Evaluation of poultry transport flooring cleaning methods for the reduction of *Salmonella* and *Campylobacter*

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science in Poultry Science

Auburn, Alabama
August 5, 2023

Keywords: *Salmonella*, *Campylobacter*, transport containers, steam, hot air, holding

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ABSTRACT

In response to unsuccessful efforts to reduce human *Salmonella* infections associated with poultry products, new approaches have been proposed by the FSIS. These include implementing risk analysis and the potential for logistical slaughter in poultry processing plants. However, the effectiveness of logistical slaughter may vary due to potential cross-contamination during transport, particularly when broilers are placed in uncleaned transport containers. To address this issue, in the first chapter the efficacy of pressurized steam followed by forced hot air was compared to conventional cleaning procedures. Fiberglass and plastic flooring pieces were contaminated with *Salmonella* Infantis and *Campylobacter jejuni*. The treatments included pressurized steam, forced hot air, pressurized steam followed by forced hot air, pressure washing, pressure washing before and after disinfectant, and no cleaning. The greatest reductions in *Salmonella*, *Campylobacter*, and *E. coli* were observed with pressurized steam followed by forced hot air and pressure washing with water before and after disinfectant. Additionally, the second chapter focused on *Salmonella* transfer from transport drawer flooring to broiler chickens during different holding times. Treatments included pressure washing before and after disinfectant, pressurized steam followed by forced hot air, and no cleaning. The results showed lower transfer of *Salmonella* to broilers placed in cleaned containers compared to non-cleaned containers. *Salmonella* transfer decreased after 6 hours in non-cleaned containers, and top drawers showed lower *Salmonella* transfer than middle or bottom drawers in cleaned containers. The application of pressurized steam and forced hot air showed comparable results to the use of water washes and disinfectant, highlighting their potential role of pressurized steam, and forced hot air in cleaning poultry transport containers.

ACKNOWLEDGMENTS

I want to express my deepest gratitude to God, my family, and my friends for their invaluable support and love. I am incredibly thankful to Dr. Bourassa for her patience and guidance during this process. To Gregory Smith, thank you for your kindness and unconditional support, life is brighter next to you. To Andrea Urrutia, Juan Figueroa, Montana Riggs, Abigail McConnell, Madalyn Jennings, Fanny Contreras, Matthew Hughes, and Wellington Arthur, I am profoundly grateful for all your help during those long experiment days. I could not have achieved this milestone without your immense support. I am truly blessed to have been given this life-changing opportunity, which has brought significant improvement to my life. My time in Auburn has been filled with precious memories that I will forever cherish. Thank you all for being a part of this incredible journey.

"*I will make you into a great nation, and I will bless you.*
I will make your name great, and you will be a blessing"
(Genesis, 12:2)

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LIST OF ABBREVIATIONS

200NAL	200 ppm of Nalidixic acid
CDC	Centers for Disease Control and Prevention
CFU	Colony-forming unit
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
ERS	Economic Research Service
FAO	Food and Agriculture Organization
FSIS	Food Safety and Inspection Service
IFSAC	Interagency Food Safety Analytics Collaboration
NACMCF	National Advisory Committee on Microbiological Criteria for Foods
NCC	National Chicken Council
ODPHP	Office of Disease Prevention and Health Promotion
PBS	Phosphate Buffered Saline
PR/HACCP	Pathogen Reduction/ Hazard Analysis and Critical Control Point
qPCR	Quantitative polymerase chain reaction
SMA	Standard Methods Agar
USDA	U. S. Department of Agriculture
USDHHS	U. S. Department of Health & Human Services
UV	Ultraviolet
WGS	Whole-genome sequencing
WHO	World Health Organization
XLT4	Xylose Lysine Tergitol 4 agar

CHAPTER 1. LITERATURE REVIEW

1.1 INTRODUCTION

The U. S. poultry industry holds a prominent position as a global leader of poultry meat production. Additionally, the U. S. ranks as the second largest exporter of poultry meat worldwide. Over the past few decades, the consumption of poultry meat has steadily increased in the U. S., resulting in a noticeable displacement in the consumption of other types of meat. Currently, the consumption of poultry meat in the U. S. surpasses the consumption of beef and pork, reflecting a significant change in consumer preference from previous generations. This trend can possibly be attributed to the combination of affordable prices and health recommendations, which have boosted the popularity of poultry meat among consumers (USDA-ERS, 2022). The production of ready-to-cook chicken in 2022 reached 46.21 billion pounds, whereas in 1972, it was only 8.15 billion pounds. This indicates that the production of ready-to-cook chicken meat has grown approximately 5.67 times larger over the span of just 50 years. It is important to note this increment in pounds produced has been mostly achieved by intensifying live production and optimizing the performance of broilers. Advancements in genetics, nutrition, health care, housing management, and technology have facilitated the adaptation of chickens to meet the growing market demand (NCC, 2023).

One of the main factors that has permitted this substantial increase in productivity is the vertical integration that occurred in the poultry industry. Vertical integration occurs when a single company coordinates every stage of the process, which includes the breeding flocks, hatcheries, feed mills, transportation, and processing plants. Vertical integration has been driven by the large economy of scale and the substantial value added after processing, making poultry processors the head coordinators of the industry, more often referred to as “integrators”. Although the

integrators have ownership of the birds within the farms, they do not own the farm facilities themselves. Instead, the integrators enter into production contracts with independent farmers, often referred to as “growers”, entrusting them with the task of growing the birds until they reach a certain market weight. The use of contracts in live production offers a reduced financial risk for both parties, and it has helped growers to access capital and has expedited the adoption of new technology. As a result, the U. S. poultry industry has enhanced its competitive position over the past decades by improving efficiency, maintaining affordable consumer prices, and significantly expanding its market share (Vukina, 2001).

The vertically integrated structure of the poultry industry increased the responsibility of integrators to ensure the safety of their final products by applying interventions throughout the entire production process. Also, as the poultry industry in the U. S. expanded, food safety regulations were adapted to address emerging public health issues associated with poultry meat consumption. Monitoring biological hazards like *Salmonella* and *Campylobacter* has become a necessary practice for all the poultry industry. Since 1996, the U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) established the *Salmonella* verification program and performance standards for whole carcasses as part of the Pathogen Reduction/ Hazard Analysis and Critical Control Point (PR/HACCP) systems program. Later versions of the program incorporated standards for *Campylobacter* and expanded the scope of products to include raw chicken parts and non-ready-to-eat comminuted poultry products (Singh and Thippareddi, 2020).

According to the FSIS’s monitoring data, after the PR/HACCP program was implemented, the prevalence of *Salmonella* in poultry products has decreased significantly. For example, the *Salmonella* prevalence for chicken parts reported by FSIS in 2022 (calendar year)

was 6.76%, which contrasts with the 24% prevalence reported in 2012 for the same category. In the case of *Campylobacter* prevalence results do not show such a substantial decrease when the same time period is compared. The prevalence of *Campylobacter* in chicken parts reported in 2012 was 21.7%, while the prevalence reported in 2022 was 16.14% (USDA-FSIS, 2012; 2022; 2023). Nonetheless, the reduction observed in *Salmonella* prevalence has not shown to be effective in reducing *Salmonella* infections across the U. S. According to the U. S. Department of Health & Human Services, Centers for Disease Control and Prevention (2022a), the incidence rate of *Salmonella* infections has remained relatively consistent from 1996 to 2021, fluctuating between 13.34 and 18.28 per 100,000 population. These indicators strongly suggest that the existing strategies implemented to reduce pathogens in poultry products have not yet produced a noticeable influence on public health. As a result, it is imperative to conduct a comprehensive reassessment of the current interventions and to adopt a fresh approach that can deliver measurable outcomes and effectively address the challenges at hand.

1.2 PUBLIC HEALTH CONCERNS OF THE POULTRY INDUSTRY IN THE U. S.

Poultry meat inspection emerging in the U. S.

Public health concerns for poultry meat products were first acknowledged in 1926, when the USDA established a voluntary inspection and grading service for poultry processors. Then, the growth in consumer demand for poultry products led to the passage of the Poultry Products Inspection Act in 1957, which mainly focused on the inspection for animal diseases before initiating interstate commerce. Gradually, this approach shifted over time to verify the wholesomeness and visible contamination of chicken carcasses, and also addressed concerns about mislabeling and product adulteration (USDA-FSIS, 2018). Later, in 1968, the Wholesome Poultry Act substituted the Poultry Products Inspection Act. This new act required states to

maintain inspection programs “at least equal to” the federal inspection program. In 1965, the federal meat and poultry inspection merged into a single program, but it was not until 1977 that the task was formally assigned to Food Safety and Inspection Service (FSIS), formerly known as the Food Safety and Quality Service (FSQS) (Ollinger and Mueller, 2003; USDA-FSIS, 2018).

In 1996, FSIS issued the Pathogen Reduction/Hazard Analysis and Critical Control Points (PR/HACCP) systems rule for all meat and poultry processing plants, focusing on preventing and reducing microbial pathogens in raw poultry products. This regulation required that each poultry processing plant develop a HACCP plan that was adapted to their processes, and after completion it would require direct approval from FSIS. This new approach considered assessing risks to effectively control pathogens instead of the traditional visual inspection performed in the industry (Crutchfield et al., 1997; Ollinger and Mueller, 2003). Moreover, this new program helped to clarify the roles of both government and industry, with the industry being responsible for ensuring the production of safe food, while the government would set appropriate safety standards, conduct inspections, and properly enforce regulations. PR/HACCP was progressively implemented across the U. S. between 1997 and the year 2000. Since then, FSIS has intensified its efforts to combat foodborne pathogens, including enhanced testing for specific pathogens and the establishment of stricter standards (Crutchfield et al., 1997; Ollinger and Mueller, 2003; USDA-FSIS, 2018).

PR/HACCP implementation

HACCP was developed by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) as an approach to ensure food safety throughout the entire production and consumption process. The seven HACCP principles were determined to focus on hazard analysis, identification of critical control points, establishment of critical limits, adoption of monitoring

procedures, application of corrective actions, implementation of verification procedures, and thorough record-keeping. The standardization of HACCP principles has helped to ensure consistent training and application within both the industry and government sectors. Successful implementation of HACCP could provide benefits such as optimized resource utilization and prompt problem resolution (USDHHS-FDA, 1997).

The food safety strategy outlined by FSIS in the PR/HACCP program considered five key components. First, the promotion of science-based process control systems for meat and poultry establishments to systematically prevent biological, chemical, and physical hazards. Second, the implementation of fitted measures to control and reduce harmful bacteria on raw meat and poultry products. Third, the adoption of microbiological performance standards to incentivize innovation and ensure accountability in achieving acceptable food safety results. Fourth, elimination of unnecessary regulatory barriers to foster innovation. Fifth and last, addressed a wider spectrum of hazards spanning from farm to table. The implementation of the final rule required that federally inspected establishments adopt HACCP to address potential hazards in their operations. Once the rule took effect, FSIS began the verification of HACCP system operations as part of its inspection program. Processing plants were required to maintain a HACCP plan encompassing all poultry products intended for human consumption (USDA-FSIS, 1996)

Verification program for raw poultry products

Since the initial rule of PR/HACCP systems program in 1996, FSIS has demonstrated a proactive approach by continuously gathering data and updating the performance standards for *Salmonella*. As a component of the *Salmonella* Verification Program, FSIS evaluates whether establishments adhere to the pathogen reduction performance standards for *Salmonella* in various

poultry products. This includes young chicken carcasses, raw chicken parts, and non-ready-to-eat (NRTE) comminuted chicken (USDA-FSIS, 2021a). However, in the case of *Campylobacter*, FSIS has plans to revise such performance standards. FSIS continues to conduct product testing for *Campylobacter*, but they do not currently evaluate whether establishments meet the performance standards or not. *Salmonella* performance standard verification samples are systematically collected within a dynamic 52-week time frame, known as a moving window, to facilitate the continuous evaluation of establishment compliance with the performance standards (USDA-FSIS, 2021b).

When assessing process control, the moving window approach involves the analysis of a consecutive series of results obtained from a single establishment over a specific period of time. FSIS initiates the evaluation by considering the number of samples acquired during this full 52-week period. Later, as the evaluation progresses, the 52-week window shifts by one week, incorporating the most recent week's testing outcomes while discarding the oldest week's results. This methodology ensures an ongoing and thorough assessment of performance standards over time. *Campylobacter* samples are also collected and evaluated in a similar manner, but as mentioned above, FSIS does not assess the compliance of the establishments under the current *Campylobacter* performance standards (USDA-FSIS, 2019; 2021a; 2021b).

Campylobacter associated with poultry meat

Campylobacter infection typically manifests with symptoms that could include bloody diarrhea, fever, abdominal cramps, and potentially nausea and vomiting, with a duration of approximately one week. In some cases, the infection may have complications such as irritable bowel syndrome, temporary paralysis, and arthritis. Additionally, infection with *Campylobacter jejuni* is a causative factor in Guillain-Barré syndrome, which is a condition characterized by

muscle weakness and paralysis. According to national statistics, in the U. S. approximately 1 in every 1,000 individuals who have contracted *Campylobacter* infection could develop Guillain-Barré syndrome. According to the U.S. Department of Health & Human Services, Centers for Disease Control and Prevention, *Campylobacter* causes an estimated of 845,024 illnesses, 8,463 hospitalizations, and 76 deaths every year in the U. S. (USDHHS-CDC, 2018; 2021; 2022b). The economic impact *Campylobacter* infections hold on the U. S. economy has an estimated value of 1.6 billion USD per year, accounting for approximately 12% of the overall economic burden associated with foodborne pathogens (Hoffman et al., 2015).

The Interagency Food Safety Analytics Collaboration (IFSAC) reported that from 1998 to 2015, 64.7% of *Campylobacter* illnesses were associated with chicken consumption. However, in a subsequent report, this information was retracted due to limitations associated with the model used for *Campylobacter*, specifically. As a result, a reassessment of the causes is currently underway (ISFAC, 2021; 2022). In a recent study presented by Hoffman et al. (2021), they evaluated the association of foodborne illnesses with daily food purchases, instead of food consumption. In their results they indicated that the consumption of chicken prepared at home did not display an elevated risk of campylobacteriosis, while consuming chicken from restaurants was associated with a higher risk. This study casts doubt on the high association of poultry products and sporadic *Campylobacter* illnesses and further research is required.

Despite this discrepancy, poultry is known to be a natural reservoir of *Campylobacter,* commonly found in the cecum and colon, with colonization typically occurring around three weeks after hatching. Horizontal transfer through feces plays a significant role in the spread of *Campylobacter* to different flocks. The sources of *Campylobacter* contamination in birds include the external environment, previous flocks, other animals, contaminated water (Sahin et al., 2001;

Schroeder et al., 2014). Factors such as the presence of viable but nonculturable *Campylobacter* in various poultry production environments and the prevalence of specific virulence genes should be considered when evaluating and enumerating *Campylobacter* populations. However, the routine isolation and detection of *Campylobacter* in poultry have been difficult. These challenges arise from the complex nutrient growth requirements and the need for the specific microaerophilic gas atmosphere to culture the pathogen. Also, due to the variability of *Campylobacter* species, more informative characterizations are necessary to obtain a comprehensive assessment of potential risks (Ricke et al., 2019; Kim et al., 2019).

