Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2023:88
Faculty of Veterinary Medicine and Animal Sciences

Foodborne bacteria in slaughterhouses

with focus on cleaning and disinfection

Madeleine Moazzami

Foodborne bacteria in slaughterhouses

with focus on cleaning and disinfection

Madeleine Moazzami
Faculty of Veterinary Medicine and Animal Sciences
Department of Biomedical Sciences and Veterinary Public Health
Uppsala

SWEDISH UNIVERSITY
OF AGRICULTURAL
SCIENCES

DOCTORAL THESIS

Uppsala 2023

Acta Universitatis Agriculturae Sueciae
2023:88

Cover illustration by Aila Erkkilä

ISSN 1652-6880
ISBN (print version) 978-91-8046-230-3
ISBN (electronic version) 978-91-8046-231-0
https://doi.org/10.54612/a.7radbcpbh7
© 2023 Madeleine Moazzami, https://orcid.org/0000-0001-7038-911X
Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health, Uppsala, Sweden
The summary chapter of this thesis is licensed under CC BY NC ND 4.0, other licences or copyright may apply to illustrations and attached articles.
Print: SLU Grafisk service, Uppsala 2023

Foodborne bacteria in slaughterhouses with focus on cleaning and disinfection

Abstract

Proper cleaning and disinfection (C&D) in slaughterhouses and meat processing premises is a prerequisite for production of safe, high-quality meat. An example of how inadequate C&D can affect human health is provided by the strong increase in number of human cases of campylobacteriosis in Sweden in 2016-2017 due to insufficiently cleaned transport crates for chicken. This thesis evaluated the efficacy of C&D procedures in slaughterhouses and adjacent meat processing premises by testing for pathogenic and hygiene indicator bacteria on equipment, facilities and carcasses. Different sampling methods used for assessing surface cleanliness were compared and slaughterhouses with associated meat processing premises in Sweden were interviewed regarding their C&D routines and monitoring activities. The results indicated that the C&D procedures used for surfaces in the slaughterhouse premises were sufficient regarding removal/inactivation of pathogenic bacteria. However, only approximately half of the surfaces were assessed as sufficiently cleaned, as the amount of hygiene indicator bacteria was high. *Campylobacter jejuni, C. coli* and *Listeria monocytogenes* were detected on food contact surfaces, indicating high risk of contamination of carcasses and meat. The same *L. monocytogenes* strain was found in a red meat slaughterhouse environment on three occasions 15 weeks apart, while a *C. jejuni* strain showed potential to spread within a poultry slaughterhouse. Extended-spectrum beta-lactamase (ESBL) *Escherichia coli* was not detected. New technologies such as 265 nm UV-C LED irradiation on chicken transport crates and ultrasound-steam treatment and chilling of broiler carcasses were shown to give significant bacterial reductions. However, these techniques require a certain time of action that is difficult to combine with high slaughter speed in an industrial setting. Dipslides and ATP-bioluminescence were found to be of varying reliability, but are commonly used by Swedish slaughterhouses with associated meat processing premises.

Keywords: ATP-bioluminescence, contact plate, dipslides, *Enterobacterales*, Extended-spectrum beta-lactamase (ESBL) *E. coli*, *Campylobacter*, *Listeria monocytogenes*, meat premises, total aerobic bacteria

