and various contamination sources at production must be considered. Therefore, more research is needed to identify these sources. The current study emphasizes again the need of regulations, control of hazards, and education program to ensure the safety of meat products in Vietnam.

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Tables and Figures

Table 4 Characteristics used to classify supermarkets (SM), indoor markets (IM), and open markets (OM) across three regions of Vietnam.

Market Characteristics	Market Type		
	SM	IM	OM
Multiple vendors		√	√
Air-conditioning	√		
Refrigeration	√		
Walls	√	√	
Roof	√	√	
Clean water availability	√	√	√

√ Existing characteristics

Table 5 Observational and environmental data collected during purchase of pork from supermarkets (SM), indoor markets (IM), open markets (OM) at the market opening (T0) and 4 h after the opening (T4) across three regions of Vietnam (Ho Chi Minh City, Da Nang, and Ha Noi).

Market Characteristics	SM		IM		OM	
	T0	T4	T0	T4	T0	T4
Outdoor temperature, °C	26.7 ± 1.1	29.3 ± 1.7	25.4 ± 0.4	27.8 ± 1.5	27.1 ± 1.7	31.2 ± 1.7
Humidity, %	68.7 ± 5.8	66.0 ± 6.5	82.7 ± 4.1	70.7 ± 5.8	73.5 ± 5.6	63.6 ± 5.5
Meat surface temperature, °C	21.7 ± 1.8	22.0 ± 4.2	27.3 ± 0.8	25.9 ± 1.1	26.5 ± 1.0	26.7 ± 1.0
Cover display, %	50.0 ± 0.2	83.3 ± 0.2	33.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Hang display, %	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 0.0	23.3 ± 0.2	16.7 ± 0.2	0.0 ± 0.0
Open display, %	50.0 ± 0.2	16.7 ± 0.2	63.3 ± 0.2	76.7 ± 0.2	83.3 ± 0.2	100.0 ± 0.0
Refrigeration, %	50.0 ± 0.2	100.0 ± 0.0	53.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Gloves, %	16.7 ± 0.2	50.0 ± 0.2	6.7 ± 0.1	16.7 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Hairnet, %	33.3 ± 0.2	83.3 ± 0.2	33.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cleaned knife before cutting, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Hot water, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fresh water, %	16.7 ± 0.1	45.0 ± 0.1	25.0 ± 0.1	8.3 ± 0.1	0.0 ± 0.0	1.7 ± 0.0

*Values were reported as means ± standard error of the means.

Table 6 Bacterial counts and the prevalence of *Salmonella* and *Listeria* in pork procured from supermarkets (SM), indoor markets (IM), open markets (OM) at the market opening (T0) and 4 h after the opening (T4) across three regions of Vietnam (Ho Chi Minh City, Da Nang, and Ha Noi).

Microbiological Measurement*	SM		IM		OM		*P* market type	*P* time	*P* interaction
	T0	T4	T0	T4	T0	T4			
APC[1], log CFU/g	11.6 ± 0.0 ^{ax}	11.6 ± 0.0 ^{ax}	11.4 ± 0.1 ^{ax}	11.6 ± 0.0 ^{ax}	11.6 ± 0.0 ^{ax}	11.6 ± 0.0 ^{ax}	0.313	0.277	0.163
E. coli[2], log CFU/g	9.1 ± 0.4 ^{ax}	9.1 ± 0.5 ^{ax}	7.4 ± 0.7 ^{ax}	10.3 ± 0.4 ^{ay}	8.6 ± 0.8 ^{ax}	10.1 ± 0.4 ^{ay}	0.613	<0.001	0.016
Coliform[3], log CFU/g	11.5 ± 0.1 ^{ax}	11.5 ± 0.1 ^{ax}	10.4 ± 0.6 ^{ax}	11.5 ± 0.1 ^{ax}	10.9 ± 0.5 ^{ax}	11.2 ± 0.4 ^{ax}	0.245	0.083	0.216
Salmonella[4] prevalence, %	74.4 ± 9.8 ^{bx}	67.5 ± 10.8 ^{bx}	71.0 ± 10.3 ^{bx}	60.5 ± 11.4 ^{bx}	42.9 ± 11.6 ^{ax}	53.4 ± 11.7 ^{ax}	0.049	0.700	0.469
Listeria[5] prevalence, %	77.7 ± 9.0 ^{ax}	77.7 ± 9.0 ^{ax}	90.8 ± 5.7 ^{ax}	84.4 ± 7.6 ^{ax}	64.0 ± 11.0 ^{ax}	81.1 ± 8.4 ^{ax}	0.162	0.817	0.319

[1] Aerobic Plate Count, enumerated using 3M™ Petrifilm™ Aerobic Plate Count (3M, St. Paul, MN).
[2] *Escherichia coli*, enumerated using 3M™ Petrifilm™ E. coli/Coliform Count Plates (3M, St. Paul, MN).
[3] Coliform, enumerated using 3M™ Petrifilm™ E. coli/Coliform Count Plates (3M, St. Paul, MN).
[4] *Salmonella,* detected using 3M™ Petrifilm™ *Salmonella* Express System (3M, St. Paul, MN).
[5] *Listeria,* detected using ALOA® media (BioMerieux, St. Louis, MI).
^{xy} Within market type, means without common letters differ, $P \leq 0.1$.
^{ab} Within sampling time, means without common letters differ, $P \leq 0.1$.
*Values were reported as estimated least squares means ± standard error of the means.

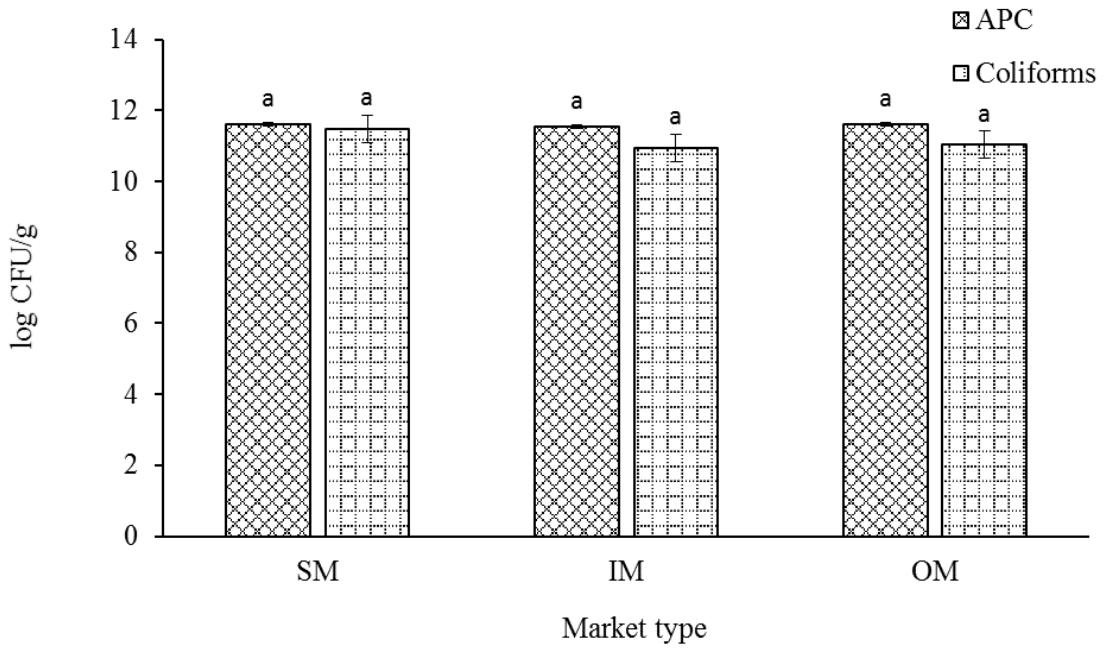

Figure 6 Aerobic bacteria and coliforms counts (log CFU/g) of pork purchased at the supermarket (SM), indoor market (IM), and open market (OM), in Ho Chi Minh City, Da Nang, and Ha Noi in Vietnam, averaged across to sampling times

Within a category of bacterial count, means without common letters differ, ($P_{market\ type}$ = 0.313 and 0.245, respectively).