At the moment, the genus *Campylobacter* includes 17 species and 6 subspecies, but the most commonly reported to cause human illnesses are *C. jejuni* (subspecies *jejuni*) and *C. coli*. While species like *C. lari* and *C. upsaliensis* have also been identified, their occurrence is less frequent (WHO, 2020). In the U. S., limited data is available regarding the specific species responsible for campylobacteriosis, as approximately 80% of the infections are not speciated. For the remaining cases where species identification was possible, *C. jejuni* has been most frequently linked to human illness, accounting for 17% of cases from 1996 to 2021 (USDHHS-CDC, 2022a). Some studies have shown that chicken carcasses could have a variety of *Campylobacter* species, which is an aspect that should be considered in the future (Dickins et al., 2002; Walker et al., 2019).

Salmonella associated with poultry meat

Consuming food products contaminated with *Salmonella* can lead to salmonellosis, characterized by symptoms such as diarrhea, fever, and abdominal cramps, typically resolving within a week. According to the U.S. Department of Health & Human Services, Centers for Disease Control and Prevention, *Salmonella* causes an estimated of 1,027,561 illnesses, 19,336

hospitalizations, and 378 deaths every year in the U. S. (USDHHS-CDC, 2018; 2023). In economic terms, *Salmonella* holds a significant burden on the country, with an estimated cost of 3.7 billion USD per year. *Salmonella* infections account for approximately 24% of the overall economic burden associated with foodborne pathogens (Hoffman et al., 2015). The Interagency Food Safety Analytics Collaboration (IFSAC) reports that 75% of *Salmonella* illnesses are attributed to just seven food categories, with chicken as the leading category responsible for 17.3% of associated outbreaks from 1998 to 2015 (ISFAC, 2022).

The transmission of *Salmonella* in poultry meat production can occur through vertical transmission from infected breeder flocks or horizontal transmission within a flock or via environmental sources. *Salmonella* may be present in several environments due to direct contact with infected birds, including hatcheries, grower houses, transportation, and processing plants. Moreover, potential vectors such as insects, rodents, wild birds, feed, and water can contribute to *Salmonella* exposure during the grow-out period. Detecting *Salmonella* contamination during live production and processing presents challenges because of the varied range of potential sources. Conventional culture methods, while capable of detecting *Salmonella*, do not provide specific serovar identification, which is crucial for evaluating public health risks (Ricke, 2021).

Serotypes are crucial for identifying the sources of *Salmonella* infections. For example, an analysis conducted by FSIS in 2014 revealed that *Salmonella* Kentucky (60.8%) and *Salmonella* Enteritidis (13.6%) were the most common serotypes found in young chicken carcasses. However, *Salmonella* Kentucky is not commonly associated with human illnesses when compared to Enteritidis. Other prominent serotypes found were Montevideo, Typhimurium, Infantis, and Dublin, which are directly associated with causing human illnesses (USDA-FSIS, 2014). Recent advancements in whole-genome sequencing (WGS) and CRISPR

technologies have revolutionized the differentiation of serovars and strains, facilitating accurate tracking of *Salmonella* isolates during outbreaks. Quantifying *Salmonella* is also of great importance, and a combination of culture methods and quantitative polymerase chain reaction (qPCR) assays has been employed (Shariat and Dudley, 2014; Ricke et al., 2018). Future technological advancements are expected to enable simultaneous identification and quantification of serovars, accompanied by reduction in assessment time (Ricke, 2021).

Proposed regulatory framework to reduce Salmonella infections

Despite the efforts to reduce *Salmonella* infections associated with poultry products, the current approach has not produced the desired outcomes. Previous targets set by the U.S. Department of Health and Human Services were not met in 2010 nor 2020. Therefore, for 2023 the Healthy People target has been set to reduce *Salmonella* infections by 25%, and FSIS has aligned their objectives, accordingly. Thus, in 2022, FSIS released the proposal of a regulatory framework with the aim of effectively controlling *Salmonella* in poultry products and consequently reducing infections associated with these products (NCHS, 2012; 2021; USDHHS-ODPHP, 2020; USDA-FSIS, 2022).

First component: Requiring incoming flocks be tested for *Salmonella* before entering an establishment. FSIS mentioned that they are currently considering implementing new regulations that would require poultry processing establishments to address *Salmonella* as a potential risk during receiving. As part of this framework, incoming flocks would go through *Salmonella* testing to ensure they meet predetermined standards for *Salmonella* levels before the broilers enter the processing plant. Documentation would be necessary to demonstrate that pre-slaughter *Salmonella* testing has been conducted. In cases where a flock does not meet the designated criteria, the establishment would be required to have protocols in place to prevent cross-

contamination and implement corrective measures to reduce the *Salmonella* load (USDA-FSIS, 2022).

Second component: Enhanced establishment process control monitoring and FSIS verification. FSIS mentioned that they are exploring modification of some existing regulations to strengthen monitoring procedures. These modifications would involve revising sampling locations and implementing statistical process control methods to improve the control of *Salmonella*. This component is proposed to establish re-hang as the pre-chill location to sample but the existing requirement to conduct sampling at post-chill would remain unchanged. Also, this component is proposed to use a standardized statistical approach to assess process control, which would establish consistent microbial data definitions and enable effective monitoring and action-taking in cases of processes outside the established limits (USDA-FSIS, 2022).

Third component: Enforceable Final Product Standard. FSIS mentioned that they are exploring the feasibility of establishing a uniform product standard for *Salmonella* across all raw poultry items. The standard would serve as an incentive for implementing *Salmonella* reduction practices throughout the production process, requiring that interventions on-farm and transportation practices meet a prescribed criterion. Within the framework, FSIS restated that the agency possesses the authority to enforce compliance and may contemplate discontinuing the existing *Salmonella* performance standards, including the moving window approach and the categorization of establishments by size (USDA-FSIS, 2022). All elements of the proposed framework outlined above are fundamentally rooted in the concept of implementing risk analysis and afterwards employing logistical slaughter in poultry processing plants.

Risk analysis and logistical slaughter

The proposed transition aims to shift from conventional food safety controls to comprehensive risk-based controls across all stages of food production, for which risk analysis plays a crucial role in assessing and managing risks associated with food safety. Risk analysis in food safety involves a structured method that includes risk assessment, risk management, and risk communication as its components. The risk analysis process starts with risk management defining the problem, setting goals, and determining the questions for the risk assessment. Risk assessment includes measuring and describing the nature of the analyzed risk through a risk profile. Then, the decision for implementation of corrective actions when required and continuous monitoring and adjustments would be based on new data or changes in the problem context. Lastly, risk communication involves interactive exchange of information among all stakeholders throughout the risk analysis process, including sharing risk-related information, factors, perceptions, and explaining assessment findings and management decisions (Attrey, 2017; FAO and WHO, 2023).

Logistical slaughter is a strategic intervention that could be implemented in poultry processing plants, where flocks would be subjected to pathogen testing shortly prior (minimum of 24 h) to their designated harvest day. Subsequently, flocks would be classified as low or high risk, with the low-risk flocks receiving priority in processing and being handled before the high-risk flocks. The primary goal of this approach is to reduce cross-contamination during processing by reducing the possibility of pathogen presence during processing of low-risk flocks. Although logistical slaughter is generally acknowledged as a favorable measure to control cross-contamination, there is limited data that clearly demonstrates its benefits (Evers, 2004).

A study conducted by Sasaki et al. (2013) provides supporting evidence on the advantages of implementing logistical slaughter. They found that the contamination rate in chicken products from *Campylobacter* positive flocks was higher (51.1%) compared to those from *Campylobacter* negative flocks (7.2%). On the other hand, when *Campylobacter*-negative flocks were slaughtered first, no contamination was observed in the corresponding chicken products. Based on these findings, Sasaki et al. (2013) concluded that the strategic use of logistical slaughter to prioritize *Campylobacter* negative flocks can effectively reduce the prevalence of contaminated chicken products. Miwa et al. (2003), Potturi-Venkata et al. (2007), and Schroeder et al. (2014) also observed similar findings in their respective studies, emphasizing the potential of logistical slaughter as an intervention to minimize cross-contamination during processing.

Nonetheless, other studies do not report the same level of success, as logistical slaughter showed limited advantages when the likelihood of cross-contamination is high between the farm and the processing plant. For example, Rasschaert et al. (2008) studied the relationship between *Salmonella* colonization in poultry flocks and carcass contamination after slaughter. In this study, discrepancies were observed between the *Salmonella* status of poultry flocks at the farm and their status at slaughter. Additionally, even when *Salmonella* positive flocks have not been processed, carcasses still showed contamination. Rasschaert et al. (2008) suggested the potential for cross-contamination from equipment or transport crates makes it challenging to achieve the benefits of logistic slaughter.

The results presented by Choi et al. (2014) offer additional support to the previous statement. Their study examined the prevalence and distribution of *Salmonella* within an integrated broiler company, by sampling broiler breeder farms, commercial broiler farms, broiler

trucks, slaughterhouses, and retail chicken meat. Choi et al. (2014) highlighted that broiler transporting trucks had the highest prevalence of *Salmonella* (71.43%), followed by the slaughterhouse (63.89%), while broiler farms showed the lowest prevalence (16.05%). The findings from both studies suggest that there is a high likelihood of cross-contamination after the broilers leave the farm, and relying solely on the *Salmonella* status of the flock before harvesting is insufficient information to adequately control the risk.

Furthermore, statistical risk models tend to agree that the benefits of logistical slaughter are directly related to cross-contamination rates in each step of the process. For example, Evers (2004) presented a mathematical model that predicted the prevalence of contamination after both logistical and random order slaughter. The analysis showed that the effectiveness of logistic slaughter depended on factors such as the probability of cross-contamination, the length of the slaughter queue, and the sensitivity of detecting contamination. The model by Evers (2004) suggested that the benefit of logistic slaughter may be limited, especially when the prevalence of contaminated flocks is very low or very high (when none of the flocks are contaminated or when all of the flocks are contaminated).

In a different study, Nauta et al. (2005) presented a quantitative microbial risk assessment for *Campylobacter* in poultry processing. The model developed suggested that the impact of reducing and removing bacteria was more important for carcasses with high initial levels of *Campylobacter*, while cross-contamination was more significant for carcasses with low initial levels of *Campylobacter*. The study also indicated that logistical slaughter as a risk mitigation strategy had minimal effects after scalding and defeathering, as low cross-contamination was expected during those steps. Both models suggest that to achieve the benefits of logistical slaughter, it is essential to minimize cross contamination at every stage of the process once the

broilers have left the farm, which opens the possibility to find interventions and better practices during catching, transportation, and lairage.

Cross-contamination during catching and transportation

In the U. S., the majority broilers on farms are manually caught by catching crew, also referred to as "catchers." During the catching process, broilers are gripped by their legs as it is the standard procedure in the industry. The National Chicken Council (2017) Animal Welfare Guidelines and Audit Checklist for broilers strictly forbid lifting, carrying, dragging, or throwing the broilers by their wings or necks. The number of broilers held in the catcher's hand depends on the bird's size, ensuring no harm is caused. For broilers weighing more than 4 pounds, the maximum limit is five birds per hand. In the case that a mechanical catching system is used, it is important to have a standard operating procedure in place to ensure that the broilers are handled according to the same guidelines as hand-caught birds. (NCC, 2017).

Prior studies have examined the potential for cross-contamination resulting from human traffic, particularly involving the catching crew (Hald et al., 2001). This concern arises due to the fact that catchers frequently move between different farms. *Campylobacter* has been isolated from clothes, hands, and boots of farm staff, managers, catchers, and drivers (Herman et al., 2003; Ramabu et al., 2004). The implementation of a robust biosecurity program and comprehensive training of personnel in these practices can significantly reduce the risk of cross-contamination during this stage.

For example, the study performed by Racicot et al. (2013) compared different hand cleaning and sanitization methods for the catching crew. The methods tested included water and soap, degreasing cream and hand wipes, both combined with alcohol-based hand gel. Then the use of only alcohol-based gel was also evaluated as well. All of the protocols showed to

effectively neutralize *Salmonella*. Racicot et al. (2013) suggested prioritizing the reduction of organic material before using alcohol-based gel, either using degreasing cream or water and soap could achieve this reduction.

In the U. S. the transportation of broilers involved the use of wooden or plastic coops before the 1970s. These coops were manually carried from the broiler houses to trucks or trailers. However, as the demand for broilers grew and the necessity for more efficient transportation systems became evident, the innovative "dump cage" system was developed and implemented in the U. S., which has persisted as the main transportation system today. The dump cage system revolutionized the process by replacing traditional coops with modular cage units, which are formed by a galvanized metal frame with multiple solid fiberglass floors. The use of forklifts has played a key role in transporting these modules to and from trailers during loading and unloading operations. Remarkably, during the unloading phase, the need for manual labor was significantly reduced as the broilers were effortlessly removed by gently tilting the modules once they are unloaded from the trailer. The dump cage has proven to be highly advantageous, leading to a significant decrease in labor (Aldridge, 2017).

In addition to the conventional dump cage system, an alternative approach uses detachable plastic drawers within a metal framework or stackable trays. The drawers or trays are designed with numerous openings on the side walls and in the floor to provide ventilation, but it is important to note that these openings easily gather debris and should be able to be thoroughly cleaned (Box, 1989; Weaver, 1999). When catching the drawers or trays are designed to be partially open, allowing the catching crew to place the broilers inside. The nature of this system provides greater adaptability, as when damaged or faulty the drawers or trays can be replaced. Upon arrival at the processing plant, the modules are unloaded and transferred onto an automated

conveyor line. Then a different mechanism pushes out each drawer onto another conveyor belt to be directed in front of the operators' shackles (Kettlewell and Turner, 1985).

Furthermore, establishments equipped with controlled atmosphere stunning can use the traditional “dump-cage” system but generally favor the use of plastic drawer or tray systems. In this setup, the drawers or trays are unstacked and individually pushed onto a conveyor, but in this case the conveyor belt leads the drawers into a gas stunning tunnel or chamber. Throughout the controlled atmosphere stunning system, the broilers are gradually exposed to increasing concentrations of CO_2. This characteristic not only ensures effective stunning but also contributes to a reduction in the overall CO_2 usage when compared to systems that use on-truck controlled atmosphere stunning systems (AVMA, 2016). Hereafter, the term "transport containers" will be used to collectively refer to both systems (the dump cage and plastic drawers).

Several previous studies have documented the relationship between transport containers and cross-contamination during transport (Heyndrickx et al., 2002; Slader et al., 2002; Berrang et al., 2003; Herman et al., 2003; Rasschaert et al., 2007; 2008; Marin and Lainez, 2009; Schroeder et al., 2014). One of the main examples is the study conducted by Berrang et al. (2003), which evaluated the risk of *Campylobacter* contamination on broiler carcasses when birds were exposed to a contaminated dump cage. In their study, after 4 h feed withdrawal broilers confirmed positive with *Campylobacter* were placed into a dump cage for 8 hours. Immediately after their removal, *Campylobacter* negative broilers were placed in the same compartments (without cleaning) and held for up to 6 hours. The results of this study showed that over 50% of the defeathered carcasses from the initial negative broilers showed *Campylobacter* prevalence after exposure to the contaminated dump cage compartments. Berrang et al. (2003) concluded

that transporting *Campylobacter* negative broilers in a contaminated container could result in carcass contamination, and that the contamination acquired during lairage could remain on the carcasses even through scalding and picking.