Livsmedelsburna bakterier på slakterier med fokus på rengöring och desinfektion

Sammanfattning

Korrekt rengöring och desinfektion (R&D) på slakterier och styckningsanläggningar är en förutsättning för produktion av säker mat av hög kvalitet. Ett exempel på hur bristande R&D på slakterier har påverkat människors hälsa var det kraftigt förhöjda antalet fall av campylobacterios i Sverige 2016-2017 på grund av otillräckligt rengjorda transportlådor till kyckling. Syftet med avhandlingen var att utvärdera effekten av olika R&D metoder genom att kartlägga förekomsten av patogena och hygienindikatorbakterier på utrustning, i slakterimiljön och på slaktkroppar. Dessutom jämfördes olika provtagningsmetoder för utvärdering av rengöringseffekten. Intervjuer genomfördes med ansvarig personal på svenska slakterier och styckningsanläggningar gällande deras R&D rutiner samt provtagningsmetoder. Resultaten visade att R&D metoderna avlägsnade/inaktiverade patogena bakterier från ytor. Däremot var cirka hälften av ytorna inte tillräckligt rengjorda, då antalet hygienindikatorbakterier var högt. *Campylobacter jejuni, C. coli* och *Listeria monocytogenes* påvisades på produktkontaktytor, vilket utgör stor risk för korskontamination av slaktkroppar och kött. Dessutom återfanns samma *Listeria monocytogenes* stam på ytor i ett gris- och nötslakteri inom ett 15-veckors intervall och en *Campylobacter jejuni* stam visade stor spridningspotential på ett kycklingslakteri. Extended-spectrum beta-lactamase (ESBL) *E. coli* påvisades inte. Utvärdering av nya tekniker såsom 265 nm UV-C LED strålning av transportlådor för kyckling och behandling av kycklingslaktkroppar med ultraljud-vattenånga samt kylning innebar en signifikant minskning av antalet bakterier. Dessa tekniker kräver dock en viss verkningstid vilket kan vara svårt att kombinera med ett högt slakttempo. Tryckplattor och ATP–bioluminescens som används för rengöringskontroll på många svenska slakterier, är av varierande tillförlitlighet.

Nyckelord: ATP-bioluminscens, tryckplattor, *Enterobacterales*, Extended-spectrum beta-lactamase (ESBL) *E. coli*, *Campylobacter*, *Listeria monocytogenes*, styckningsanläggningar, totalantal aeroba bakterier

Dedication

To all of you who are responsible for cleaning, your work is very important as it helps protecting human health.

Contents

List of publications 11

List of tables 13

List of figures 15

Abbreviations 17

1. Introduction 19
   - 1.1 Contamination at slaughter 19
   - 1.2 Microbiological hazards in raw meat 21
     - 1.2.1 *Campylobacter* 21
     - 1.2.2 *Listeria monocytogenes* 22
     - 1.2.3 ESBL *Escherichia coli* 24
   - 1.3 Meat spoilage bacteria 25
   - 1.4 Residential bacteria in slaughterhouses 25
   - 1.5 Cleaning and disinfection as a control measure in slaughterhouses 26
     - 1.5.1 Food legislation and guidelines.. 26
     - 1.5.2 General cleaning and disinfection procedures.. 27
     - 1.5.3 Cleaning and disinfection of equipment and facilities…..29
     - 1.5.4 Cleaning and disinfection of chicken transport crates.. ..30
     - 1.5.5 Decontamination of carcasses 31
   - 1.6 Monitoring of cleaning and disinfection procedures 32
     - 1.6.1 Selection of pathogens and hygiene indicators.. 32
     - 1.6.2 Selection of sampling methods and sampling points...... 33
     - 1.6.3 Interpretation of sampling results.. 33

2. Main aims of the thesis 35

3. Considerations on materials and methods 37
   - 3.1 Selection of slaughterhouses 37

3.1.1 Descriptive study.. 37
3.1.2 Cleaning and disinfection (Papers I & II) and decontamination studies (Paper IV)……………………………… 38
3.2 Study design and data collection………………………………….. 39
3.2.1 Descriptive study……………………………………………... 39
3.2.2 Cleaning and disinfection studies on equipment and facilities (Papers I & II)……………………………….……….…. 39
3.2.3 UV-C LED light source (Disinfection study, Paper III)..... 42
3.2.4 Ultrasound-steam equipment (Decontamination study, Paper IV)...44
3.3 Microbiological and ATP-bioluminescence analyses…….…….. 45
3.4 Statistical analysis…………………………………………………...47