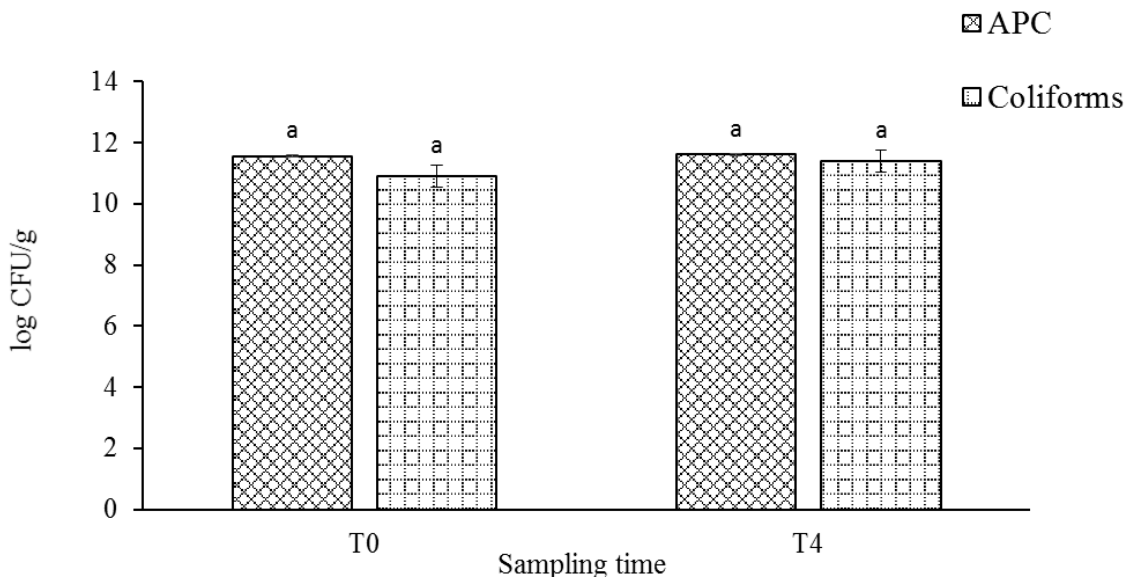

Figure 7 Aerobic bacteria and coliform counts of pork purchased at two sampling times (opening - T0 and 4 h after opening - T4) in Ho Chi Minh City, Da Nang, and Ha Noi of Vietnam, averaged across supermarkets, indoor markets, and open markets

Within a category of bacterial count, means without common letters differ, ($P_{sampling\ time}$ = 0.277 and 0.083, respectively).

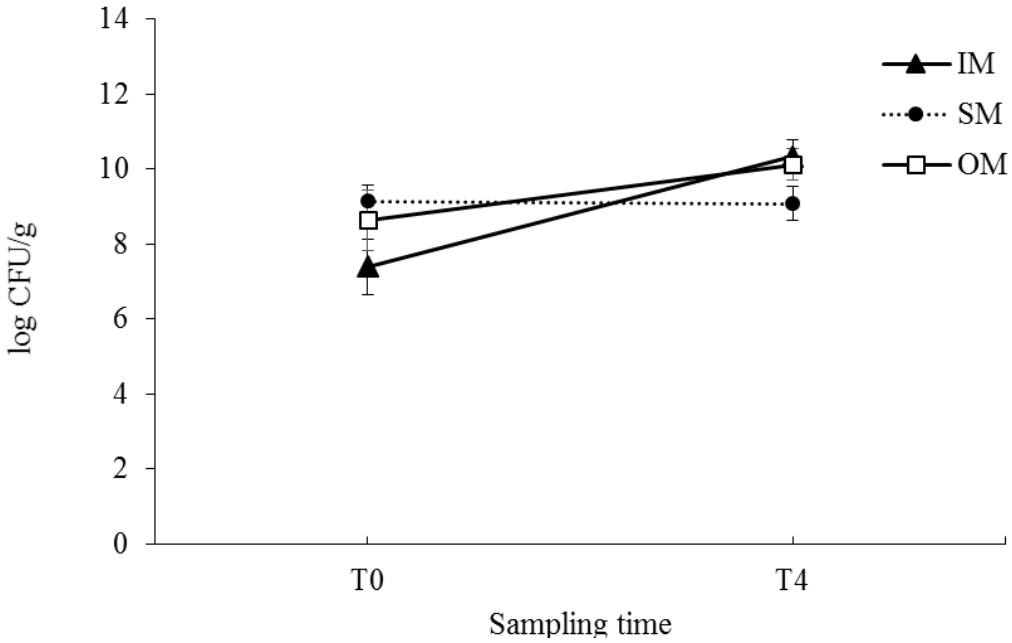

Figure 8 *E. coli* counts at opening (T0) and 4 h after opening (T4) in indoor markets (IM, $P < 0.001$) and open markets (OM, $P = 0.04$), varied by market type × sampling time interaction ($P_{market\ type\ x\ sampling\ time} = 0.016$).

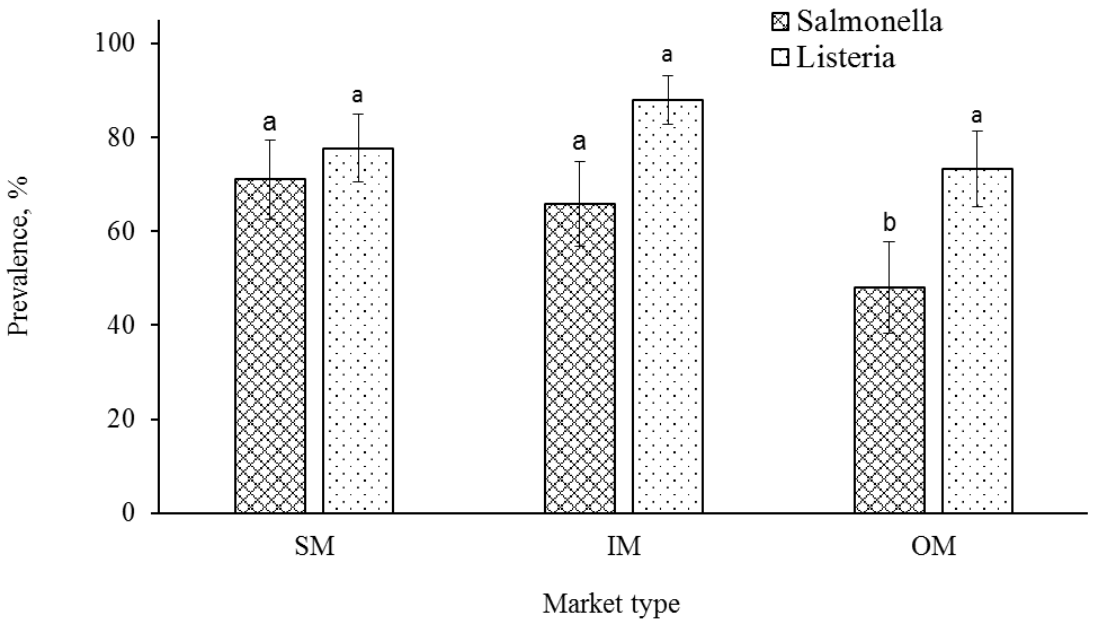

Figure 9 *Salmonella* and *Listeria* prevalence in pork purchased from supermarkets (SM), indoor markets (IM), and open markets (OM)) in Ho Chi Minh City, Da Nang, and Ha Noi of Vietnam, averaged across two sampling times.