These mentioned studies collectively highlight that implementing effective measures to prevent cross-contamination during transport is necessary to reduce the prevalence of foodborne pathogens throughout the processing line (Heyndrickx et al., 2002; Slader et al., 2002; Berrang et al., 2003; Herman et al., 2003; Rasschaert et al., 2007; 2008; Marin and Lainez, 2009; Schroeder et al., 2014). However, the economic and environmental implications associated with such decision-making are multifaceted and they should be considered before implementing a new cleaning procedure.

Water usage in poultry processing plants

The availability of water is essential for poultry processing, as it is a necessary component for various interventions and cleaning procedures that ensure the production of safe poultry products. In 2004, a survey on water usage in broiler processing facilities in the U. S. reported that processing plants use 26.0 liters of water per bird. Based on the survey findings, it was mentioned that the implementation HACCP resulted in an average increase of around 5.4 liters per bird in water usage. This increase could possibly be attributed to the adoption of new practices or the intensification of their current interventions. Additionally, the survey noted that only 28.4% of the facilities surveyed reported using a truck or coop washing station. However, the majority of processing plants that reported implementing this practice were categorically small facilities, and they represented 11.9% of all establishments surveyed (Northcutt and Jones, 2004).

It is necessary to acknowledge that the decision to adopt a new cleaning procedure involves significant economic factors. For example, in a different survey conducted by Kiepper et al. (2003), it was reported that in 2003 the chicken processing plants had an average water consumption of 1.46 million gallons per day, and an average water cost was 1.64 USD per 1000 gallons of water. Nonetheless, the challenges faced by poultry processors encompass a broad spectrum of possible scenarios, with the highest reported water consumption reaching 4.50 million gallons per day and the highest cost reported at 6.75 USD per 1000 gallons of water.

When considering the cleaning of transport cages, different approaches have been explored to avoid the use of water. For example, a study conducted by Berrang et al. (2004) evaluated the effect of extended storage as an intervention to reduce *Campylobacter* in a dump cage. The experiment involved *Campylobacter* positive broilers placed in new, unused cages and then the broilers were held for 8 hours before removal. Then, the empty cages were stored under a shed and sampled at various time intervals (up to 48 h) to assess viable *Campylobacter*. The results of this study showed that there was no decrease in *Campylobacter* numbers during the first 8 hours of storage. However, after 24 hours, reductions in levels and prevalence of *Campylobacter* were observed. Then after 48 hours of storage, *Campylobacter* reached undetectable levels in the dump cage. Berrang et al. (2004) suggested in their study that storing soiled transport cages for 48 hours between uses could reduce *Campylobacter* levels, but it may not completely eliminate the bacteria. Unfortunately, due to the cost of each cage and space requirements, this approach might not be a practical intervention to adopt for poultry processors.

Additionally, there is a significant distinction between *Campylobacter* and *Salmonella*, and it is important to recognize that extended storage may not be an effective measure for controlling both pathogens, as their survival rates in the environment can differ significantly.

Topalcengiz et al. (2020) evaluated the survival of *Salmonella* in the fecal samples from waterfowl across four different states in the U. S. over a period of one year. During their study, *Salmonella* was detectable in the stored waterfowl fecal samples for a duration ranging from 84 days to 308 days differentiated based on their origin location. Topalcengiz et al. (2020) stated that factors affecting microorganism survival in fecal matter could include diet, moisture content, pH, and chemical composition.

A different study evaluated the survival and persistence of *Campylobacter* and *Salmonella* on different food contact surfaces using different media for the suspension of bacteria. The study clearly showed that *Salmonella* had a higher resistance to the environment when compared to *Campylobacter*. When suspended in phosphate-buffered saline solution, *Salmonella* was 1.4 to 22 times more robust than *Campylobacter*, while in the trypticase soy broth, *Salmonella* was 2 to 130 times more robust than *Campylobacter*. Regardless of the suspension medium used, *Salmonella* showed significantly greater resilience than *Campylobacter* when subjected to various contact surfaces, including Formica, stainless steel, ceramic tile (De Cesare et al., 2003). Therefore, effective interventions that focus on physically removing feces from poultry transport containers and directly reducing pathogen loads (both *Salmonella* and *Campylobacter*) should be explored.

Current cleaning systems for poultry transport containers

As previously mentioned, in the U. S. cleaning transport containers is not a widespread practice in the poultry industry (Northcutt and Jones, 2004). However, there are some transport container cleaning systems available (Northcutt and Berrang, 2006; Dzieciolowski et al., 2022; Morgan et al., 2022). In the U. S., the efficacy of a partially automated cleaning system for dump cages was documented by Northcutt and Berrang in 2006. The system was comprised of two

stages. First, the cages underwent an automated spray wash using stationary nozzles with water from the chiller overflow. Then, in the second stage, an employee utilized a high-pressure hose to rinse the cages with regular water. The results showed limited reductions for aerobic bacteria (1.3 $\log_{10}$ CFU/cm^2), coliforms (1.6 $\log_{10}$ CFU/cm^2), and *E. coli* (1.5 $\log_{10}$ CFU/cm^2), evidencing the low efficacy of such system. Following the implementation of the washing system at the processing plant, Northcutt and Berrang (2006) proceeded to apply a chlorine-based sanitizer to the cages. This extra step resulted in additional reductions in bacteria levels and the prevalence of *Salmonella* and *Campylobacter*. However, it is important to note that even after this extra step, bacteria levels on the fiberglass flooring could still be regarded as high (for aerobic bacteria: 7.0; coliforms: 5.6; *E. coli*: 5.2 $\log_{10}$ CFU/cm^2) and the counts for *Salmonella* and *Campylobacter* were not assessed in this study.

Outside of the U. S., Dzieciolowski et al. (2022) documented the efficacy of a 5-step automated washing system for plastic drawers. The process included prewashing with water spray, followed by soaking in a tank filled with cold water and 0.5% (v/v) sodium hypochlorite, then undergoing three high-pressure washing modules. After that, the crates were dried using cold air blades and finally disinfected with sodium hypochlorite (0.5% v/v) using spray nozzles. Even though the process for the plastic drawers was more intensive than the process documented for the dump cage (Northcutt and Berrang, 2006), the reductions were shown to be limited as well. Under this method used for plastic drawers, in average aerobic bacteria was reduced 2.2 $\log_{10}$ CFU/mL and *Enterobacteriaceae* reduction was 1.6 $\log_{10}$ CFU/mL. Then, Dzieciolowski et al. (2022) replaced the high-pressure cold air drying and used low-pressure hot air drying. This change in the process led to greater reductions in aerobic bacteria (3.4 $\log_{10}$ CFU/mL) and

Enterobacteriaceae (3.8 $\log_{10}$ CFU/mL). Unfortunately, no pathogens counts were assessed in this study.

Another study performed outside the U. S. was conducted by Morgan et al. (2022) and documented the results for an automated cleaning tunnel for loose plastic crates. In the cleaning tunnel, the process started with a water spray, followed by the application of a chlorinated alkaline detergent (undisclosed concentration), rinsing with water spray, and finishing with the application of 1% benzalkonium chloride-based disinfectant. In this study, the prevalence of *Campylobacter* was evaluated and quantified using the most probable number method. The results by Morgan et al. (2022) indicated that the cleaning tunnel did not result in a decrease in *Campylobacter* levels. However, reductions in *Campylobacter* levels and prevalence were observed after 24 hours of natural drying, which aligns with the findings reported by Berrang et al. (2004).

The aforementioned studies (Northcutt and Berrang, 2006; Dzieciolowski et al., 2022; Morgan et al., 2022) collectively suggest that the existing systems utilized in processing plants are inadequate in achieving substantial reductions in bacterial loads, highlighting the need for exploring alternative approaches. Furthermore, the findings from Berrang et al. (2004), Dzieciolowski et al. (2022), and Morgan et al. (2022) emphasize the potential significance of drying as a critical factor in reducing bacterial load and potentially mitigating the presence of pathogens.

Research exploring conventional approaches to clean poultry transport containers

In the present study, the term "conventional" refers to interventions that involve water washes (in the form of low-pressure or high-pressure sprays) and the use of disinfectants, which are commonly preferred but require a significant amount of water for their implementation. For

example, in 2005, Berrang and Northcutt evaluated the effectiveness of a low-pressure water spray (10 PSI) combined with or without an immersion dip in a chemical sanitizer. In this lab scale experiment, 5 x 5 cm fiberglass flooring was used, and two types of disinfectants at 200 ppm were evaluated (quaternary ammonium chloride and sodium hypochlorite) using different dipping times (15 seconds, 30 seconds, and 5 min). The findings from this experiment indicated that regardless of the duration of dipping in the sanitizers, the flooring samples did not show additional reductions in coliforms, *E. coli*, and *Campylobacter* when compared to the samples that only underwent a water rinse. Berrang and Northcutt (2005) stated that high pressure water spray could result in higher reductions and the possibility of a second water spray should be considered. However, they emphasized that the economic and environmental costs are limiting for the implementation of additional water sprays.

A different study performed by Ramesh et al. (2002) indicated that higher concentrations of disinfectants might be required to effectively reduce bacterial loads. In their study a total of 13 commercial disinfectants were selected and assessed for their ability to reduce *Salmonella* in galvanized steel surfaces. Out of the thirteen disinfectants tested, two of them completely eliminated *Salmonella*, achieving reductions of 7.18 $\log_{10}$ per coupon within just 2 min. One of the effective disinfectants contained sodium hypochlorite at a concentration of 500 ppm (vol/vol), while the other disinfectant was an alkaline peroxide compound at a concentration of 10,000 ppm (wt/vol). Both of these studies (Ramesh et al., 2002; Berrang and Northcutt, 2005) clearly illustrate that the effectiveness of disinfectants varies depending on the type of compound and its concentration, for which continuous monitoring during their application may be required.

In 2015 and 2018, Hinojosa et al. have evaluated the efficacy of foaming disinfectants and cleaners, as well as the impact of high-pressure and low-pressure water rinses, in reducing

bacterial loads in dump cage flooring. In the 2015 study, the results indicated that the application of a foamed disinfectant containing peroxyacetic acid (5.9%) and hydrogen peroxide (27.3%), diluted at a ratio of 1:32, resulted in a significant reduction of 4.45 $\log_{10}$ CFU/mL for aerobic bacteria. However, when the foamed disinfectant was combined with a high-pressure water rinse, no additional reductions were observed in the dump cage flooring. Then, in 2018, Hinojosa et al. conducted a similar experiment but, on this occasion, they evaluated peroxyacetic acid (1:32 dilution ratio) combined with a foaming agent (1% concentration) to reduce bacterial loads in dump cage flooring.

Similar to the study in 2015, the results in 2018 by Hinojosa et al. showed that foamed disinfectant (peroxyacetic acid in this case) had reduction of 4.71 to 4.77 log10 CFU/mL for aerobic bacteria and 4.12 to 4.22 $\log_{10}$ CFU/mL for *Salmonella*. Hinojosa et al. (2018) also reported the prevalence of *Salmonella* after selective enrichment. According to the results, the use of foam cleansers resulted in a 100% prevalence of *Salmonella*, whereas when a foamed disinfectant (containing peroxyacetic acid) was applied, the prevalence of *Salmonella* decreased to 82%. However, it should be noted that the authors did not perform a statistical analysis since the use of foamed cleaners and foamed disinfectants were evaluated in separate trials.

Furthermore, consistent with the findings from previous studies on established cleaning systems for transport containers (Northcutt and Berrang, 2006; Dzieciolowski et al., 2022; Morgan et al., 2022), the laboratory-scale experiments conducted using conventional methods (Ramesh et al., 2002; Berrang and Northcutt, 2005; Hinojosa et al., 2015; 2018) have not effectively mitigated *Salmonella* or *Campylobacter* while still consuming considerable amount of water and disinfectants, which makes it imperative to explore new alternatives.

Novel approaches for cleaning poultry transport containers

For the purpose of the present study, the term "novel" will be employed to refer to approaches that go beyond the conventional methods previously discussed. Exploring alternatives that do not depend exclusively on water washes (high-pressure and low-pressure) or the use of disinfectants as their primary approach for reducing bacterial levels. For instance, unconventional techniques could include utilizing ultrasonic treatments (Allen et al., 2008), applying cornstarch for drying feces (Berrang et al., 2011b), utilizing slightly acidic electrolyzed water (Zang et al., 2019), or employing ultraviolet light (Moazzami et al., 2021). In addition, Berrang et al. (2011a; 2020) have evaluated certain thermal interventions due to the lower probability of bacterial vegetative cells in developing resistance to heat-based treatments (Cebrián et al., 2017).

Previously mentioned studies conducted by Berrang et al. (2004), Dzieciolowski et al. (2022), and Morgan et al. (2022) have shown that *Campylobacter* is highly susceptible to drying. However, the extended storage of transport containers, as suggested by Berrang et al. (2004) and Morgan et al. (2022), may not be a practical solution due to associated costs, storage space requirements, and uncertainty regarding its effectiveness against other pathogens such as *Salmonella*. For this reason, Berrang et al. (2011a) evaluated the use of hot air, as a measure of reducing the time needed to dry transport cage fiberglass flooring. In this study, different drying methods were compared, including forced air (at 26.1°C), forced hot air (at 50.2°C), and static hot air (at 53.6°C), both with and without a water rinse prior to drying. Following a 15-min application of all methods, the results showed that samples subjected to washing and drying with forced air at 26.1°C had low levels of coliforms, *E. coli*, and *Campylobacter*. However, the most significant reductions were observed in samples that were washed and dried with forced hot air.

Remarkably low levels of coliforms, *E. coli*, and *Campylobacter* (0.48, 0.18, and 0.00 $\log_{10}$ CFU/sample, respectively) were achieved under this treatment when compared to the unwashed control samples (6.30, 6.18, and 6.81 $\log_{10}$ CFU/sample, for coliforms, *E. coli*, and *Campylobacter,* respectively).

In a subsequent study conducted in 2020, Berrang et al. investigated the effectiveness of using flowing steam to decrease bacterial contamination on fiberglass flooring. The motivation for exploring this method surged from previous research suggesting that steam could serve as a practical option for surface sanitization (Chaine et al., 2013; Ban et al., 2014; Berrang et al., 2014). In this study, flooring samples underwent four distinct treatments to examine their effects on bacterial loads. The treatments included an untreated control, a low-pressure water rinse, the application of flowing steam (100°C), and a low-pressure water rinse followed by flowing steam. The fiberglass flooring temperature was raised to 94°C when steam was applied for 15 seconds. The results demonstrated reductions of 2.4, 2.6, and 2.1 $\log_{10}$ CFU/coupon for coliforms, *E. coli*, and *Campylobacter*, respectively. Similar to the previously discussed study conducted by Berrang et al. (2011a), the samples subjected to a combination of washing and thermal treatment (in this case steam) had the most substantial reductions in this study. Reductions of 3.9, 4.0, and 4.7 $\log_{10}$ CFU/coupon were observed for coliforms, *E. coli*, and *Campylobacter*, respectively. Despite not achieving a complete bacterial inactivation, both studies conducted by Berrang et al. (2011a; 2020) showed higher reductions in bacterial loads compared to established cleaning procedures in processing plants (Northcutt and Berrang, 2006; Dzieciolowski et al., 2022; Morgan et al., 2022) all without depending on the use of disinfectants.