4. Main results and discussion 49
4.1 Descriptive study...50
4.1.1 Cleaning and disinfection procedures.. 50
4.1.2 Monitoring activities.. 53
4.1.3 Cleaning and disinfection procedures used by the slaughterhouses (Papers I, II and IV)…………………………….. 56
4.2 Control of pathogenic and hygiene indicator bacteria in slaughterhouses by cleaning and disinfection measures (Paper I-IV)...57
4.2.1 Efficacy of cleaning and disinfection procedures of equipment and facilities (Papers I & II)…………………………… 57
4.2.2 Disinfection study on chicken transport crates (Paper III)……………………………………………………………….. 64
4.2.3 Decontamination study on broiler carcasses (Paper IV)…….. 66
4.2.4 Assessment of monitoring methods to evaluate surface cleanliness (Paper I)…….. 67
4.3 Antimicrobial resistance – AMR (Paper II).................................70

5. Main conclusions 73

6. Future perspectives 75

References 77

Popular science summary 99

Populärvetenskaplig sammanfattning 103

Acknowledgements 107

Appendix I 111

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Moazzami M., Bergenkvist E., Boqvist S., Frosth S., Langsrud S., Møretrø T., Vågsholm I., Hansson I. (2023). Assessment of ATP-bioluminescence and dipslide sampling to determine the efficacy of slaughterhouse cleaning and disinfection compared with total aerobic and *Enterobacterales* counts. Journal of Food Protection, 86(10), 100155.

II. Moazzami M., Bergenkvist E., Boqvist S., Frosth S., Langsrud S., Møretrø T., Vågsholm I., Hansson I. Occurrence of *Campylobacter*, *Listeria monocytogenes*, and extended-spectrum beta-lactamase *Escherichia coli* in slaughterhouses before and after cleaning and disinfection. (manuscript)

III. Moazzami M., Fernstrom L-L., Hansson I. (2021). Reducing *Campylobacter jejuni, Enterobacteriaceae* and total aerobic bacteria on transport crates for chickens by irradiation with 265-nm ultraviolet light (UV–C LED). Food Control, 119, 107424.

IV. Moazzami M., Bergenkvist E., Fernström L-L., Rydén J., Hansson I. (2021). Reducing *Campylobacter jejuni, Enterobacteriaceae, Escherichia coli*, and total aerobic bacteria on broiler carcasses using combined ultrasound and steam. Journal of Food Protection, 84(4), 572-578.

Papers I, III-IV are reproduced with the permission of the publishers.

The contribution of Madeleine Moazzami to the papers included in this thesis was as follows:

I. Determined sampling plan and laboratory analyses in cooperation with the co-authors. Collected the samples and performed the labwork. Analysed and visualised the data. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

II. Determined sampling plan and laboratory analyses in cooperation with the co-authors. Collected the samples and performed the labwork. Analysed and visualised the data. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

III. Took major responsibility in planning the study and performing the labwork. Analysed and visualised the data. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

IV. Took major responsibility in planning the study and performing the labwork together with the co-authors. Analysed and visualised the data. Drafted the manuscript and finalised it together with the co-authors. Corresponded with the journal.

List of tables

Table 1. Hygiene indicators and pathogenic bacteria analysed with each sampling method………………………………………………………….…..... 40

Table 2. Data from interviews with quality assurance managers at 10 of the largest slaughterhouses in Sweden, slaughtering cattle, swine, sheep, poultry and horses, about their general cleaning and disinfection procedures………………………………………………………………….….. 52

Table 3. Data obtained in interviews with quality assurance managers at 10 of the largest slaughterhouses in Sweden, slaughtering cattle, swine, sheep, poultry and horses, about their monitoring activities………………..…………………………………………………….. 54

Table 4. Observational data on measured temperature (Temp) and relative humidity (RH) at the time of sampling in the slaughter area and processing area in the red meat and poultry slaughterhouses, before and after cleaning and disinfection..............…………………………………………………... 57