Within a pathogen category, means without common letters differ ($P_{market\ type}$ = 0.049 and 0.162, respectively).

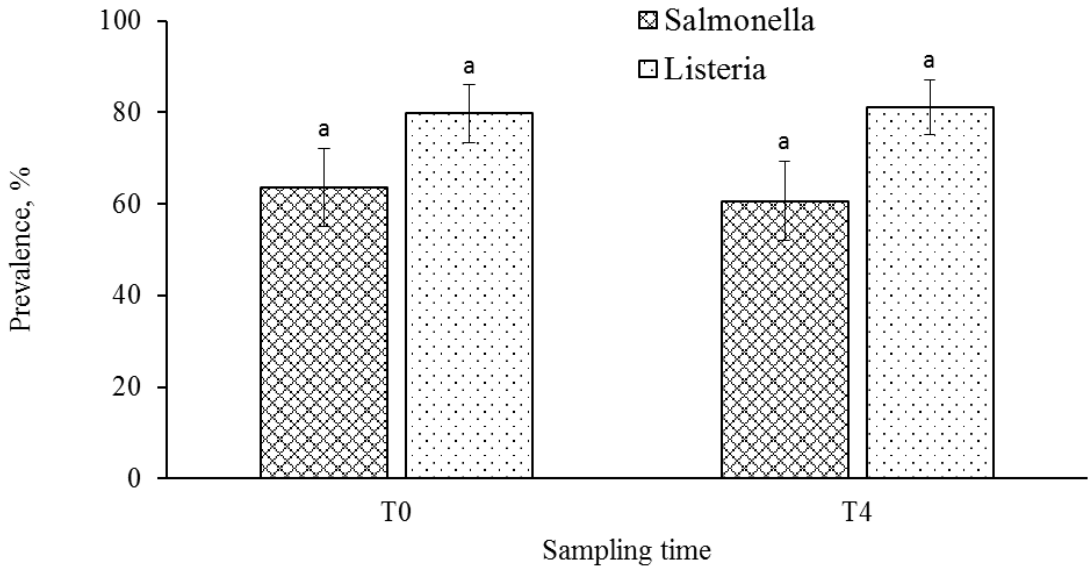

Figure 10 *Salmonella* and *Listeria* prevalence in pork purchased at opening (T0) and 4 h after opening (T4) in Ho Chi Minh City, Da Nang, and Ha Noi of Vietnam, averaged across supermarkets, indoor markets, and open markets.

Within a pathogen category, means without common letters differ, ($P_{sampling\ time}$ = 0.700 and 0.817, respectively).

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CHAPTER IV

INFLUENCE OF MARKET SETTINGS AND TIME OF PURCHASE ON COUNTS OF AEROBIC BACTERIA, *ESCHERICHIA COLI*, AND COLIFORM AND PREVALENCE OF *SALMONELLA* AND *LISTERIA* IN CHICKEN IN VIETNAM.

Abstract

This objective of the current study was to determine the influence of market setting and sampling time on the prevalence of *Salmonella* and *Listeria* in and the microbiological quality of 180 whole chicken carcasses collected in 6 supermarkets (SM), 6 indoor markets (IM), and 6 open markets (OM) in Vietnam at the opening (T0) and 4 h after the opening (T4). *Salmonella* and *Listeria* prevalence were greater than 30.4 and 56.6%, respectively. Chicken carcasses had more than 10, 7, and 9 logs of aerobic bacteria, *E. coli*, and coliforms, respectively. Sampling did not influence counts of aerobic bacteria, *E. coli,* and coliforms nor did it affect *Salmonella* and *Listeria* prevalence ($P \geq 0.113$). Both *E. coli* and coliform counts were greater in IM than in SM ($P = 0.002$ and 0.006, respectively). However, only *E. coli* counts differed between SM (7.7 log CFU/g) and OM (8.3 log CFU/g; $P = 0.024$). Whole birds in IM had greater *Salmonella* prevalence than birds from both SM and OM by 28.37 and 22.97% ($P =$ 0.006 and 0.022, respectively). *Listeria* prevalence was less in SM, at 56.6%, than in IM and OM (78.6 and 73.2%, $P = 0.024$ and 0.089, respectively). There was no market type

× sampling time interaction for all microbiological measurements ($P > 0.118$). Market characteristics such as display, refrigeration, hot water, and hygienic conditions varied greatly among vendors in SM, IM, and OM. These results highlighted high levels of bacterial loads and incidence in whole chicken in retail establishments in Vietnam, which posed great danger to public health because whole birds are much more popular than parts and boneless meat.

Keywords: Chicken, *Salmonella, Listeria, Escherichia coli,* coliforms, retail, developing countries, safety, quality, Vietnam.

Introduction

Chicken meat is the second most popular animal protein in Vietnam after pork. Approximately 621.1 thousand tons of poultry meat were produced in 2010 (General Statistics Office of Vietnam, 2010). The annual average poultry meat per capita consumption in Vietnam is 7.1 kg per person (General Statistics Office of Vietnam, 2010). Therefore, ensuring the microbiological safety and quality of poultry supply is important.

Poultry processing includes bleeding, scalding, defeathering, evisceration, washing and chilling. The whole process can be divided into two areas, the "dirty zone" including stunning, bleeding, scalding, defeathering, and evisceration and the "clean zone" including washing and chilling (Gonzalez-Miret et al., 2006). These stages are common in most processing plants and countries. However, not all processing operations have the capacity to decontaminate and chill carcasses rapidly, which can make a difference in controlling microbial loads (Belluco et al., 2016). Most European poultry processing plants use air-based chilling, whereas water immersion chilling is standard in

the U.S. (Sanchez et al., 2002). Chlorine is also used widely in the U. S. for washing (Northcutt et al., 2003). The final step in poultry processing must be chilling because it is an important step to suppress microbial growth for maximum product safety and shelf life (Allen et al., 2000; Carrol and Alvarado, 2008). However, in developing countries such as Vietnam, most vendors in open markets and indoor markets slaughter their own birds, however, have neither intention nor resources to chill carcasses. On the contrary, vendors in supermarkets receive frozen and packaged whole chickens, thereby having better probability to prevent cross-contamination. When purchasing whole chickens in Vietnam, consumers prefer to keep the internal organs with the carcass in the same bag. This can pose serious microbiological safety implications because bacterial pathogens such as *Salmonella* survive in the intestines of infected birds throughout their lifetime. Moreover, during slaughter, fecal contamination from the internal organs can occur (Adeyanju and Ishola, 2014). Throughout poultry processing, interventions are applied at critical control point to lower overall bacterial counts. Studies have shown that as the poultry carcass is further processed bacterial loads decrease (Mead, 2004; Lues et al., 2007; Svobodová et al., 2012). No research has conducted to quantify effects of bird processing interventions on microbiological safety in retail establishment. In the U.S., *Salmonella*-positive incidence in young chicken has been at 3.8 % in 2013 and 2014. *Salmonella* prevalence in ground chicken has been steadily at 44.6%. There has not been any comprehensive study on *Listeria* levels in whole chicken. However, few researchers such as Kuan et al. (2013) reported *Listeria* was found at consistently high levels of 20.8 to 33.3% in chicken offal. This is of particular importance because of culinary culture of consuming offal in developing countries such as Vietnam. Few authors have investigated

bacterial pathogens on whole chickens in Vietnam (Luu et al., 2006; Van et al., 2007a; Ta et al., 2012), however, influence of market setting, time of purchase, and meat merchandising has never been evaluated. Therefore, the objective of this study was to investigate *Salmonella* and *Listeria* prevalence, microbiological quality, and vendors' practices in various chicken markets at two sampling times in Ho Chi Minh City (HCMC), Da Nang (DN), and Ha Noi (HN) in Vietnam.