1.3 SUMMARY

Efforts to reduce *Salmonella* infections associated with poultry products have been ineffective, leading to the development of new strategies. FSIS has proposed a regulatory framework to control *Salmonella* in poultry processing plants, suggesting incorporating risk analysis and implementing logistical slaughter. However, the effectiveness of logistical slaughter could vary due to factors like the likelihood of cross-contamination after the broilers leave the farm. To achieve the advantages of such intervention, it is required to perform a comprehensive risk assessment throughout the production process, including catching, transportation, and lairage. Studies have emphasized the need for preventive measures to address cross-contamination during transport, especially for uncleaned transport containers. As *Salmonella* and *Campylobacter* survival rates differ, proposed interventions for cleaning transport containers should consider the assessment of both pathogens. The proposal of new cleaning procedures should focus on feces removal, effective pathogen inactivation, low water usage, and possible automation. Studies have shown that conventional cleaning methods using water washes and disinfectants have limited efficacy when removing or inactivating aerobic bacteria, coliforms, *E. coli*, *Salmonella*, and *Campylobacter*, while consuming a significant amount of resources. Novel approaches, such as ultrasonic treatments, cornstarch for drying feces, slightly acidic electrolyzed water, ultraviolet light, hot air, and flowing steam, have been explored as alternatives to rely less on water washes and disinfectants. Some of these methods show promise in reducing bacterial loads, particularly thermal interventions like forced hot air and steam. Studies have shown that when combined with a water wash, steam or forced hot air effectively reduce coliforms, *E. coli*, and *Campylobacter*. There is a necessity to investigate new alternatives and improve the effectiveness of cleaning methods for poultry transport containers.

1.4 KNOWLEDGE GAP IN LITERATURE SPECIFICALLY FOR CLEANING PROCEDURES FOR POULTRY TRANSPORT CAGE FLOORING

Based on all the information presented, studies have evidenced the ongoing challenge of finding an effective solution for cleaning poultry transport containers. However, it is clear that a satisfactory solution is yet to be found. One significant limitation of these studies is their failure to account for the diverse range of transportation systems available, each with its own unique materials and designs. This factor is crucial as it could greatly impact on the effectiveness of the cleaning approaches proposed. Furthermore, the majority of the studies have concentrated on a limited set of microbial indicators and most of the time only assessing either *Salmonella* or *Campylobacter* within the same study. Notably, a significant number of effective treatments discussed have relied on water wash interventions. However, it is worth mentioning that there is a lack of exploration and comparison regarding the use of only thermal interventions, as well as their comparison to conventional cleaning methods. The limited scope of the studies presented has hindered the comprehensive understanding of the overall efficacy of steam and forced hot air as cleaning methods for poultry transport containers. Considering these knowledge gaps, two studies were conducted with the following titles:

- Application of pressurized steam and forced hot air for cleaning broiler transport container flooring.
- Quantification of *Salmonella* Infantis transfer from transport drawer flooring to broiler chickens during holding.

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CHAPTER 2: APPLICATION OF PRESSURIZED STEAM AND FORCED HOT AIR FOR CLEANING BROILER TRANSPORT CONTAINER FLOORING

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2.1 ABSTRACT

In the United States, cleaning poultry transport containers prior to arrival at the broiler grow out farm is not a widely adopted practice in the industry today. However, previous studies have shown that transport containers have an important role in cross-contamination before the broilers arrive at the processing plant. The objective of this study was to evaluate the efficacy of pressurized steam followed by forced hot air to clean transport container flooring and compare it to conventional cleaning procedures. Fiberglass and plastic flooring were cut into even pieces and inoculated with chicken intestinal contents containing *Salmonella* Infantis or *Campylobacter jejuni*. The cleaning treatments were pressurized steam, forced hot air, pressurized steam followed by forced hot air, water pressure washing, water pressure washing before and after disinfectant, and no cleaning. Counts for *Salmonella*, *Campylobacter*, *E. coli*, coliforms, and aerobic bacteria were assessed. Forced hot air applied by itself was not efficient in reducing

Campylobacter, coliforms, and *E. coli* when compared to samples from non-cleaned flooring, but limited reductions (less than 1 $\log_{10}$ CFU/cm^2) were observed for *Salmonella* and aerobic bacteria. Pressurized steam applied by itself showed greater reductions (2.4 to 3.5 $\log_{10}$ CFU/cm^2) than hot air alone for all bacteria evaluated. Further reductions (4.0 to 4.6 $\log_{10}$ CFU/cm^2) were observed when samples were cleaned with one single pressure water wash for all bacteria types. For *Salmonella*, *Campylobacter*, and *E. coli,* the greatest reductions were observed when samples were cleaned with pressurized steam followed by forced hot air (4.3 to 6.1 $\log_{10}$ CFU/cm^2) or water washed before and after disinfectant (4.5 to 6.2 $\log_{10}$ CFU/cm^2), and these treatments did not differ from each other. Pressurized steam followed by forced hot air was shown to be an efficient cleaning procedure to reduce poultry associated pathogens on transport cage flooring, with the benefit of using less water than conventional water cleaning. Processors may be able to adapt this process to reduce potential cross-contamination and lessen the level of pathogens entering the processing plant with the broilers.

2.2 INTRODUCTION

In the United States, raw chicken products that have been mishandled or under cooked have been strongly associated with cases of foodborne illnesses. In 2021, the Interagency Food Safety Analytics Collaboration (IFSAC) reported that chicken products have led the *Campylobacter* and *Salmonella* outbreaks (64.7% and 16.8%, respectively) from 1998 to 2019 (IFSAC, 2021). The U. S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) has approved numerous antimicrobials, like peracetic acid, to be used during poultry processing, which makes focuses the main interventions for controlling foodborne pathogens to generally be performed post-slaughter (USDA-FSIS, 2018). During processing the typical practice is to spray or to dip the carcass or parts in antimicrobials but the outcome will

vary depending on concentration, contact time, and the type of antimicrobial compound used (Zhang et al., 2019; De Villena et al., 2022). Nonetheless, FSIS released in 2022 their new proposed framework to reduce human *Salmonella* infections related to poultry raw meat products. Their intention was to identify key points to improve current procedures and discover innovative opportunities for intervention, including measures taken prior to the broilers' arrival at the processing plant for slaughter. Under the proposal of this framework, FSIS is encouraging researchers, processors, and producers to find the best practices to be implemented to reduce *Salmonella* as a hazard in breeding facilities, hatcheries, grow out, and throughout transport and holding (USDA-FSIS, 2022).

Before the release of the proposed framework, some researchers had already started to work on identifying risk factors and exploring quantitative methods to provide a more accurate risk profile of the processing line (Hue et al., 2010; Akil and Ahmad, 2019; De Villena et al., 2022). This could lead to the application of more suitable solutions by categorizing certain scenarios as low risk or high risk (Rosenquist et al., 2002; Evers, 2004). However, pre-slaughter interventions, like the use of logistic slaughter, are challenged by the high likelihood of cross contamination before broilers reach the processing plant (Berrang et al. 2003; Hue et al., 2010) because broilers that are considered low risk could become contaminated during harvest, transportation, or holding. Multiple previous studies have highlighted that the utilization of non-cleaned transport containers is a contributing factor for cross-contamination before the broilers enter the processing plant (Slader et al., 2001; Berrang et al., 2003; 2004; Rasschaert et al., 2007).

In the case of the poultry industry in the United States, the high volume of production and the related costs of additional cleaning procedures have limited the disposition to clean poultry

transport containers. As reported in 2004 by Northcutt and Jones, 72% of poultry processors surveyed did not have an established procedure for washing transport cages (Northcutt and Jones, 2004). Besides the economic implications, the lack of interest of processors to adopt this practice could be related to the insufficient effectiveness of existing cleaning procedures, as they have not been shown to successfully mitigate pathogens (Slader et al., 2001; Hansson et al., 2005; Northcutt and Berrang, 2006). For example, Berrang and Northcutt (2005) tested the application of water spray and disinfectant on a lab-scale experiment for transport cage flooring. Their findings indicated a significant decrease in the number of bacteria, but complete disinfection was not achieved. Furthermore, they observed varying degrees of effectiveness based on different contact times and different types of disinfecting compound. In a subsequent study, Berrang et al. (2011b) evaluated spray washing combined with different drying times. For this study, they observed that *Campylobacter* was reduced to undetectable levels after 24 h of drying time, regardless of whether the cage flooring was cleaned or not. However, washing the cage flooring expedited this process, as samples subjected to spray washing showed undetectable *Campylobacter* levels after only 2 h.

The argument continues, as environmental impacts are also taken into consideration. A survey conducted by Northcutt and Jones (2004) highlights that water use in poultry processing plants increased 20% after HACCP implementation. Other studies have taken this environmental aspect into account, looking for cleaning procedures that utilize less water or no water at all. For example, Berrang et al. (2011a) evaluated forced hot air as a cleaning procedure for fiberglass flooring from poultry transport cages. In their study, they were able to reduce *Campylobacter* to undetectable levels when samples were first water rinsed and then followed by 15 min of drying time using forced hot air. In a different study, Berrang et al. (2020) evaluated the application of a

water rinse and steam to reduce *Campylobacter* in fiberglass flooring. In their study, when fiberglass flooring was cleaned using a water rinse and then steam, *Campylobacter* was reduced by approximately 5 $\log_{10}$ CFU/5 × 5 cm squares. However, the levels of *Campylobacter* persisted above the threshold of detection and complete disinfection was not achieved. For both of these methodologies evaluated by Berrang et al. (2011a; 2020), it was observed that when wet cleaning is combined with a thermal process *Campylobacter* recovery was significantly reduced. It is important to note that both of these studies relied on the use of water rinsing as a first step (Berrang et al., 2011a), which could deter processors from adopting this method due the requirement to contain and treat the additional wash and rinse water.

Nonetheless, the studies previously mentioned have certain limitations (Berrang and Northcutt, 2005; Berrang et al., 2004; 2011a; 2011b; 2020). As there is an array of options available for handling and transporting poultry, it is important to consider different flooring materials. In the U. S., modular systems have replaced the use of loose stacked crates since the early 1980's. Currently, one of the most widely adopted systems is the "dump-cage", which consists of a metal cage frame with multiple floor decks composed of solid fiberglass. Due to its popularity, fiberglass flooring has been the most commonly evaluated in the research conducted in U. S. (Kettlewell and Turner, 1985; Bilgili, 1999). However, there are other modular systems that employ a comparable concept of multi-floor module but featuring detachable drawers instead. These drawers are usually made of plastic (polyethylene or polypropylene) with perforated grid floors for better ventilation, and these systems are typically preferred for processors that use controlled atmosphere stunning (Box, 1989; Weaver, 1999; AVMA, 2016). Furthermore, another limitation in the mentioned studies is the absence of the assessment of

Salmonella and aerobic bacteria (Berrang and Northcutt, 2005; Berrang et al., 2011a; 2011b; 2020).

Therefore, the objective of this study was to evaluate the efficacy of pressurized steam followed by forced hot air as a possible cleaning procedure for transport container flooring, taking into consideration different flooring types (fiberglass and plastic), the assessment of the most commonly poultry meat associated pathogens (*Salmonella* and *Campylobacter*), and general microbial indicators (aerobic bacteria, coliforms, *E. coli*).

2.3 MATERIALS AND METHODS

Experimental design

For this experiment 2 flooring materials were studied, fiberglass and plastic. Both materials were challenged by 6 different cleaning treatments, which consisted of A: pressurized steam, B: forced hot air, C: the combination of pressurized steam followed by forced hot air, D: a water wash with pressure washer, E: a water wash followed by the application of a commercial disinfectant, then a second water wash, and F: no cleaning. Five samples of each flooring type were evaluated for each treatment, generating a total of 60 samples per repetition. The experiment had 6 repetitions in total, the microbiological tests performed for the first three repetitions were aerobic bacteria, coliforms, *Escherichia coli*, and *Salmonella* counts. Later, in the following three repetitions, only *Campylobacter* counts were assessed. In total, 180 observations were generated for each type of bacteria evaluated. For each repetition, the treatments and type of flooring were randomized before application to prevent skewing by order of execution.

Sample preparation

The flooring was measured and cut into pieces to provide a surface area of 25 cm^2. In the case of fiberglass flooring, the application area was a square of 5.0 x 5.0 cm. However, for the plastic flooring the application area was a square of 5.4 x 5.4 cm due to small perforations per its design, which were taken into consideration to calculate surface area. Before each repetition, all flooring pieces were hand washed and autoclaved at 121°C at 15 PSI with an exposure time of 15 min. To obtain intestinal contents to be applied onto the pieces, viscera packs were collected from a commercial processing facility the day before each experiment and kept refrigerated overnight. On the morning of the experiment 100 g of intestinal contents were manually expressed from the ceca, colon, and ilium into a sterile beaker.

To ensure presence of pathogens, the intestinal contents were inoculated with field strains of *Salmonella* Infantis for the first three repetitions and *Campylobacter jejuni* for the last three repetitions. To prepare the *Salmonella* Infantis inoculum, the field strain was incubated for 24 h at 37°C in Xylose Lysine Tergitol 4 agar (XLT4), then one colony was transferred onto Standard Methods Agar (SMA) and incubated for 24 h at 37°C. The morning of the experiment some colonies were scraped from the SMA surface and suspended in Phosphate Buffered Saline (PBS) to produce cell suspension of 10^8 per mL according to its optical density of 0.15 at a wavelength of 540 nm. A similar process was performed to prepare the inoculum of *Campylobacter*, but in this case the field strain was incubated on Cefex agar for 48 h at 42°C in a microaerobic atmosphere (5% O_2, 10% CO_2, balance N_2). Then, on the day of the experiment, some colonies were scraped directly from the Cefex agar surface and suspended in PBS to produce a cell suspension of 10^8 per mL when an optical density greater than 0.50 was achieved at a wavelength of 540 nm.

After reaching these specifications for each inoculum, 1 mL of *Salmonella* or *Campylobacter* inoculum (depending on the repetition) was applied to 100 g of intestinal contents and mixed thoroughly. For each repetition, the final concentrations of each pathogen were confirmed by plating the serial dilutions of the inoculated intestinal contents on their corresponding selective media. Final concentrations for *Salmonella* were 6.0, 6.1, and 6.2 $\log_{10}$ CFU/g for the first, second, and third repetition, respectively. For *Campylobacter* the final concentrations were 7.9, 8.6, and 8.9 log10 CFU/g for the fourth, fifth, and sixth repetition, respectively. These results represent the inoculated and naturally present pathogens in the intestinal contents used. All flooring samples were sanitized with 70% ethanol and allowed to dry before applying the intestinal contents. Once dry, 1 g of intestinal slurry was applied to each flooring square and evenly spread across the application area. After inoculation, all the flooring pieces remained at room temperature (20°C) for 60 min before any treatment was applied.