Table 5. Occurrence (%) of *Campylobacter* spp. and *Listeria monocytogenes* in the red meat and poultry slaughterhouses before and after cleaning and disinfection……………………..…... 60

Table 6. Number of samples in which *Campylobacter* spp., *Listeria monocytogenes* and *Enterobacterales* were detected before and after cleaning and disinfection (C&D), mean before and after C&D, and reduction in total aerobic bacteria………... 61

Table 7. Mean bacteria levels and mean reduction (log CFU/mL) on chicken transport crates before and after irradiation with UV-C light for 1 and 3 min... 65

Table 8. Mean bacteria levels and mean reduction (log CFU/g) on chicken neck skins before and after carcass decontamination in a poultry slaughterhouse………………………………………………………........... 66

List of figures

Figure 1. Contaminated cutting blade in a slaughterhouse…………..…….. 19

Figure 2. Number of notified human cases of campylobacteriosis and incidence (per 100,000 inhabitants) in Sweden, 2002-2022……………….. 22

Figure 3. Number of notified human cases of listeriosis and incidence (per 100,000 inhabitants) in Sweden, 2002-2022, with trend curve…………….. 23

Figure 4. General steps in cleaning and disinfection procedures in slaughterhouses.. 28

Figure 5. Sinner's circle summarising the four interacting factors in the cleaning and disinfection procedure……………………………….………. 29

Figure 6. Sampling material: wiping cloth, sponge, dipslide and ATP-bioluminescence swab……………………..……………………………….. 40

Figure 7. Autoclaved stainless steel frames (100 cm^2 and 25 cm^2) used to delineate an exact sampling area……..………………………………………. 41

Figure 8. Light-emitting diode (LED) light cabinet for irradiation of chicken transport crates…………………………………………………………….... 42

Figure 9. Simulated contamination of a piece of a chicken transport crate……………………………………………………...……………. 43

Figure 10. Chicken carcasses entering and leaving the treatment chamber with ultrasound and steam installed in the poultry slaughterhouse studied in Papers I, II and IV………………………………..……………………….. 44

Figure 11. Characteristic *Listeria monocytogenes* colonies surrounded by opaque zones on a chromogenic Listeria agar plate................................ 46

Figure 12. ATP-bioluminescence swab and device………………………… 47

Figure 13. Measures for prevention of spread of pathogenic and hygiene indicator bacteria by cleaning and disinfection at slaughterhouse level evaluated in the studies included in this thesis……………………….……… 49

Figure 14. Contaminated salt injector needles…………………………….… 59

Figure 15. Contaminated drain in a slaughterhouse………………………... 63

Figure 16. Proportion (%) of acceptably clean food contact surfaces (FCS) and non-food contact surfaces (NFCS) in the red meat and poultry slaughterhouses when using swabbing (total aerobic bacteria, TAB), dipslides (total viable count, TVC) and ATP-bioluminescence (relative light units, RLU)…………………………………..………………………….. 68

Abbreviations

Ac	Accuracy
AMR	Antimicrobial resistance
ATP	Adenosine-triphosphate
BPW	Buffered peptone water
CC	Clonal complex
C&D	Cleaning and disinfection
CFU	Colony-forming units
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ESBL	Extended-spectrum beta-lactamase
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
FBO	Food business operator
FCS	Food contact surface
GHP	General hygiene practices
HACCP	Hazard Analysis and Critical Control Points
ISO	International Organization for Standardization
κ	Cohen's kappa agreement coefficient
LED	Light-emitting diode
MALDI-TOF	Matrix-assisted laser desorption ionization-time flight mass spectrometry
MIC	Minimum inhibitory concentrations
MLST	Multilocus sequence typing
NFCS	Non-food contact surface