Materials and Methods

Sample Collection and Preparation

The supermarkets (SM), indoor markets (IM), and open markets (OM) in Ho Chi Minh City, Da Nang, and Ha Noi, were selected to achieve adequate representation of regional variation in poultry processing and merchandising in Vietnam. Markets were classified by their infrastructure in Table 7. Within each region, two of the most popular grocery markets per market type were selected, resulting in six markets per region. Locally raised and processed whole chickens were purchased in each market at two sampling times, the opening time (T0) and 4 h after opening (T4). Briefly, five whole chickens averaging 1000-g each, were purchased individually from various vendors in each market at both sampling times, resulting in 180 samples. Vendors randomization was performed as described in chapter II for all markets and both sampling times. If a market had less than five vendors, at least one vendor was sampled repeatedly. There was no vendor randomization in SM because each SM was the sole poultry vendor; however, samples were purchased separately by different buyers. Moreover, whole chickens in SM were individually overwrapped in Styrofoam™ trays and displayed on refrigerated shelves. Vendors in IM and OM processed their own birds at time of

purchase, defeathered, and rinsed birds in water before being collected aseptically. The samples were placed individually in sterile Nasco Poultry Rinse Bags (Nasco, Fort Atkinson, WI). Carcass surface temperature was recorded by a Fisher Scientific™ Traceable™ Infrared Thermometer Gun (Fisher Scientific, Waltham, MA). Bags were sealed, stored in an Igloo Super Tough Sportsman ice chest (Igloo, Katy, TX) with frozen ice packs, and transported to a local university for further preparation.

Weight of whole chickens were recorded, 90 mL of Buffered Peptone Water broth (BPW; 25.5 g/L; 3M, St. Paul, MN) was added to Nasco Poultry Rinse Bags (Nasco, Fort Atkinson, WI; Vipham et al., 2012), and bags were shaken for 60 s. Volume of BPW used for the whole chicken rinse was evaluated by using food color solution to ensure that it was sufficient to wash of surface and body cavity. Two sterile 15-mL polypropylene tubes (Greiner Bio-One, Monroe, NC) of BPW rinsate were collected and stored on ice to be transported to HCMC University of Technology for further analyses.

Microbiological Analysis

Salmonella was analyzed as described in chapter II. In a sterile Whirl-Pak® bag (Nasco, Fort Atkinson, WI), 2.5 mL of BPW rinsate and 22.5 mL of Salmonella Enrichment Broth (3M, St. Paul, MN) were combined and incubated at 45°C for 24 h. After incubation, 1 mL of solution was combined with 10 mL of Rappaport-Vassiliadis R10 Broth (RVR10; 3M, St. Paul, MN) and incubated again at 41.5°C for 24 h. A 10-µL streak of the incubated RVR10 solution was made onto 3M™ Petrifilm™ of the Salmonella Express System. The Petrifilm™ was incubated at 41.5°C for 24 h.

Presumptive positive *Salmonella* spp. colonies were isolated and identified by a red color with yellow halo.

Listeria was identified as described in chapter II. The BPW rinsate (2.5 mL) was combined with Demi-Fraser Listeria Enrichment Broth (22.5 mL; 3M, St. Paul, MN) in a sterile Whirl-Pak® bag (Nasco, Fort Atkinson, WI), incubated at 30°C for 24 h, and subsequently spread onto an ALOA® agar petri dish. The dish was inverted and incubated at 37°C for 24 h. Presumptive positive *Listeria spp.* colonies were isolated and identified by a blue to green color with or without halo.

Microbiological quality analyses were performed as described in chapter II. Original BPW rinsate was serially diluted with sterile BPW by a 10^{-2} factor to a final volume of 1.5mL. One mL of each dilution was spread separately onto APC Petrifilm™ and *E. coli*/Coliform Petrifilm™ and incubated at 35°C for 24 h. Colony forming units (CFU) for aerobic bacteria (Aerobic Plate Counts, APC), *E. coli,* and coliforms were counted according to the 3M interpretation guides (3M, 2015c; 3M, 2015d).

Except for sterile sampling bags, all pipettes, tips, tubing, and solutions were autoclaved before microbiological analyses. Blank enrichment, isolation, and incubation on all solutions including sterile water were performed for all microbiological assays to ensure no contamination to samples.

Market Characteristics

Outdoor temperature (ºC), relative humidity (%), meat surface temperature (ºC), type of retail display, availability of refrigeration, use of gloves and hairnets, cleaning of

knife before cutting meat, and water availability were recorded for individual samples on data collection forms.

Calculation and Statistical Analysis

The incidence of *Salmonella* and *Listeria* was reported as percentage of positive samples estimated by statistical model. Bacterial counts were reported as log CFU/g, calculated from CFU as follows:

$$\log \text{CFU/g} = \log \left(\frac{N}{V} \times DF \times V_0 \times \frac{1}{m} \right) \quad (3)$$

with N, V, DF, V_0, and m being number of colony forming units, volume of a spread (1 mL), dilution factor, original volume of BPW rinsate (90 mL), and carcass weight (g), respectively. Market characteristic data were reported as crude percentage without statistical analysis.

All statistical analyses were performed using generalized linear mixed model estimated by the GLIMMIX procedure in SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). The prevalence of *Salmonella* and *Listeria* were analyzed as a 3 × 2 factorial arrangement in a randomized complete block design with region as block, market type and sampling time as two factors, and a specific market at a specific sampling time as experimental unit, using logistic regression. However, in the same design, linear regression was used to analyze microbiological quality data with whole chicken being the experimental unit. Market type, sampling time, and their interaction were the fixed effects and region was the random effect. Means were separated by the protected t-test by using the LSMEANS statement with the PDIFF option in the GLIMMIX procedure. Statistical significance was determined at $P \leq 0.10$.

Results and Discussion

Microbiological Quality

There was no difference in bacterial counts of APC, *E. coli*, or coliforms between two sampling times in all markets ($P = 0.170$, 0.281, 0.874, respectively; Table 9 and Figure 12). Many APC Petrifilm™ were too numerous to count (TNTC) at 10^{-6} dilution with pink color in the entire growth area (3M, 2015b) and were estimated at 10^8 CFU. Whole chickens purchased in SM, IM, and OM markets were contaminated with greater than 10.5, 7.7, and 9.5 log CFU/g of APC, *E. coli,* and coliforms, respectively (Figure 11). *E. coli* and coliform counts were greater in IM than in SM ($P = 0.002$ and 0.006, respectively; Figure 11). Furthermore, *E. coli* counts were also greater in OM than in SM (8.3 and 7.7 log CFU/g, respectively; $P = 0.024$), whereas both OM and SM had the same level of coliform count ($P = 0.170$). The high bacterial counts indicated that whole chickens in these markets had much more bacterial loads than what is normally observed in developed countries. It is important that good management practices must be used during slaughtering and processing stages to minimize bacterial contamination (Buncic and Sofos, 2012). Carcass hygiene is very important to identify critical control points and correctly manage poultry processing (Belluco et al., 2016). Aerobic bacteria and *E. coli* are commonly used hygienic indicator organisms throughout poultry production process (Adeyanju and Ishola, 2014). *E. coli* counts are usually more correlated with *Enterobacteriaceae* counts, the levels of which in poultry carcasses have been routinely linked to processing hygiene, handling, and storage conditions (Whyte et al., 2004; Williams et al., 2015). Market observations during this study in IM and OM revealed that conditions of cages used to store chickens before slaughter and water used to defeather

birds and rinse carcasses could provide an insight into increased levels of APC, *E. coli*, and coliforms. The cages and water were unsanitary with abundance of fecal materials. Water used to rinse live chickens was used for final rinse of carcasses. In IM and OM, chilling was not available to all vendors, although some did have access to refrigeration to store final products. Allen et al. (2000) observed 1.28 log CFU/carcass reduction when water chilling was used. However, water chilling can also be a primary vehicle for foodborne pathogens (Demirok et al., 2013). Extensive bird-to-bird contact by water chilling can result in pathogen cross-contamination to other carcasses (Bilgili et al., 2002). There is currently not much research in the U.S. or other developed countries on aerobic bacteria and *E. coli* enumeration in poultry, primarily because *Salmonella* is a more challenging problem in the poultry industry. Moreover, there has not been any research on *E. coli* counts in whole chickens to be used as a hygienic indicator for market types in Vietnam. Therefore, the data in the current study provides important baseline information for the meat and poultry industries in Vietnam.