Treatment application

For the first repetition (preliminary cleaning procedures), the first treatment was pressurized steam (**A**) applied for 15 seconds and held directing at the center of the application area of the flooring piece at approximately 6 cm of distance. For this treatment, a commercial steam cleaner was used (Goodway, Item: 793Z51, Mfr. Model: GVC-1100) with a boiler working pressure of 105 PSI and 171°C. The second treatment was forced hot air (**B**) applied for 15 seconds directed at the center of the application area of the flooring piece at approximately 6 cm of distance. For this treatment, a heat gun pistol-style was used (Westward, Item: 4HWK4, Mfr. Model: 4HWK4) with an average airflow of 7 cfm and air temperature of 171°C. The third treatment was the application of pressurized steam followed by forced hot air (**C**), both were employed in the same manner as previously described for treatment A and B.

The fourth treatment was one water wash (**D**) applied using a pressure washer with a 4-stroke pattern (up to down, down to up, left to right, and right to left) at approximately 30 cm distance. The pressure washer was used only with cold tap water at an operating pressure of 1700 PSI with a 15-degree nozzle and flow rate of 1.7 gpm (AR Blue Clean, Item: 61HL16, Mfr. Model: BC142HS). For the fifth treatment, the flooring pieces were water washed and disinfected (**E**) with a quaternary ammonium compound disinfectant (United Laboratories, United 262 Hepacide). For this treatment, the first step was one water wash as previously described, followed by the application of the disinfectant at a dilution rate of 1:64 until surface saturation. Then, the disinfectant was allowed to have a contact time of 10 min as suggested by the manufacturer. The treatment was finished with a second water wash to rinse off the disinfectant. Lastly, for the control (**F**), the flooring pieces were not cleaned at all.

After the first repetition, the cleaning procedures were revised and some parameters were adjusted based on this data, then such changes were applied for all subsequent repetitions. All pressurized steam was applied in an up and down pattern instead of directing the steam only at the center of the flooring piece. Also, all water washes were applied in an up and down pattern to mimic the same pattern used for the pressurized steam. For the forced hot air, application was extended to 60 seconds instead of 15 seconds. All other parameters remained unchanged from what had been previously described in the first repetition.

Microbiological assessment

After treatment, each sample surface was immediately swabbed with a sterile sponge premoistened with 10 mL of PBS, using a 4-stroke pattern (2 strokes up to down and 2 strokes from left to right). Then samples were kept chilled and transported to the laboratory in the same day. Once in the laboratory, an additional 10 mL of PBS was added to each sample and

homogenized in a stomacher for 30 seconds. Afterwards, serial dilutions were prepared, and the microbiological assessment was conducted.

The tests performed for general indicators were counts for aerobic bacteria, *E. coli*, and coliforms, for which 1 mL of selected dilutions were plated in duplicate onto their corresponding 3M Petrifilms. For *Salmonella* and *Campylobacter* counts, 0.1 mL of selected dilutions were plated in duplicate on their corresponding selective media (XLT4 and Cefex agar, respectively). For all *Salmonella* and *Campylobacter* samples without counts (presumptive negatives), the flooring pieces were enriched in their corresponding media (Buffered Peptone Water for *Salmonella* or 3M *Campylobacter* Enrichment Broth for *Campylobacter*), incubated for 24 h at 37°C for *Salmonella* and 42°C for *Campylobacter*, and then confirmed positive or negative through the 3M Molecular Detection System.

Statistical analysis

For data analysis, all counts were transformed into $\log_{10}$ CFU/cm^2, then the analysis was performed using SAS OnDemand for Academics software. The data obtained for each microbiological test were analyzed per treatment, type of flooring, and repetition using the General Linear Model procedure with means separated by Tukey's Honest Significant Difference with significance at P-value ≤ 0.05.

2.4 RESULTS AND DISCUSSION

Preliminary cleaning procedures (first repetition)

Due to modifications in treatment application procedures after the first repetition, data from the first repetition were analyzed separately. All counts are reported in $\log_{10}$ CFU/cm^2 and

results for the first repetition are shown in Table 2.1. For the first repetition, the microbiological assessment conducted included aerobic bacteria, coliforms, *E. coli*, and *Salmonella*.

Aerobic bacteria. For the first repetition of the experiment, non-cleaned flooring pieces (**F**: 6.27) and samples treated only with hot air (**B**: 6.13) had the highest aerobic counts among all the treatments and they did not differ from each other. However, for both of these treatments (**B**: P=0.0006; **F**: P=0.0097), fiberglass pieces (**B**: 6.52; **F**: 6.55) had higher aerobic counts than plastic pieces (**B**: 5.75; **F**: 6.01), when treated with only hot air or non-cleaned at all. Although, the results for non-cleaned samples were fairly similar to the initial concentrations reported for the controls used in previous studies (Hinojosa et al., 2015; Moazzami et al., 2021), the application of hot air did not decrease aerobic bacteria levels during this repetition. When the present study was compared to other studies that have used hot air within their treatments, it can be observed that other studies have had longer drying times, which could vary from 15 min up to 24 h (Berrang et al., 2011a; 2011b; Dzieciolowski et al., 2022). No observable effect was detected from the application of hot air in the first repetition, which led to considerations of extending the application time for future repetitions, for which the application time of hot air was extended from 15 seconds to 60 seconds. Differences shown between fiberglass and plastic flooring were not detected in subsequent repetitions.

Reductions of aerobic bacteria were observed in treatments that included steam application. Samples treated with only steam (**A**: 5.36) and samples treated with steam followed by hot air (**C**: 5.61) had lower aerobic counts than non-cleaned samples (**F**: 6.27). Unfortunately, there is a lack of data reported for the effects of steam and/or hot air on aerobic bacteria on transport cage flooring, however a certain degree of reduction was expected but not achieved under current conditions. Based on the reductions in coliforms, *E. coli*, and *Campylobacter*

reported by Berrang et al. (2011a; 2020), it can be emphasized that the set up used for hot air did not show any reduction for the first repetition of this study, as samples treated with only steam and samples treated with steam followed by hot air did not differ.

Further aerobic bacteria reductions were observed when flooring pieces were water washed once (**D**: 4.28). Samples water washed before and after the application of disinfectant (**E**: 3.42) resulted in the lowest aerobic bacteria counts of all the treatments. These results could be contrasted to the study conducted by Hinojosa et al. in 2015. They reported a reduction of 4 to 5 $\log_{10}$ CFU/mL of aerobic bacteria when transport cage flooring was cleaned using a single high-pressure water rinse and disinfectant. However, the reduction observed in the present study for the samples that were water washed before and after the disinfectant was only 2.85 $\log_{10}$ CFU/cm^2 of aerobic bacteria. These differences may be attributed to variations in application time, operating pressure, and type of disinfectant. Hinojosa et al. (2015) used 1 min of pressure washing to clean their cage flooring, as opposed to only using 4 strokes as indicated in the current repetition. In addition, their pressure washer had a greater operating pressure, which was 3,000 PSI instead of 1,700 PSI.

Coliforms and E. coli. As the results for *E. coli* were analyzed separately for further specificity. It is important to note that *E. coli* is one of the members of the coliform group, along with *Enterobacter* and *Klebsiella*. Both microbiological indicators shared the same trend for the first repetition. Samples treated only with hot air (**B**: 5.59 and 5.50 for coliforms and *E. coli*, respectively) and samples non-cleaned (**F**: 5.76 and 5.66 for coliforms and *E. coli*, respectively) had the highest counts among all treatments and were not different from each other. However, within both treatments, fiberglass and plastic flooring differed from each other in their counts for coliforms (**B**: P=0.0127; **F**: P=0.0003) and *E. coli* (**B**: P=0.0202; **F**: P=0.0003). When samples

were treated with only hot air or non-cleaned, the fiberglass flooring (coliforms for **B**: 6.04 and **F**: 6.01; *E. coli* for **B**: 5.93 and **F**: 5.88) had higher counts than plastic flooring (coliforms for **B**: 5.17 and **F**: 5.53; *E. coli* for **B**: 5.09 and **F**: 5.46). Non-cleaned sample results are similar to the controls used in the studies conducted by Berrang et al. (2011a; 2011b). However, similar to what was previously mentioned for aerobic bacteria, reductions in coliforms and *E. coli* were not observed following hot air treatment even though the hot air used for this study had a higher temperature (171°C) than the hot air applied by Berrang et al. in 2011a (50°C). The temperature differential did not compensate for the short application time used in the first repetition of this study. For comparison, Berrang et al. (2011a) used 15 min of drying time. The differences shown between fiberglass and plastic flooring were not observed once the drying time was extended to 60 seconds.

Coliform and *E. coli* reductions were observed when samples were treated with only steam (**A**: 4.56 and 4.49 for coliforms and *E. coli*, respectively) and with steam followed by hot air (**C**: 4.73 and 4.61 for coliforms and *E. coli*, respectively). Similar to what was discussed previously for aerobic bacteria, an effect of hot air was not observed, as the addition of hot air did not represent any additional reduction to only using steam. Even though reductions were observed when steam was applied, the reductions were less than when compared to the results reported by Berrang et al. (2020). They observed a reduction of 2.40 $\log_{10}$ CFU/5 × 5 cm squares for coliforms and a reduction of 2.60 $\log_{10}$ CFU/coupon for *E. coli*. In contrast, the first repetition of the present study observed limited reductions of 1.20 $\log_{10}$ CFU/cm^2 for coliforms and of 1.17 $\log_{10}$ CFU/cm^2 for *E. coli*. It was noted that for the first repetition, flooring samples remained visually dirty after the application of steam, and it was inferred that this could be due to the application pattern. Unfortunately, application pattern details were not reported in the study

performed by Berrang et al. (2020), but for the present study it was established to use an up to down pattern to clean the flooring pieces for all subsequent repetitions.

Further reductions were observed for samples treated with a single water wash (**D**: 3.53 and 3.55 for coliforms and *E. coli*, respectively). However, the lowest coliforms and *E. coli* levels were observed when samples were water washed before and after disinfectant (**E**: 2.59 and 2.45 for coliforms and *E. coli*, respectively). These results differ from Berrang and Northcutt (2005) who reported reductions between 1 to 2 $\log_{10}$ CFU/5 × 5 cm squares of coliforms and *E. coli* when samples were only water washed or water washed and disinfected. For both coliforms and *E. coli* in this repetition, a single water wash led to a reduction of 2 $\log_{10}$ CFU/cm^2, and for samples that were washed and disinfected had reductions of 3 $\log_{10}$ CFU/cm^2. However, a difference between the present study and the research conducted by Berrang and Northcutt (2005) was the pressure they used for the water washes (10 PSI). Greater reductions were expected because higher water pressure was applied, and this effect was observed for coliforms and *E. coli*. Nevertheless, none of the samples under these treatments achieved undetectable levels of bacteria even after the application of disinfectant, indicating that using only 4-strokes was not sufficient to achieve the desired reduction. In the subsequent repetitions, the water washes were modified to be applied in an up to down pattern for 15 seconds.

***Salmonella*:** For the first repetition, samples treated with only steam (**A**: 3.73), only hot air (**B**: 4.17), and non-cleaned (**F**: 4.28) had the highest counts among all treatments and were not different from each other. Based on these results observed in the present study when steam and hot air were applied separately, the configuration used for both steam and hot air during the first repetition was insufficient to reduce *Salmonella* counts. However, when steam and hot air were sequentially applied resulted in lower *Salmonella* levels (**C**: 3.46). Also, counts observed on

fiberglass and plastic flooring differed from each other within this treatment (P=0.0055). Fiberglass flooring had lower *Salmonella* counts than plastic flooring (3.23 and 3.71, respectively) when samples were treated with steam followed by hot air and this trend persisted for all subsequent repetitions.

When samples were treated with one water wash (**D**: 2.62), further *Salmonella* reductions were observed, similar as previously described in aerobic bacteria, coliforms, and *E. coli*. The lowest *Salmonella* levels were observed when transport flooring samples were water washed before and after disinfectant (**E**: 1.52), which aligns with the results previously discussed for aerobic bacteria, coliforms, and *E. coli*. These results can be compared to the study conducted by Hinojosa et al. in 2018, with reported reductions in *Salmonella* Typhimurium counts between 4 to 5 $\log_{10}$ CFU/mL when samples (fiberglass flooring) were water washed and disinfected, but in the present study only a 2.76 $\log_{10}$ CFU/cm^2 reduction was observed when samples were water washed before and after disinfectant. These differences may be attributed to the time of application, operating pressure, and type of disinfectant applied (Hinojosa et al., 2018).

For this first repetition, fiberglass flooring had lower *Salmonella* counts than plastic flooring (0.88 and 2.18, respectively) when samples where water washed before and after disinfectant (P<0.0001), but this difference was not observed for any subsequent repetition. Lastly, after enrichment all the flooring pieces remained positive for *Salmonella* regardless of their treatment, which led to revision of the procedures in the subsequent repetitions.

Revised cleaning procedures (second to sixth repetition)

After modifications in treatment application procedures, changes were maintained for all subsequent repetitions. For the second and third repetition, the microbiological assessment conducted included aerobic bacteria, coliforms, *E. coli*, and *Salmonella*, results are shown in

Table 2.2. For the fourth, fifth, and sixth repetitions only *Campylobacter* was assessed, and results are shown in Table 2.3.

Aerobic bacteria. For the second and third repetition, non-cleaned samples (**F**: 6.72) had the highest counts of all treatments. Samples treated with only hot air (**B**: 5.76) had the second highest aerobic bacteria counts of all treatments. The non-cleaned samples were similar to the first repetition and comparable to the non-cleaned controls from other studies (Hinojosa et al., 2015; Moazzami et al., 2021). In contrast to the first repetition, forced hot air reduced aerobic bacteria once the application time was extended to 60 seconds. Based on the results of the present study, the application of forced hot air by itself had limited efficacy to reduce aerobic bacteria under the presented experiment configuration. It is important to note that heat inactivation is a complex process with a wide range of potential configurations that can significantly impact effectiveness. The efficacy of a thermal treatment will depend on the modification of at least one crucial element above a specific threshold that determines its lethality (Cebrian et al., 2017). Extending the drying time while using a temperature of 171°C is a possible configuration to explore in future studies.

A greater reduction was observed when only steam was applied (**A**: 4.28). Once the application pattern was modified, lower aerobic bacteria counts were observed in comparison to the first repetition. Also, for these repetitions, samples treated with steam appeared visually clean after 15 seconds when using an up to down cleaning pattern. Samples treated with one water wash (**D**: 2.51) and samples treated with steam followed by hot air (**C**: 3.10) had even lower aerobic bacteria counts, and both of these treatments were comparable to each other. In comparison to the first repetition, greater reductions were achieved once the application pattern and the drying time was extended. The lowest aerobic count was observed when samples were

water washed before and after the application of disinfectant (**E**: 0.66). However, within the treatment, fiberglass flooring had lower aerobic counts than plastic flooring (0.23 and 1.11, respectively) when disinfectant and a second water wash was added to the cleaning procedure (P=0.0218). Greater reductions were observed in the present study than the cleaning procedures evaluated by Hinojosa et al. (2015). When the samples of this study were water washed before and after disinfectant, a reduction of approximately 6 $\log_{10}$ CFU/cm^2 was observed. In comparison, Hinojosa et al. (2015) reported reductions of aerobic bacteria between 4 and 5 $\log_{10}$ CFU/mL when their samples were water washed and disinfected. The differences observed could be attributed to the modification of the cleaning pattern in the current study, as well as the application of a second water wash, whereas the study conducted by Hinojosa et al. (2015) only used one water wash.