Prevalence of *Salmonella*

The prevalence of *Salmonella* was 30.4, 58.77, and 35.8% in SM, IM, and OM, respectively (Figure 13). Among market types, IM had 28.4 and 23.0% greater *Salmonella* prevalence than SM and OM ($P = 0.006$ and 0.022; Figure 13). Sampling time had no effect on *Salmonella* incidence rate ($P = 0.515$) and there was no market type × sampling time interaction ($P = 0.822$). Presently, Vietnam does not have a foodborne disease surveillance system to monitor annual incidence of human salmonellosis (Ta et al., 2012). *Salmonella* is a major cause of foodborne disease worldwide, especially in Southeast Asia (Ta et al., 2012; CDC, 2015a). *Salmonella* is isolated more from raw

poultry than from other foods (CDC, 2007) because of the bacteria can survive in intestinal tract of birds.

Few studies published in Vietnam investigated *Salmonella* in chicken; however, they are limited in geographical variation and sample size (Phan, 2005; Luu et al., 2006; Van et al., 2007b; Ta et al., 2012). These authors reported 48.9, 53.3, 21.0, and 45.9% incidence rate, respectively, which are comparable to the levels found in whole chickens in the current study. However, the data collected throughout the country from 2005 to 2012 indicated some degree of variation, as Van et al (2007) observed only 21.0% incidence rate, compared with 53.3 % reported by Luu et al (2006). Ta et al. (2012) conducted a more similar study to the current study in Ha Noi, Da Nang, and Ho Chi Minh City. These authors revealed 51.1 (N = 239), 45.5 (N = 33), and 44.7% (N = 264) prevalence, respectively. Averaging across all markets and sampling times for each region, the current study showed that *Salmonella* prevalence in Ha Noi, Da Nang, and Ho Chi Minh City was at 47.1, 25.0, and 55.0%, respectively. Market types in Vietnam vary in poultry processing and handling. SM receives frozen retail products that may have undergone decontamination and chilling. Chilling is a crucial step to prevent microbial growth (Demirok et al., 2013). Vendors in IM and OM markets did not have infrastructure and financial resources to decontaminate or chill chicken carcasses. This might explain greater bacterial loads and pathogenic incidence rate than SM. A study conducted in open markets in China reported 52.2% *Salmonella* prevalence, approximately 16.4% greater than the results in the current study (Yang et al. 2011). Recent retail surveys have revealed that *Salmonella* prevalence on raw chicken carcasses varied among countries, at 68.2% in Ethiopia (Tibaijuka et al., 2003), 66.0% in Thailand

(Jerngklinchan et al., 1994), 60.0% in Portugal (Antunes et al., 2003), 55.0% in Spain (Dominguez et al., 2002; Capita et al., 2003), but only 4.2% in the U.S. (Zhao et al., 2001).

Water washing is also important in poultry processing. This step can either decrease or increase the bacterial load. Increased bacterial loads, especially those of *E. coli* could lead to increased *Salmonella* incidence rate (Gill and Baker, 1998). A Spearman rank correlation conducted in the current study revealed a correlation between *Salmonella* prevalence and *E. coli* counts ($r = 0.52$; $P = 0.001$). Moreover, proper washing with clean water has been shown to decrease *Salmonella* prevalence by the physical removal of injured or semi-attached cells (Cox et al., 2010). However, in IM and OM, clean water was not always readily available. Carcasses were rinsed in the same water throughout the day. This practice can lead to cross-contamination among poultry carcasses (Kuan et al., 2013). Yang et al. (2011) observed wet markets in China, similar to open markets in Vietnam, where the supply of potable water is limited. These authors reported that the eviscerated birds were rinsed with minimal amounts of water or dipped in a tank without frequent change of water. The vendors at these Chinese wet markets were so busy that they seldom had time to wash their hands, scales, and other tools; therefore, it was suggested that cross-contamination between chicken carcasses with *Salmonella* was likely to be the cause of increased *Salmonella* incidence (Yang et al., 2011). This could also be attributed to the prevalence of *Salmonella* found in the current study, in which up to 26.7 and 6.7% of IM and OM vendors, respectively, wore gloves. Many consumers are not aware of safety risks associated with contamination of raw chicken with *Salmonella* because chicken is usually cooked thoroughly by boiling

before consumption (Othman, 2003). However, chicken meat is usually associated with direct hand-to-mouth exposure to pathogens and cross-contamination to food preparation area (Yang et al., 2011). In the U.S., an estimated 11% of human *Salmonella* infections annually is attributed to exposure to live poultry (USDA, 2014b) and 17% of all foodborne illnesses are associated with poultry (Painter et al., 2013). Moreover, undercooking of chicken is a major source of salmonellosis (Yang et al., 2016). The potential risks of foodborne illnesses to consumers at retail markets could be reduced by implementing proper processing practices throughout the poultry production chain.

Prevalence of *Listeria*

Listeria prevalence for each market at a specific sampling time was reported in Table 9. Averaging across two sampling times, *Listeria* prevalence in whole chicken samples was at 56.6, 78.6, and 73.2% in SM, IM, and OM, respectively. The incidence rate of *Listeria* was less in SM than in IM and OM ($P = 0.024$ and 0.089, respectively; Figure 13). There was no effect of sampling time or market type × sampling time interaction on *Listeria* prevalence ($P = 0.113$ and 0.415, Table 9). Variation of 10 and 27% in *Salmonella* incidence rate between T0 and T4 in IM and SM was not different compared with virtually no variation between T0 and T4 in OM; i.e., no interaction (Table 9). This could be explained by a great within-market variation in *Listeria* contamination. The widespread occurrence of *Listeria* spp. in the environment can result in the contamination of food products, including poultry carcasses in processing facilities (Chiarini et al., 2009). *Listeria* has been shown to survive in the environment of food processing plants for an extended time (Lunden et al., 2003; Loura et al., 2005). Moreover, *L. monocytogenes* can colonize floor drains and persist for years (Berrang et

al., 2013). Recent research on prevalence of *Listeria* in whole chickens is not widely available in both developed and developing countries. *Salmonella* is studied more because it is present in the intestines of birds, whereas *Listeria* is mostly from environmental contamination. Frequencies of *Listeria* presence in raw broiler carcasses was reported from 41 to 84% (Uyttendaele et al., 1999). Studies have indicated that the improper cleaning and disinfecting of equipment in poultry processing facilities can lead to contamination of the poultry carcasses (Loura et al., 2005; Adeyanju and Ishola, 2014). Uytttendaele et al. (1999) reported an increase in *Listeria* contamination rate as carcasses moved through cutting and further processing of poultry carcasses. Contamination rates of whole carcasses, carcasses with parts, and retail products were 41.3%, 46.7%, and 61.0% (Uytttendaele et al., 1999). Furthermore, additional handling of poultry carcasses during processing, especially after chilling, has shown to be responsible for an increase in prevalence at the end of the processing line (Genigeorgis et al., 1989). This could be explain the prevalence in SM because SM vendors received prepackaged frozen or refrigerated whole chickens, which remained refrigerated. Since *Listeria* is psychrotrophic, this mode of distribution might explain the increased levels of *Listeria* prevalence in SM compared with normal levels (Chiarini et al., 2009). *Listeria* has been isolated from raw poultry (Miettinen et al., 2001); however, prevalence is greatly varied. Pini and Gilbert (1988) found 60% of *Listeria*-contaminated raw chickens in the United Kingdom, whereas, Bailey et al (1989) only observed 23% prevalence in raw poultry carcasses in retail establishments in the U.S. Moreover, Loncarevic et al. (1994) reported 0 to 64% prevalence of *Listeria* in raw broiler meat. The results from the current study were slightly greater than the previously mentioned studies. The current study was

conducted in retail setting, whereas others were in processing facilities. Miettinen et al (2001) observed *Listeria* contamination in processing facilities as low as 1 to 11%, whereas the prevalence at the retail was 62%. The increased prevalence at retail could be attributed to poor vendor hygiene. Genigeorgis et al (1989) observed workers' hygiene in poultry processing facilities and reported that 46.7% of the workers harbored *Listeria* spp. in their hands and gloves. Moreover, Loura et al. (2005) sampled bare hands of food handlers and reported that 60% of samples had *Listeria.* Hygienic conditions in all markets in Vietnam was poor with only 16.7, 16.7, and 6.7% of SM, IM, and OM vendors at T0 and 16.7, 26.7, and 3.3% of SM, IM, and OM vendors at T4 using gloves. Hygienic conditions of IM and OM vendors could be the reason for the 22.0 and 16.6% increase in *Listeria* prevalence compared with SM. Poultry products are recommended to be cooked to 74°C (FSIS, 2014), thereby assuming a low risk of *Listeria.* However, opportunities of cross-contamination to other foods consumers' food preparation areas should be considered (Loura et al., 2005; Voidarou et al., 2011).

Market Characteristics

Characteristics of markets and vendors in Vietnam were summarized in Table 8. Cover displays for whole chickens were overwrapped packaging or display case as a physical barrier between the products and consumers, which were only used at some SM and IM vendors. At T0, 50.0 and 73.3% of the SM and IM used cover displays. However, at T4, 83.3 and 20.0% SM and IM used cover displays. Moreover, 100.0% of OM vendors exposed whole chickens to open air at both sampling times. From observations during sampling, if the chickens were slaughtered at market, whole chickens were hung by feet after processing and displayed openly without wrapping until closing.

If not sold by the end of the day, the carcasses were overwrapped and stored for sale the next day. Likewise, in SM, chickens were processed at a central location, overwrapped, and frozen for transportation to SM. Refrigeration was used by 50.0, 66.7, and 6.7 % of SM, IM, and OM vendors at T0 and by 100.0, 23.3, and 0.0% of SM, IM, and OM vendors at T4, respectively. Refrigeration in SM at T0 was used for display and at T4 used for storage. Moreover, refrigeration in IM and OM was used for storage purposes only. As mentioned previously, hygienic conditions in all market was poor with only 16.7, 16.7, and 6.7% of SM, IM, and OM vendors at T0 and 16.7, 26.7, and 3.3% of SM, IM, and OM vendors at T4 using gloves. Chickens carcasses were rarely further processed because they are sold as whole birds. Furthermore, only 20.0% of OM vendors at T0 and 30.0 and 10.0% of OM and IM at T4 cleaned knives. Because whole chickens were shipped to SM frozen and packaged, there was no cutting necessary in SM (0.0%). Hot water was used by SM and IM only at T0, at 3.3 and 16.7%, respectively, whereas 10.0 and 23.3% of OM vendors used hot water at T0 and T4, respectively. Fresh water was available and used by 21.7, 25.0, and 10.0% of SM, IM, and OM vendors at T0 and 41.7, 20.0, and 5.0% at T4 by SM, IM, and OM vendors, respectively.

Vendors in IM and OM provide reasonably priced and conveniently available poultry products for lower income population. Chamhuri and Bratt (2013) reported that consumers in Malaysia still preferred to shop at open and street vendor markets even though they were informed that meat in those markets were not as safe as meat in supermarkets were safer (Chamhuri and Batt, 2013). This attitude is important for the policymakers in Vietnam to consider because whole chicken is the most popular poultry product in Vietnam and much of poultry supply was processed in poor hygienic

conditions. Cross-contamination to other foods and risks of salmonellosis and listeriosis will increase tremendously if whole chicken is not cooked properly.

Conclusion

Whole chickens were determined with high counts of bacteria and incidence of *Salmonella* and *Listeria*. In addition to *Salmonella,* the current study provides essential data of *Listeria*, which is lacking in not only developed countries but also developing countries such as Vietnam. The incidence of *Salmonella* and *Listeria* on whole chickens in various market types in Vietnam was increased compared with current data in literature. Furthermore, there were 7.5 to 10.9 logs of indicator organisms such as aerobic bacteria, *E. coli,* and coliforms, among which *E. coli* counts were correlated with *Salmonella* prevalence. Refrigeration, cleanliness, water usage, and good hygienic conditions can improve the status of microbiological safety and quality of poultry products in Vietnam. Much research is needed to establish a baseline for contamination at critical control points of poultry processing in Vietnam's meat markets. These data justify an enhanced enforcement of food safety regulations and a creation of education programs for both consumers and vendors in Vietnam's meat markets.

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Tables and Figures

Table 7 Characteristics used to classify supermarkets (SM), indoor markets (IM), and open markets (OM) across three regions of Vietnam.

Market Characteristics	Market Type		
	SM	IM	OM
Multiple vendors		√	√
Air-conditioning	√		
Refrigeration	√		
Walls	√	√	
Roof	√	√	
Clean water availability	√	√	√

√ Existing characteristics

Table 8 Observational and environmental data collected during the purchase of whole chickens from supermarkets (SM), indoor markets (IM), and open markets (OM) at the market opening (T0) and 4 h after the opening (T4) across three regions of Vietnam (Ho Chi Minh City, Da Nang, and Ha Noi).

Market Characteristics	SM		IM		OM	
	T0	T4	T0	T4	T0	T4
Outdoor temperature, °C	27.8 ± 1.0	29.0 ± 1.7	25.2 ± 0.5	28.7 ± 0.7	27.4 ± 1.7	31.2 ± 1.6
Humidity, %	68.2 ± 5.6	59.3 ± 3.8	83.3 ± 4.6	70.5 ± 5.7	73.3 ± 5.7	63.1 ± 5.7
Meat surface temperature, °C	15.4 ± 1.6	14.5 ± 2.6	24.2 ± 1.8	21.8 ± 2.0	30.7 ± 5.5	25.9 ± 1.3
Cover display, %	50.0 ± 0.2	83.3 ± 0.1	73.3 ± 0.2	20.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Hang display, %	0.0 ± 0.0	13.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Open display, %	50.0 ± 0.2	30.0 ± 0.2	26.7 ± 0.2	80.0 ± 0.2	100.0 ± 0.0	100.0 ± 0.0
Refrigeration, %	50.0 ± 0.2	100.0 ± 0.0	66.7 ± 0.2	23.3 ± 0.2	6.7 ± 0.1	0.0 ± 0.0
Gloves, %	16.7 ± 0.2	16.7 ± 0.2	16.7 ± 0.2	26.7 ± 0.2	6.7 ± 0.1	3.3 ± 0.0
Hairnet, %	33.3 ± 0.2	66.7 ± 0.2	50.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cleaned knife before cutting, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	30.0 ± 0.2	20.0 ± 0.2	10.0 ± 0.0
Hot water, %	3.3 ± 0.0	0.0 ± 0.0	16.7 ± 0.2	0.0 ± 0.0	10.0 ± 0.1	23.3 ± 0.1
Fresh water, %	21.7 ± 0.1	41.7 ± 0.1	25.0 ± 0.1	20.0 ± 0.1	10.0 ± 0.1	5.0 ± 0.0

*Values were reported as means ± standard error of the means.