Coliforms and E. coli. For the second and third repetition, both microbiological indicators shared a similar trend with each other. Samples that were non-cleaned (**F**: 5.90 and 5.85 for coliforms and *E. coli*, respectively) and samples treated only with hot air (**B**: 5.34 and 5.23 for coliforms and *E. coli*, respectively) were the highest among all treatments and were not different from each other. Coliforms and *E. coli* levels for non-cleaned samples were similar to the results reported for the controls used in other studies (Berrang and Northcutt, 2005; Berrang et al., 2011a; 2011b; 2020). Unfortunately, extending the application time for hot air did not show reductions when it was applied by itself. While forced hot air was shown to be effective in reducing aerobic bacteria, it did not have the same results when coliforms and *E. coli* were used as indicators. Coliforms and *E. coli* counts continued to show the same pattern and reductions were observed once the samples were treated with only steam (**A**: 3.28 and 3.11 for coliforms and *E. coli*, respectively). The results for coliforms and *E. coli* in the present study were similar

to Berrang et al. (2020). For flooring samples treated only with steam, they reported reductions of 2.4 to 2.6 $\log_{10}$ CFU/5 × 5 cm squares, while this study demonstrated reductions of 2.6 to 2.7 $\log_{10}$ CFU/cm^2 for coliforms and *E. coli*.

Following, further reductions were observed in samples treated with one water wash (**D**: 1.89 and 1.71 for coliforms and *E. coli*, respectively). These results are lower than what was reported from the first repetition, which shows that the application pattern for the water wash has direct effect on the reduction of bacteria. However, the results from the current study differ from results reported in previous studies (Berrang and Northcutt, 2005; Berrang et al., 2011a, 2020), as they report limited reductions or no reductions at all in coliforms or *E. coli* when flooring samples were cleaned with a water wash. This difference could be attributed to the use of the different operating pressures and different cleaning patterns. In their studies (Berrang and Northcutt, 2005; Berrang et al., 2020), they used a water pressure of 10 PSI, while in the present study used a water pressure of 1700 PSI.

The trend between coliforms and *E. coli* started to diverge once the steam and hot air was compared to water washing before and after disinfectant. For coliforms counts, the steam and hot air (**C**: 1.08) had higher counts than water washing before and after disinfectant (**E**: 0.11). For *E. coli* counts, the use of steam and hot air (**C**: 0.79) was equivalent to the application of water washes and disinfectant (**E**: 0.08). The results of the present study are consistent with the findings of Berrang et al. (2011a; 2020), where they employed a water rinse prior to utilizing steam or hot air in their cleaning procedures. In contrast, the water rinse was not included for this study, but instead the effect of steam followed by forced hot air was evaluated. The results shown in this study demonstrate that the methodology proposed has the ability to reduce fecal indicators (coliforms and *E. coli*) to levels comparable to those achieved through water washing and

disinfection. On the other hand, the implementation of total coliforms or *E. coli* as an indicator will depend on the level of specificity required. The general consensus is that *E. coli* has higher specificity as a fecal indicator than total coliforms. However, both of these microbiological indicators only provide a broad overview, and they are not able to confirm the presence or absence of pathogens (Odonkor and Ampofo, 2013).

***Salmonella*:** For the second and third repetition, non-cleaned samples had the highest *Salmonella* counts (**F**: 4.53) among all treatments. Samples treated only with hot air (**B**: 3.81) showed a reduction of *Salmonella* counts when compared to non-cleaned samples. Unfortunately, there were no previous studies found that were directly comparable to the present research. In comparison to the first repetition, *Salmonella* counts were reduced once the application time for hot air was extended to 60 seconds. Then, greater reductions were observed when samples were treated with only steam (**A**: 1.67), which also differed from the first repetition, as it was not different from non-cleaned samples in the preliminary cleaning procedures. It is important to note again, that once the application pattern for steam was revised the flooring samples were visibly clean, which shows that the application pattern has an effect in the reduction of *Salmonella*.

The greatest reduction of *Salmonella* counts was observed in samples that were treated with steam followed by hot air (**C**: 0.23), in samples that were water washed once (**D**: 0.48), and for samples were treated with water washes before and after disinfectant (**E**: 0.03), as none of these treatments differ from each other. These results are similar to the reductions reported by Hinojosa et al. (2018), who reported reductions of about 4 to 5 $\log_{10}$ CFU/mL of *Salmonella* when flooring cages were water washed and disinfected. In the current study, the reductions shown ranged from 4.05 to 4.50 $\log_{10}$ CFU/cm^2 for *Salmonella* counts. The application of steam

and hot air was equivalent to water washing and disinfecting indicating that possible modifications can be implemented to achieve mitigation of *Salmonella* in transport cage flooring.

Additionally, a difference between fiberglass and plastic flooring was observed for samples treated with steam followed by hot air (P=0.0431). Within this treatment, fiberglass flooring had lower *Salmonella* counts than plastic flooring (0.06 and 0.42, respectively) when treated with steam and hot air. Despite the modification of the cleaning procedures, this trend persisted in all three repetitions of the study. This difference could possibly be attributed to the different types of surfaces, while fiberglass flooring is flat, the plastic flooring had indentations and a patterned texture, making plastic flooring more difficult to clean, which was detectable once the *Salmonella* levels were low. For *Salmonella* prevalence, all of treatments had 20 out of 20 positive samples after enrichment except for samples treated with disinfectant, for which only 13 out of 20 were positive. Hinojosa et al. (2018) also reported the prevalence of *Salmonella* after enriching samples that had been water washed and disinfected, which were similar to the prevalence found in this study for samples that were water washed before and after disinfectant. They reported 72.5% of *Salmonella* positive samples for their study, while the present study shows 65.0% of *Salmonella* positive samples that were water washed before and after disinfectant.

***Campylobacter*:** For the fourth, fifth, and sixth repetitions only *Campylobacter* was assessed, and results are shown in Table 2.3. For these repetitions, samples that were non-cleaned (**F**: 6.72) and samples treated with only with hot air (**B**: 6.15) had the highest *Campylobacter* counts of all treatments and did not differ from each other. The non-cleaned samples are comparable to the controls used in the studies conducted by Berrang et al. (2011a; 2020), but again the forced hot air did not show any effect when it was applied by itself. Berrang et al

(2011a) reported a reduction of 4.51 $\log_{10}$ CFU/5 × 5 cm squares in *Campylobacter* counts when unwashed flooring samples were dried with hot air for 15 min. Therefore, based on the results presented in the current study, it can be stated that applying hot air alone (at 171°C) for 60 seconds is not an effective method to reduce *Campylobacter* on cage flooring.

A *Campylobacter* reduction was observed when samples were treated with only steam (**A**: 3.24), but within this treatment, fiberglass flooring had lower *Campylobacter* counts than plastic flooring (2.78 and 3.72, respectively). The results for samples treated with steam in the current study showed slightly greater reductions than what was reported by Berrang et al. (2020). The reductions of *Campylobacter* observed in the study were 3.48 $\log_{10}$ CFU/cm^2, while the reductions reported by Berrang et al. (2020) were 2.10 $\log_{10}$ CFU/5 × 5 cm squares for samples treated with only steam. It is possible that the application pattern of the steam could have affected the reductions observed, but this information was not provided in the methodology outlined by Berrang et al. (2020). Further reductions of *Campylobacter* were observed when samples were treated with one water wash (**D**: 2.12). This reduction was greater than what was previously reported in other studies (Berrang and Northcutt, 2005; Berrang et al., 2011a, 2020). For example, Berrang and Northcutt (2005) reported a *Campylobacter* reduction of 2.70 $\log_{10}$ CFU/coupon, while in the current study the reduction observed is 4.6 $\log_{10}$ CFU/cm^2. Similar to what was discussed for coliforms and *E. coli*, this difference could be attributed to the use of different operating pressures and cleaning patterns. The effect of the operating pressure can be observed, as applying a higher water pressure resulted in greater bacterial removal in the current study.

The lowest *Campylobacter* counts were observed when steam followed by hot air was applied (**C**: 0.67) and when samples were treated with water washes before and after disinfectant

(**E**: 0.49). These results are consistent with what was reported previously by Berrang et al (2011a; 2020). Once again, it is important to emphasize that pressurized steam followed by forced hot air has the ability to reduce *Campylobacter* to levels similar to those achieved through the use of water washing and disinfectant. Unfortunately, when *Campylobacter* prevalence is considered none of the treatments utilized were entirely effective. All the samples remained positive after enrichment with 30 out of 30 positive samples regardless of the cleaning procedure applied.

2.5 CONCLUSIONS

Given that current cleaning methods for transport containers are unable to entirely remove or inactivate pathogens, it is important to continue to explore novel interventions. In this study, pressurized steam followed by forced hot air showed the possibility to achieve low levels of *Salmonella* and *Campylobacter* in transport cage flooring, and in consequence could reduce the risk of cross contaminating the broilers before their arrival at the processing plant. Additionally, it is important to note that when designing thermal interventions, the target microorganism should be considered. Based on this study, when forced hot air was applied by itself, it was able to reduce *Salmonella* but not *Campylobacter*. In addition, the effects of cleaning patterns, times of application, operating pressures, types of disinfectant, and even amount of water used are all intrinsically connected and will display an array of efficacies. Further research is suggested to help optimize current cleaning procedures, which can then be applied to an industrial scale.

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Table 2.1 Bacteria counts and *Salmonella* Infantis prevalence under preliminary cleaning procedures of transport cage flooring for the first repetition.

Treatment	Flooring type	log_{10} CFU/cm^2				Prevalence[n]
		Aerobic bacteria	Coliforms	*E. coli*	*S.* Infantis	*S.* Infantis
A[1] Pressurized steam	Fiberglass	5.23 ± 0.07	4.58 ± 0.11	4.52 ± 0.11	3.65 ± 0.08	10/10
	Plastic	5.50 ± 0.12	4.56 ± 0.34	4.47 ± 0.35	3.83 ± 0.08	
	Average	**5.36 ± 0.07** [c]	**4.56 ± 0.14** [b]	**4.49 ± 0.15** [b]	**3.73 ± 0.05** [ab]	
B[2] Forced hot air	Fiberglass	6.52 ± 0.14 [x]	6.04 ± 0.12 [x]	5.93 ± 0.11 [x]	4.22 ± 0.06	10/10
	Plastic	5.75 ± 0.08 [y]	5.17 ± 0.11 [y]	5.09 ± 0.11 [y]	4.14 ± 0.05	
	Average	**6.13 ± 0.14** [ab]	**5.59 ± 0.15** [a]	**5.50 ± 0.15** [a]	**4.17 ± 0.03** [a]	
C[3] Pressurized steam and forced hot air	Fiberglass	5.79 ± 0.27	4.54 ± 0.27	4.41 ± 0.28	3.23 ± 0.12 [y]	10/10
	Plastic	5.45 ± 0.24	4.94 ± 0.05	4.82 ± 0.06	3.71 ± 0.09 [x]	
	Average	**5.61 ± 0.15** [bc]	**4.73 ± 0.13** [b]	**4.61 ± 0.13** [b]	**3.46 ± 0.10** [b]	
D[4] Water wash	Fiberglass	4.02 ± 0.24	3.19 ± 0.22 [y]	3.38 ± 0.23	2.24 ± 0.47	10/10
	Plastic	4.57 ± 0.22	3.89 ± 0.20 [x]	3.75 ± 0.21	3.02 ± 0.20	
	Average	**4.28 ± 0.16** [d]	**3.53 ± 0.17** [c]	**3.55 ± 0.14** [c]	**2.62 ± 0.25** [c]	
E[5] Water washes and disinfectant	Fiberglass	3.19 ± 0.26	2.33 ± 0.24	2.21 ± 0.30	0.88 ± 0.09 [y]	10/10
	Plastic	3.66 ± 0.29	2.87 ± 0.13	2.72 ± 0.15	2.18 ± 0.05 [x]	
	Average	**3.42 ± 0.17** [e]	**2.59 ± 0.14** [d]	**2.45 ± 0.16** [d]	**1.52 ± 0.21** [d]	
F[6] No cleaning	Fiberglass	6.55 ± 0.17 [x]	6.01 ± 0.15 [x]	5.88 ± 0.15 [x]	4.33 ± 0.07	10/10
	Plastic	6.01 ± 0.08 [y]	5.53 ± 0.09 [y]	5.46 ± 0.06 [y]	4.24 ± 0.07	
	Average	**6.27 ± 0.11** [a]	**5.76 ± 0.10** [a]	**5.66 ± 0.09** [a]	**4.28 ± 0.04** [a]	

[a-e] Values within a column with different superscripts are significantly different ($P \leq 0.05$).

[x-y] Values with different superscripts are significantly different between types of flooring ($P \leq 0.05$).

[1]Pressurized steam directed at center for 15 s.

[2]Forced hot air directed at center for 15 s.

[3]Pressurize steam and forced hot air (as previously described).

[4]Water wash using 4-strokes (left-right, right-left, up-down, down-up).

[5]Water wash (as previously described), disinfectant (10 min contact time), and final water wash.

[6]No cleaning (control).

n = 5 per flooring type by treatment.

Table 2.2 Bacteria counts and *Salmonella* Infantis prevalence under revised cleaning procedures of transport cage flooring for second and third repetition.