Table 9 Bacterial counts and the prevalence of *Salmonella* and *Listeria* in whole chickens procured from supermarkets (SM), indoor markets (IM), open markets (OM) at the market opening (T0) and 4 h after the opening (T4) across three regions of Vietnam (Ho Chi Minh City, Da Nang, and Ha Noi).

Microbiological Measurement*	SM		IM		OM		*P* *market type*	*P* *time*	*P* *interaction*
	T0	T4	T0	T4	T0	T4			
APC[1], log CFU/g	10.8 ± 0.1 ^{ax}	10.8 ± 0.1 ^{ax}	10.87 ± 0.0 ^{ax}	10.9 ± 0.0 ^{ax}	10.9 ± 0.0 ^{ax}	10.1 ± 0.5 ^{ax}	0.233	0.170	0.118
E. coli[2], log CFU/g	7.5 ± 0.5 ^{ax}	8.0 ± 0.5 ^{ax}	8.39 ± 0.5 ^{bx}	8.7 ± 0.5 ^{bx}	8.3 ± 0.7 ^{bcx}	8.3 ± 0.7 ^{bcx}	0.006	0.281	0.619
Coliform[3], log CFU/g	9.4 ± 0.4 ^{ax}	9.7 ± 0.3 ^{ax}	10.1 ± 0.3 ^{bx}	10.23 ± 0.3 ^{bx}	10.1 ± 0.3 ^{abx}	9.6 ± 0.4 ^{abx}	0.024	0.874	0.162
Salmonella[4] prevalence, %	25.5 ± 9.8 ^{ax}	35.8 ± 11.3 ^{ax}	57.0 ± 11.8 ^{bx}	60.5 ± 11.6 ^{bx}	35.8 ± 11.3 ^{acx}	35.8 ± 11.3 ^{acx}	0.013	0.515	0.822
Listeria[5] prevalence, %	42.7 ± 15.8 ^{ax}	69.61 ± 13.9 ^{ax}	73.2 ± 13.03 ^{bx}	83.2 ± 9.8 ^{bx}	73.2 ± 13.0 ^{bx}	73.18 ± 13.0 ^{bx}	0.060	0.113	0.415

[1] Aerobic Plate Count, enumerated using 3M™ Petrifilm™ Aerobic Plate Count (3M, St. Paul, MN).
[2] *Escherichia coli*, enumerated using 3M™ Petrifilm™ E. coli/Coliform Count Plates (3M, St. Paul, MN).
[3] Coliform, enumerated using 3M™ Petrifilm™ E. coli/Coliform Count Plates (3M, St. Paul, MN).
[4] *Salmonella,* detected using 3M™ Petrifilm™ *Salmonella* Express System (3M, St. Paul, MN).
[5] *Listeria,* detected using ALOA® media (BioMerieux, St. Louis, MI).
^{xy} Within market type, means without common letters differ, $P \leq 0.1$.
^{ab} Within sampling time, means without common letters differ, $P \leq 0.1$.
*Values were reported as estimated least squares means ± standard error of the means.

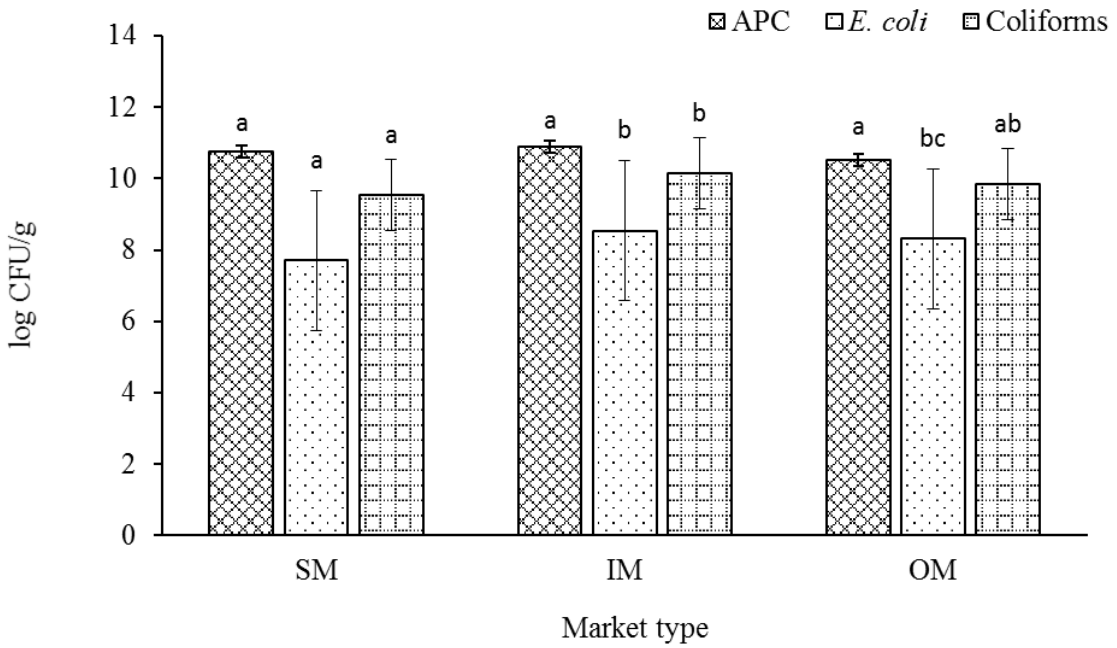

Figure 11 Aerobic Plate Count (APC) and *E. coli* and coliform counts (log CFU/g) of whole chickens purchased from supermarkets (SM), indoor markets (IM), and open markets (OM) in Ho Chi Minh City, Da Nang, and Ha Noi of Vietnam, averaged across two sampling times.

Within a category of bacterial count, means without common letters differ, ($P_{market\ type}$ = 0.233, 0.006, 0.024, respectively).