Treatment	Flooring type	log_{10} CFU/cm^2				Prevalence[n]
		Aerobic bacteria	Coliforms	*E. coli*	*S.* Infantis	*S.* Infantis
A[1] Pressurized steam	Fiberglass	4.36 ± 0.23	3.42 ± 0.24	3.19 ± 0.28	1.60 ± 0.18	20/20
	Plastic	4.21 ± 0.20	3.15 ± 0.33	3.04 ± 0.38	1.75 ± 0.38	
	Average	**4.28 ± 0.13** [c]	**3.28 ± 0.18** [b]	**3.11 ± 0.21** [b]	**1.67 ± 0.19** [c]	
B[2] Forced hot air	Fiberglass	5.38 ± 0.66	5.32 ± 0.22	5.15 ± 0.26	3.62 ± 0.27	20/20
	Plastic	6.16 ± 0.11	5.38 ± 0.11	5.32 ± 0.12	4.01 ± 0.14	
	Average	**5.76 ± 0.32** [b]	**5.34 ± 0.11** [a]	**5.23 ± 0.13** [a]	**3.81 ± 0.14** [b]	
C[3] Pressurized steam and forced hot air	Fiberglass	2.90 ± 0.14	0.95 ± 0.27	0.65 ± 0.30	0.06 ± 0.07 [y]	20/20
	Plastic	3.31 ± 0.19	1.22 ± 0.32	0.95 ± 0.37	0.42 ± 0.17 [x]	
	Average	**3.10 ± 0.11** [d]	**1.08 ± 0.19** [d]	**0.79 ± 0.21** [d]	**0.23 ± 0.09** [d]	
D[4] Water wash	Fiberglass	2.77 ± 0.52	2.07 ± 0.52	1.83 ± 0.44	0.60 ± 0.31	20/20
	Plastic	2.27 ± 0.41	1.73 ± 0.33	1.61 ± 0.35	0.37 ± 0.21	
	Average	**2.51 ± 0.30** [d]	**1.89 ± 0.28** [c]	**1.71 ± 0.25** [c]	**0.48 ± 0.17** [d]	
E[5] Water washes and disinfectant	Fiberglass	0.23 ± 0.14 [y]	0.07 ± 0.08	0.05 ± 0.06	0.00 ± 0.00	13/20
	Plastic	1.11 ± 0.35 [x]	0.17 ± 0.14	0.13 ± 0.14	0.06 ± 0.07	
	Average	**0.66 ± 0.19** [e]	**0.11 ± 0.07** [e]	**0.08 ± 0.06** [d]	**0.03 ± 0.03** [d]	
F[6] No cleaning	Fiberglass	6.77 ± 0.08	5.95 ± 0.05	5.90 ± 0.06	4.59 ± 0.03	20/20
	Plastic	6.70 ± 0.07	5.86 ± 0.10	5.82 ± 0.10	4.50 ± 0.06	
	Average	**6.72 ± 0.04** [a]	**5.90 ± 0.04** [a]	**5.85 ± 0.05** [a]	**4.53 ± 0.03** [a]	

[a-e] Values with different superscripts are significantly different among treatment averages ($P \leq 0.05$).

[x-y] Values with different superscripts are significantly different between types of flooring ($P \leq 0.05$).

[1] Pressurized steam applied in up-down pattern for 15 s.

[2] Forced hot air directed at center for 60 s.

[3] Pressurize steam and forced hot air (as previously described).

[4] Water wash applied up-down pattern for 15 s.

[5] Water wash (as previously described), disinfectant, water wash.

[6] No cleaning (control).

n = 10 per flooring type by treatment.

Table 2.3 *Campylobacter jejuni* counts and prevalence under revised cleaning procedures of different transport cage flooring type for fourth, fifth, and sixth repetition.

Treatment	*Campylobacter jejuni* $\log_{10}$ CFU/cm^2			Prevalence[n]
	Fiberglass	Plastic	Average	
A[1] Pressurized steam	2.78 ± 0.25 [y]	3.72 ± 0.30 [x]	**3.24 ± 0.19** [b]	30/30
B[2] Forced hot air	6.30 ± 0.30	6.04 ± 0.33	**6.15 ± 0.21** [a]	30/30
C[3] Pressurized steam and forced hot air	0.70 ± 0.25	0.66 ± 0.24	**0.67 ± 0.16** [d]	30/30
D[4] Water wash	1.95 ± 0.40	2.31 ± 0.40	**2.12 ± 0.26** [c]	30/30
E[5] Water washes and disinfectant	0.18 ± 0.14	0.82 ± 0.34	**0.49 ± 0.18** [d]	30/30
F[6] No cleaning	6.76 ± 0.24	6.70 ± 0.29	**6.72 ± 0.17** [a]	30/30

[a-d] Values within a column with different superscripts are significantly different ($P \leq 0.05$).

[x-y] Values with different superscripts are significantly different between types of flooring ($P \leq 0.05$).

[1] Pressurized steam applied in up-down pattern for 15 s

[2] Forced hot air directed at center for 60 s

[3] Pressurize steam and forced hot air (as above)

[4] Water wash applied up-down pattern for 15 s

[5] Water wash (as above), disinfectant, water wash

[6] No cleaning (control)

n = 15 per flooring type by treatment.

CHAPTER 3: QUANTIFICATION OF *SALMONELLA* INFANTIS TRANSFER FROM TRANSPORT DRAWER FLOORING TO BROILER CHICKENS DURING HOLDING

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3.1 ABSTRACT

Transportation is a potential point of cross-contamination before broiler chickens arrive at the processing plant for slaughter. Previous studies have associated the use of uncleaned transport containers to the introduction of pathogenic bacteria onto uncontaminated broilers. The objective of this study was to quantify the transfer of *Salmonella* from transport drawer perforated flooring to broiler chickens during different holding times. For traceability, the flooring of each drawer was inoculated with fecal content slurry containing a marker strain of *Salmonella* Infantis. Three drawers per treatment were used, and each drawer was subjected to one of the following treatments: pressure wash, disinfectant, and pressure wash (A), pressurized steam followed by forced hot air (B), and no cleaning (C). Drawers were classified as top, middle, or bottom based on the relative position with each other. After treatment, broilers were introduced to each drawer and held for 2, 4, or 6 h. At each timepoint, broilers were removed from drawers, euthanized, and carcasses rinsed to obtain *Salmonella* counts. Samples under the

limit of direct plating detection were enriched, plated and later confirmed positive or negative. Differences were observed per treatment, holding time, and drawer relative position ($P<0.0001$). Broilers placed in transport containers that underwent a cleaning procedure (A and B) had lower levels of *Salmonella* when compared to broilers placed in non-cleaned containers. However, most of samples under the limit of detection were positive after enrichment, indicating that both procedures evaluated need improvement for efficient pathogen inactivation. A decrease in *Salmonella* transfer was observed after 6 h in rinsates obtained from broilers placed in non-cleaned containers (C). Rinsates obtained from top drawers had less *Salmonella* than the middle or bottom drawers when broilers were placed in transport containers that underwent a cleaning procedure (A and B). The application of pressurized steam and forced hot air was comparable to the use of water washes and disinfectant indicating a potential role in cleaning poultry transport containers.

3.2 INTRODUCTION

In 2022, The U. S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) released a proposed framework to reduce human foodborne *Salmonella* infections associated with poultry meat products. This initiative has been launched as a result of identifying the limitations in the monitoring and verification programs that currently operate in poultry processing facilities (USDA-FSIS, 2022). While the existing monitoring programs show that the established interventions to control microbial loads have been able to reduce *Salmonella* prevalence over the years, they have not yet led to any reduction in the national *Salmonella* infection rate since the year 2000, as the Healthy People objectives in reducing *Salmonella* infections transmitted through food were not met in 2010 nor 2020 (USDHHS-ODPHP, 2020; USDA-FSIS, 2022).

The first component of the proposed framework is to require incoming flocks to be tested for *Salmonella* before being processed. The intent of this first component is to implement a prevention and risk-based approach to adequately adapt processes and to take special considerations before the broilers enter the processing plant (USDA-FSIS, 2022). While there are studies assessing the risk of cross-contamination for poultry products during processing and after retail purchase (Nauta et al., 2005; Hue et al., 2010; Jeong et al., 2018), there is not enough information reported to conduct a comprehensive risk assessment for all the steps prior to arrival of the broilers at the processing facilities in the United States (Parsons et al., 2005; McCrea et al., 2006). It is important to note that the risk assessment only provides insight into the degree of factors as potential risks but allows for implementation of customized preventative measures to adequately handle specific scenarios when they are considered out of control (Attrey, 2017).

One potential step for improvement mentioned in the proposed framework for reducing *Salmonella* infections could be the inclusion of measures to address cross-contamination during transportation. Poultry transport containers have been reported as a source of cross-contamination in multiple studies (Slader et al., 2001; Berrang et al., 2003; Rasschaert et al., 2007). Unfortunately, the vast majority of poultry processors in the United States do not have an established procedure to clean and disinfect these transport containers, as reported by Northcutt and Jones in 2004. The uncertain effectiveness and benefits of the current transport cage handling methodologies make it challenging for processors to justify the potential economic costs of implementing a new cleaning procedure (Northcutt and Jones, 2004).

Previous studies have explored a range of mechanisms to reduce microbial loads on transport containers, including wet cleaning procedures, the use of different sanitizers and foaming agents, and the application of UV light to transport container flooring (Berrang et al.,

2011; Hinojosa et al., 2015; 2018; Moazzami et al., 2021). However, a satisfactory solution for this complex issue is yet to be determined. For poultry processors to willingly adopt a new cleaning practice, the procedure must demonstrate its effectiveness in reducing the target pathogens, while also being fast and cost-effective (Northcutt and Jones, 2004). One possible alternative to save water and time is the application of a thermal intervention as part of the cleaning process for transport containers. Previous studies have evaluated the application of forced hot air or pressurized steam after a water rinse as an option for cleaning fiberglass cage flooring. In both studies they observed that the combination of water rinsing followed by a thermal intervention resulted in the greatest decrease in bacterial loads within their treatments, and in some cases reaching undetectable levels (Berrang et al., 2011; 2020).

Nonetheless, most of the studies previously mentioned have only assessed *Campylobacter* as their target microorganism. Few studies have reported the efficacy of such cleaning procedures in reducing *Salmonella* levels (Hinojosa et al., 2018), and unfortunately, they cannot be directly compared with the reductions observed for *Campylobacter*. Both of these pathogens have different morphologies, different growing requirements, and even different survivals rates outside their natural hosts (Jay, 1998, Cebrian et al., 2017; Topalcengiz et al., 2020). Consequently, these different factors result in the creation of a unique risk profile for each one of these pathogens (Slader et al., 2002; De Cesare et al., 2003; McCrea et al, 2006).

For example, De Cesare et al., (2003) evaluated the persistence and survival of *Salmonella* and *Campylobacter* on different food contact surfaces and their findings indicate that *Salmonella* could be up to 25 times more persistent than *Campylobacter* depending on the type of food contact surface. In a separate study conducted by Berrang et al. (2004), they reported undetectable levels of *Campylobacter* on transport cage flooring after a drying period of 48 h

(placed under a shed at temperature: 24-25°C). This finding is in stark contrast to the survival period of *Salmonella* outside their host, which can last up to 308 days in waterfowl feces (stored at room temperature: 22°C) as reported by Topalcengiz et al. (2020).

Therefore, the objective of this study is to quantify the amount of *Salmonella* transferred from transport drawer flooring to broiler chickens while determining the role of wet cleaning procedures, thermal interventions, and no cleaning in reducing *Salmonella* transfer. Additionally, the effect of different contact times and the relative position of the transport drawers on cross-contamination were compared.

3.3 MATERIALS AND METHODS

Experimental design

For this study, perforated plastic transport drawers (1.20 x 1.27 x 0.23 m) and their corresponding metal modules typical for controlled atmosphere stunning systems were used (Baader, UniLoad Live Bird Handling System). The drawers' floors were inoculated with *Salmonella* Infantis and then subjected to one of the following treatments, **A**: the application of water washes and a commercial disinfectant, **B**: the application of pressurized steam followed by forced hot air, or **C**: no-cleaning. Each treatment was placed in a separate metal module containing three drawers, in total 9 drawers and 3 metal modules were used in each of three repetitions. The drawers were placed in the top three slots of their module and classified as top, middle, or bottom in relation to each other. After treatment, 15 (6-week-old) broilers were introduced in each drawer and held for 2, 4, or 6 h. At each timepoint, 5 broilers were removed from each drawer and sampled. This generated a total of 135 samples per repetition, and after completing three full repetitions a total of 405 observations were obtained.

Drawer flooring inoculation

The total internal area of each drawer was 1.34 m^2, but this area was reduced to approximately 1 m^2 using a wire divider placed lengthwise across the drawer. This application area allowed for accommodation of 15 broilers at 42 days of age and to provide an even exposure of 100 g of inoculated intestinal contents per m^2. To obtain the intestinal contents, viscera packs were collected from a commercial processing facility the day before the experiment. On the same day, 900 g of intestinal contents from the ceca, colon, and ileum were manually expressed into a sterile beaker and kept refrigerated until the following morning.

To ensure the presence and traceability of *Salmonella*, the intestinal contents were inoculated with a marker strain of *Salmonella* Infantis resistant to 200 ppm of Nalidixic acid. To prepare the inoculum, the *Salmonella* marker strain was incubated for 24 h at 37°C in Xylose Lysine Tergitol 4 agar with 200 ppm of Nalidixic acid (XLT4-200NAL), then one colony was transferred onto Standard Methods Agar (SMA) and incubated for 24 h at 37°C. The morning of the experiment some colonies were scraped from the SMA surface and suspended in 100 mL Phosphate Buffered Saline (PBS) to produce a cell suspension of 10^8 CFU per mL when an optical density of 0.15 was achieved at a wavelength of 540 nm.

Then, the 100 mL of *Salmonella* inoculum was added to the 900 g of intestinal contents and mixed thoroughly to obtain a homogenous mixture. The final concentrations of the inoculated intestinal contents were later confirmed by spread plating the serial dilutions on XLT4-200NAL, which were 7.04, 7.77, and 7.78 $\log_{10}$ CFU/g for the first, second, and third repetition, respectively. Before inoculation, the surface of the application area was dry wiped, sprayed with 70% ethanol, and allowed to dry. Once ready, 100 g of inoculated intestinal contents were applied to each drawer only to the floor surface and dispersed evenly with a 3-in

disposable paint brush (Project Source, Item: 104125, Model: 150030) across all the application area. After inoculation, all the drawers remained at room temperature (23°C) for 1 h before applying any treatment.

Treatment application

For the first treatment, the drawer floors were water washed and disinfected (**A**) with a quaternary broad-spectrum disinfectant (United Laboratories, United 262 Hepacide). The first step was one water wash applied with a pressure washer using an up-down pattern at approximately 30 cm distance. All water washes were standardized to be applied for 3 min to achieve a visually clean drawer floor surface. The pressure washer was used only with cold water at an operating pressure of 1,700 PSI with a 15-degree nozzle and flow rate of 1.7 gpm (AR Blue Clean, Item: 61HL16, Mfr. Model: BC142HS). Then, the disinfectant was applied at a dilution rate of 1:64 until saturating the drawer floor surface, and a contact time of 10 min was allowed as suggested by the manufacturer. To conclude this treatment, a second water wash was applied as previously described to rinse off the disinfectant.

For the second treatment, pressurized steam followed by forced hot air (**B**) was applied to clean the drawer's floor surfaces. The first step was to apply pressurized steam in an up-down pattern at approximately 6 cm of distance. However, due to noticeable pressure differences between the pressure washer and the steam cleaner, the time of application differed to achieve a visually clean drawer floor. The pressurized steam was applied in cross sections of approximately 10 cm in an up-down pattern and cleaned from left to right. This resulted in a prolonged application time but was standardized to 25 min for each drawer. A commercial steam cleaner (Goodway, Item: 793Z51, Mfr. Model: GVC-1100) was used with a boiler working pressure of 80 PSI and 171°C. After this step, forced hot air was applied at approximately 30 cm

of distance using a heat blower (Master Appliance, Item: 5PYR0, Mfr. Model: AH-301) with an average airflow of 47 cfm at 149°C. The forced hot air was applied in an S-shape pattern across all the drawer floor area for 15 min to achieve even drying. Although the time of application applied in this study may not be practical in a commercial setting, it is anticipated that large scale systems purpose-designed for the cleaning of transport containers could achieve the same cleanliness within a shorter timeframe.

Lastly, for the control of this experiment, the drawer floors were not cleaned at all (**C**). Treatments were performed simultaneously to prevent skewing the results by order of application.

Microbiological assessment

After treatment, 15 broilers at 42 days of age were immediately introduced to the drawers and held for 2, 4, or 6 h. At each time point, 5 birds were removed from each drawer, euthanized, and the entire carcasses (including the feet) were rinsed with 400 mL of Buffered Peptone Water (BPW) for 60 seconds. Samples were kept chilled and transported to the laboratory for the quantification of *Salmonella*. Once in the laboratory, serial dilutions were prepared (10^{-1}), and 0.1 mL of 10^{0} and 10^{-1} were spread plated in duplicate on XLT4-200NAL. For samples without *Salmonella* counts (presumptive negatives), 30 mL were extracted from the original carcass rinse (10^{0}), placed in a conical tube, and incubated for 24 h at 37°C. Then, 0.1 mL was plated on XLT4-200NAL to confirm the presence or absence of the *Salmonella* Infantis that was spread within feces onto the floors of the drawers.

Statistical analysis

For data analysis, all counts were transformed into $\log_{10}$ CFU/mL, then the analysis was performed using the SAS OnDemand for Academics software. The data obtained for each microbiological test were analyzed per treatment, time, drawer position, repetition, and their interactions using the General Linear Model procedure with means separated by Tukey's Honest Significant Difference with significance at P-value ≤ 0.05.

3.4 RESULTS AND DISCUSSION

Effect of treatment on Salmonella transfer

Differences were found among treatments ($P<0.0001$) and are presented in Table 3.1. Carcass rinses from broilers that were placed in non-cleaned drawers (**C**: 2.31 $\log_{10}$ CFU/mL) had higher amounts of *Salmonella* than carcass rinses collected from broilers placed in drawers that underwent a cleaning procedure. Both cleaning procedures, **A** (water wash, disinfectant, and water wash) and **B** (pressurized steam followed by hot air) were not different from each other (**A**: 1.39; **B**: 1.34 $\log_{10}$ CFU/mL). With the exception of one single carcass rinse, all other rinses tested positive for *Salmonella* after enrichment (total prevalence: 404/405). This specific sample was obtained from a broiler placed in a top drawer of treatment **A** (water wash, disinfected, water wash), and it was collected after 6 hours during the second repetition.

The inoculation of the drawer flooring with an artificially high amount of *Salmonella* presented the opportunity to have a deeper understanding of the potential transfer. However, other studies that have documented real life scenarios reported that initial loads of *Salmonella* were substantially lower than the results presented in this study. For example, Chavez-Velado (2022) evaluated the initial load of *Salmonella* at live receiving in three different processing

plants. In that study, the *Salmonella* loads observed before the broilers enter each processing plant were 2.39, 2.83, 2.78 $\log_{10}$ CFU/30 mL (of a 400mL carcass rinsate) for the first, second, and third processing plant, respectively.

In a different study, De Villena et al. (2022) reported similar results to Chavez-Velado (2022). This study assessed the levels of *Salmonella* for a single processing plant during live receiving, reporting 2.63 $\log_{10}$ CFU/30 mL. When adjusting the results of the present study to a volume of 400 mL (volume of BPW used per each carcass rinse), the *Salmonella* loads obtained were notably higher even for the broilers placed in drawers that underwent a cleaning procedure (**A**: 3.42; **B**: 3.44; **C**: 4.90 $\log_{10}$ CFU/30 mL). Nonetheless, only a broad comparison could be made since neither Chavez-Velado (2022) nor De Villena (2022) disclosed information on whether the sampled broilers were placed in cleaned or non-cleaned transport containers.

Borges et al. (2019) conducted a study to quantify *Salmonella* across the slaughtering process. Despite subjecting the transport containers to a cleaning and disinfecting procedure, no reductions in *Salmonella* were observed on transport flooring (2.77 $\log_{10}$ CFU/mL before cleaning and 2.96 $\log_{10}$ CFU/mL after cleaning). This lack of effectiveness in cleaning and disinfecting the transport containers allowed for a comparison between the results of carcass rinses obtained before scalding by Borges et al. (2019) and those obtained from broilers placed in non-cleaned drawers in the current study. In their study, Borges et al. (2019) reported the loads of *Salmonella* from broiler carcasses before scalding were 3.04 $\log_{10}$ CFU/mL, which aligns to the loads observed in this study for broilers placed in non-cleaned drawers (**C**: 2.31 $\log_{10}$ CFU/mL). This comparison reinforces that broilers placed in containers that were either non-cleaned or inadequately cleaned could present comparable recovery level.

It is worth emphasizing that the *Salmonella* loads observed during holding prior to slaughter could remain at similar levels even after scalding and picking. As an example, Chavez-Velado (2022) documented that one of the three processing plants included in their study did not demonstrate a reduction in *Salmonella* loads when evaluating the levels at the rehang (1.85 $\log_{10}$ CFU/400 mL) compared to the levels observed during live receiving (2.39 $\log_{10}$ CFU/400 mL). Also, in the study conducted by Borges et al. (2019) a similar trend was reported. In this study, there was a Salmonella reduction observed after plucking (1.16 $\log_{10}$ CFU/mL) but then this reduction was not observed when sampled after the initial carcass wash (3.64 $\log_{10}$ CFU/mL). However, it is important to note that the results can vary depending on specific circumstances. De Villena (2022) and Chavez-Velado (2022) also documented cases in which *Salmonella* loads considerably decreased before reaching rehang.

In the case of *Salmonella* prevalence, the present study is comparable to the results presented by other studies (Chavez-Velado, 2022; De Villena et al., 2022). In the present study, the overall prevalence of *Salmonella* was 99.8%, while De Villena (2022) reported a prevalence of 94% for a single processing plant and Chavez-Velado (2022) documented *Salmonella* prevalence ranging from 87% to 98% in three separate processing plants. As mentioned earlier, the loads observed in both of those studies (Chavez-Velado, 2022; De Villena et al., 2022) were considerably lower compared to the loads observed in the current study. This serves as a clear example that relying solely on prevalence does not provide comprehensive insight into the *Salmonella* status of an incoming flock. The findings of the current study indicate that the process of cleaning transport containers reduces the transfer potential of *Salmonella* during holding.

The results of the carcass rinses obtained from treatment **A** (water wash, disinfectant, and water wash) and **B** (pressurized steam followed by hot air) indicate that the cleaning procedures evaluated reduce the transfer of *Salmonella* but did not achieve a complete inactivation or removal from the transport drawers, which concurs with previous studies that have reported remaining levels of bacteria after evaluating cleaning and disinfecting procedures for transport container flooring (Ramesh et al., 2002; Berrang and Northcutt, 2005; Hinojosa et al., 2015; 2018). Although reductions in transfer were not as distinctively perceptible in a logarithmic scale, when analyzing the transfer on an arithmetic scale (**A**: 122; **B**: 65; **C**: 394 CFU/mL), it showed that the incoming *Salmonella* loads could decrease by 69 to 83% when broilers are held in drawers that have undergone a cleaning procedure. Nauta et al. (2005) created a model for a quantitative microbiological risk assessment which favored the addition of arithmetic means to provide a deeper understanding of cross-contamination rates and incoming loads to the processing plant.

Effect of holding time on Salmonella transfer

As differences among treatments have been previously reported above, a comparison within each treatment was performed to observe the effect of holding time on *Salmonella* transfer, with results presented in Table 3.2. No effect of holding time is observed for the carcass rinses obtained from treatment **A** (water wash, disinfectant, and water wash) and **B** (pressurized steam followed by hot air), as 2, 4, or 6 h did not differ from each other within each treatment. For treatment **C** (no cleaning), a higher amount of *Salmonella* was observed from carcass rinses collected at 2 h (2.58 $\log_{10}$ CFU /mL) than those collected at 6 h (2.05 $\log_{10}$ CFU/mL). The carcass rinses obtained after 4 h (2.32 $\log_{10}$ CFU /mL) were comparable to those collected at either 2 or 6 h.

While no previous studies were found to be directly comparable to the present study, other research that has used food matrices and food contact surfaces have reported similar trends. For example, Moore et al. (2007) evaluated the transfer of *Salmonella* Typhimurium from different domestic food contact surfaces to cucumber slices with a 10 second contact time. The results reported by Moore et al. (2007) showed that transfer of *Salmonella* Typhimurium decreased over a period of 6 h regardless of the type of food contact surface inoculated (stainless steel, Formica, polypropylene, and wood). Furthermore, these studies reported a higher transfer from all contact surfaces when bacteria were suspended in high protein media, although variations were observed depending on the type of surface inoculated. For the specific case of polypropylene surfaces inoculated with *Salmonella* Typhimurium suspended in a high protein media showed a rapid decreased of transfer (with 10 seconds contact time) over a period of 5 h, reaching undetectable levels.

When the previous scenario is compared to the samples obtained from broilers placed in non-cleaned drawers, a slight resemblance can be observed (Moore et al., 2007). However, the transfer of *Salmonella* from the plastic drawers to the broilers in the present study did not decrease to undetectable levels, this outcome could potentially be attributed to the favorable type of matrix used for inoculation (fecal contents) as previous studies have shown the lengthy resilience of *Salmonella* in animal feces (Topalcengiz et al., 2020).

Additionally, the trend of *Salmonella* transfer rate reducing over time has been reported and supported by other studies that have evaluated different combinations contaminated surfaces and food matrices. However, all the matrices evaluated have been inanimate objects that were placed onto inoculated surfaces for a delimited contact time at specific times after inoculation (Kusumaningrum et al., 2003; Moore et al., 2003; Dawson et al., 2007). This differs from the

present study where a live animal was present and held for hours within a transport container, and a certain degree of movement is expected within the container.

Effect of drawer relative position on Salmonella transfer

As differences among treatments have been previously established above, a comparison within each treatment was performed to observe the effect the drawers' relative position on *Salmonella* transfer, with result presented in Table 3.3. For treatment **C** (no cleaning), no differences were observed based on the drawers' relative position. Both cleaning procedures, **A** (water wash, disinfectant, and water wash) and **B** (pressurized steam followed by hot air) shared a similar trend where the carcass rinses collected from the broilers placed in the top drawers (**A**: 0.92; **B**: 0.68 $\log_{10}$ CFU/mL) had lower counts than those collected from the broilers placed underneath them (**A**: 1.60 and 1.65 $\log_{10}$ CFU/mL for middle and bottom drawer, respectively; **B**: 1.57 and 1.76 $\log_{10}$ CFU/mL for middle and bottom drawer, respectively).

While it is possible to attribute the observed effect to the perforated floor design of the transport drawer, there were no existing studies directly comparable to the results presented in this study. The plastic drawers used for this experiment have perforated floors to enhance ventilation within the module, but droppings from the top drawers passed through acting as a vehicle for cross-contamination within the module. If a transport drawer becomes contaminated with *Salmonella*, it poses a risk not only to the birds placed in that specific drawer but also to all the birds placed underneath it. Alm et al. (2014) have documented a similar trend but evaluating the collection of droppings from a furnished 8-hen cage set up in three tiers. Alm et al. (2014) consistently collected more droppings from the bottom tiers of the cages when compared to top tiers of the cages. However, in their study a manure belt was placed under each level, and they

did not report manure moving from the top to the bottom. For which, the cause for this effect was left unknown for their experiment (Alm et al., 2014).

3.5 CONCLUSIONS

The application of pressurized steam followed by forced hot air was comparable to the application of water washes and disinfectant for cleaning plastic transport drawers. Both cleaning procedures effectively decreased the transfer of *Salmonella* to the broilers when compared to non-cleaned drawers, however, neither achieved complete pathogen inactivation or removal from flooring of the drawers. Moreover, it was observed that the transfer of *Salmonella* from the plastic drawers' flooring to the broilers could be influenced by the duration of holding and the relative position of the drawer within the module, which could result in points of interest to lessen cross-contamination during transport. The results of this study indicate that the application of pressurized steam and forced hot air have a potential role in cleaning poultry transport drawers and adaptations could be considered for a larger scale.

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Table 3.1 Prevalence and transfer of *Salmonella* Infantis from transport drawer flooring to broilers and the effect of using different cleaning procedures.

Treatment	$\log_{10}$ CFU/mL	Prevalence[n]
A: Water wash, disinfectant, and water wash	1.39 ± 0.07^{b}	134/135
B: Pressurized steam and forced hot air	1.34 ± 0.06^{b}	135/135
C: No cleaning	2.31 ± 0.05^{a}	135/135

[a-b] Values within a column with different superscripts are significantly different ($P \leq 0.05$).
n = 135

Table 3.2 Transfer of *Salmonella* Infantis from transport drawer flooring to broilers and the effect of different cleaning procedures measured at different timepoints during holding.

Treatment	$\log_{10}$ CFU/mL		
	2 h	**4 h**	**6 h**
A: Water wash, disinfectant, water wash	1.55 ± 0.13	1.39 ± 0.14	1.23 ± 0.11
B: Pressurized steam and forced hot air	1.57 ± 0.11	1.19 ± 0.12	1.25 ± 0.10
C: No cleaning	2.58 ± 0.06 ^{x}	2.32 ± 0.08 ^{xy}	2.05 ± 0.08 ^{y}

$^{x-y}$ Values within a row with different superscripts are significantly different ($P \leq 0.05$).
n = 45

Table 3.3 Transfer of *Salmonella* Infantis from transport drawer flooring to broilers and the effect of different cleaning procedures by drawer relative position.

Treatment	$\log_{10}$ CFU/mL		
	Top	**Middle**	**Bottom**
A: Water washes and disinfectant	0.92 ± 0.12^{y}	1.60 ± 0.14^{x}	1.65 ± 0.10^{x}
B: Pressurized steam and forced hot air	0.68 ± 0.10^{y}	1.57 ± 0.09^{x}	1.76 ± 0.07^{x}
C: No cleaning	2.18 ± 0.09	2.31 ± 0.08	2.45 ± 0.08

$^{x\text{-}y}$ Values within a row with different superscripts are significantly different ($P \leq 0.05$).
n = 45

CHAPTER 4: THESIS CONCLUSION AND FUTURE IMPLICATIONS

The inadequacy of current cleaning methods for transport containers highlights the need for the development of improved interventions to reduce the risk of cross-contamination during poultry transportation. Future research in this topic should focus on exploring and refining novel techniques that can effectively remove feces and inactivate pathogens. The present study indicates that pressurized steam followed by forced hot air has the potential to be an effective cleaning procedure for poultry transport containers and significantly reduce poultry associated pathogens (*Salmonella* and *Campylobacter*). These results open possibilities for further investigation and optimization of the method presented in this study for practical application in real-world settings. Exploring different parameters, such as steam pressure, hot air temperature, and exposure times, to enhance the efficacy of this cleaning approach could be considered. Also, this study presented the variations in response between different pathogens to the same cleaning procedure, such as the difference of the effectiveness of pressurized steam followed by forced hot air against *Salmonella* versus *Campylobacter*. Cleaning patterns, application times, operating pressures, disinfectant types, and water usage are all factors intrinsically connected impacting cleaning efficacy. Future research could focus on identifying the optimal combinations and conditions of these factors to maximize the effectiveness of cleaning procedures for poultry transport containers. The findings of the present study could guide industry stakeholders, regulatory entities, and researchers in implementing more effective measures to ensure the safety of poultry products.