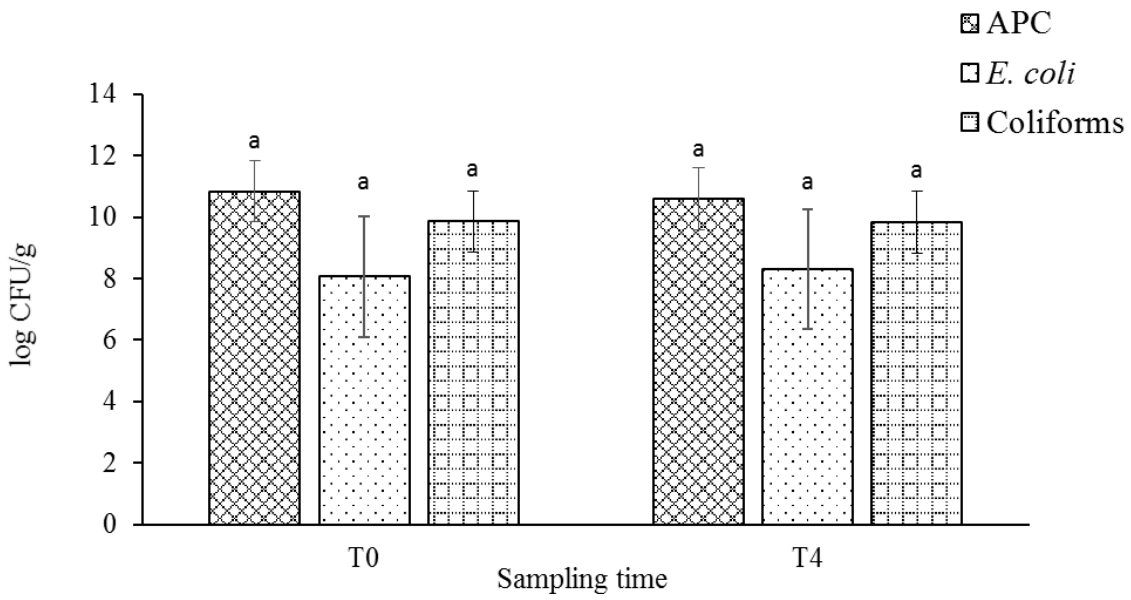

Figure 12 Aerobic Plate Count (APC) and *E. coli* and coliform counts of whole chickens purchased at two sampling times (opening - T0 and 4 h after opening - T4) in Ho Chi Minh City, Da Nang, and Ha Noi of, averaged across supermarkets, indoor markets, and open markets

Within a category of bacterial count, means without common letters differ, ($P_{sampling\ time}$ = 0.170, 0.281, 0.874, respectively).

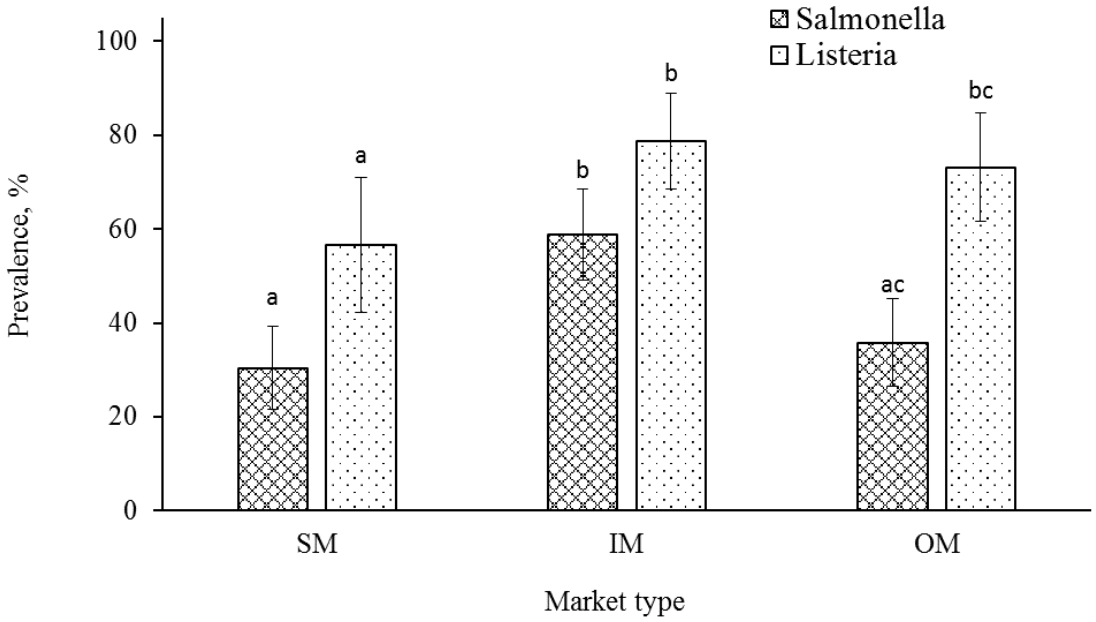

Figure 13 *Salmonella* and *Listeria* prevalence in whole chickens purchased from supermarkets (SM), indoor markets (IM), and open markets (OM)) in Ho Chi Minh City, Da Nang, and Ha Noi of Vietnam, averaged across two sampling times

Within a pathogen category, means without common letters differ, ($P_{market\ type}$ = 0.013 and 0.060, respectively).

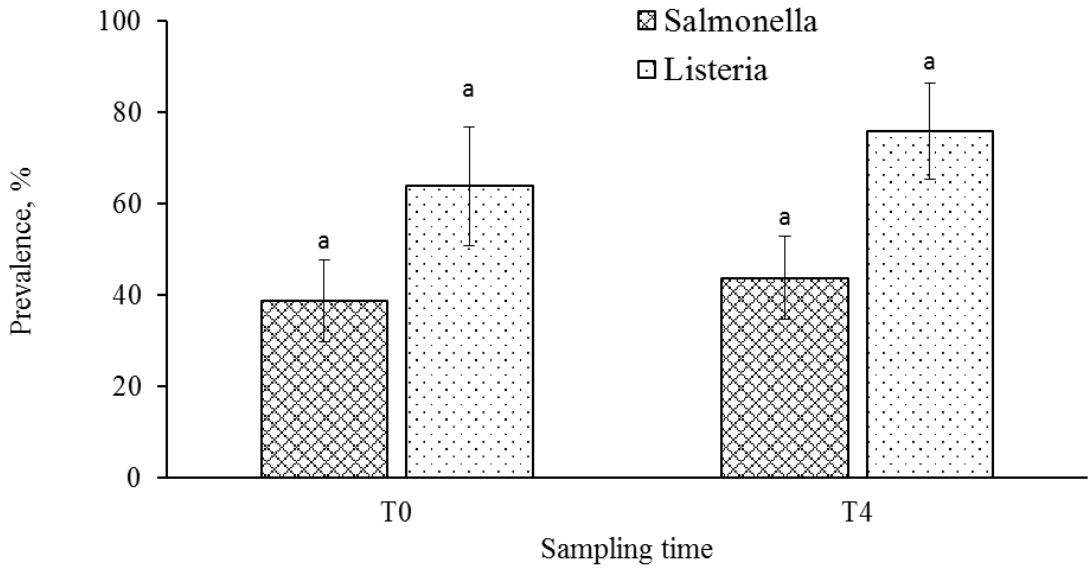

Figure 14 *Salmonella* and *Listeria* prevalence in whole chickens purchased at opening (T0) and 4 h after opening (T4) in Ho Chi Minh City, Da Nang, and Ha Noi of Vietnam, averaged across supermarkets, indoor markets, and open markets)

Within a pathogen category, means without common letters differ, ($P_{sampling\ time}$ = 0.515 and 0.113, respectively).

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CHAPTER V

CONCLUSION

This study was the first comprehensive study on the microbiological quality and safety of beef, pork, and chicken in Vietnam. Ho Chi Minh City, Da Nang, Ha Noi, and their surrounding areas were selected to achieve adequate representation of regional variation in meat merchandising in Vietnam. Three market types, including supermarkets, indoor markets, and open markets, were investigated. Two most popular grocery markets were selected for each market type in each region, resulting in six markets per region. Sampling was conducted at opening time of individual markets and at 4 h after opening. At each sampling time, five beef, pork, and chicken samples were purchased to determine counts of total aerobic bacteria, *E. coli,* and coliforms and presumptive positive colonies of *Salmonella* and *Listeria*.

There were effects of market type and sampling time on bacterial counts and prevalence of *Salmonella* and *Listeria* in beef, pork, and chicken in Vietnam. Bacterial counts and pathogen incidence in the current study were much greater than those reported in the U.S. and various developed countries. This could be attributed to lack of good manufacturing practices and standard operating procedures in packing plants and market vendors.

Therefore, more research is needed in mapping pathogen contamination and mitigating associated risks in developing countries. Moreover, mandatory training for

vendors and education programs pertaining food safety must be implemented. Furthermore, additional food safety regulations must be implemented and enforced.