NMKL	Nordisk Metodikkommitté för Livsmedel
NPV	Negative predictive value
PPV	Positive predictive value
QAC	Quaternary ammonium compound
RLU	Relative Light Units
RTE	Ready-to-eat
Se	Sensitivity
SLU	Swedish University of Agricultural Sciences
Sp	Specificity
Spp	Species
ST	Sequence type
STEC	Shiga toxin-producing *Escherichia coli* (may also be referred to as verotoxin-producing *E. coli* (VTEC))
TAB	Total aerobic bacteria
TVC	Total viable count
UV	Ultraviolet
WGS	Whole-genome sequencing
WHO	World Health Organization

1. Introduction

1.1 Contamination at slaughter

During slaughter, the slaughter equipment and facilities are continually contaminated with *e.g.* intestinal content, blood and residues of muscle and fat (Figure 1). There is risk of contamination at several steps in the slaughter process, so it is important that these steps are properly performed. In poultry slaughter, contamination can occur during steps such as bleeding, scalding, defeathering and evisceration (Buncic & Sofos 2012; Seliwiorstow et al. 2016; Boubendir et al. 2021). In pig slaughter, a risk of contamination arises *e.g.* during scalding, dehairing and evisceration (Spescha et al. 2006; De Busser et al. 2013; Wheatley et al. 2014; Bakhtiary et al. 2016). In cattle slaughter, proper execution of dressing, evisceration and the meat inspection process is crucial to avoid contamination (Zweifel et al. 2014; Nakamura et al. 2023).

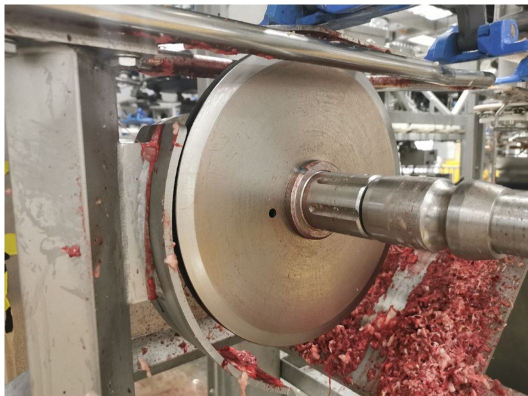


Figure 1. Contaminated cutting blade in a slaughterhouse (photo by author).

An important difference in these processes is that large amounts of water are used in poultry slaughter, which facilitates the survival and spread of bacteria (Adams & Moss 1995; Ninios et al. 2014; National Advisory Committee on Microbiological Criteria for Foods 2022).

When meat comes into contact with dirty surfaces, it can become contaminated with bacteria resulting in a risk of causing human disease or meat spoilage. Many studies have focused on bacterial contamination of carcasses (Lindblad et al. 2006; Reich et al. 2008; Hansson et al. 2010; Manage et al. 2019; Peruzy et al. 2021; Hauge et al. 2023), but fewer on surface contamination in meat processing premises. Among the few studies that have investigated microbiological hazards on surfaces in slaughterhouses, bacteria such as *Campylobacter*, *Listeria monocytogenes*, *Salmonella* and extended-spectrum beta-lactamase producing *Escherichia coli* (ESBL *E. coli*) have been detected (Olsen et al. 2003; Peyrat et al. 2008; Piras et al. 2014; Sala et al. 2016; Schäfer et al. 2017; Atlaw et al. 2022). In addition to bacteria, other microbiological hazards such as Hepatitis E can contaminate surfaces during slaughter (Di Bartolo et al. 2012), and there is also possibility of contamination by parasites, although this is rarely studied (EFSA & ECDC 2021).

Microbiological hazards pose potential risks of foodborne disease and outbreaks, resulting in severe consequences such as loss of lives and closure of food businesses (Farber & Peterkin 1991; Tam 2003; Hussain & Dawson 2013). Aside from contamination originating from the slaughtered animals, other sources of contamination include workers, who may carry pathogenic bacteria on their hands or clothing, and the slaughter environment itself, which may harbour residential bacteria (Todd et al. 2010; Ivbule et al. 2017; Møretrø & Langsrud 2017). Additional hazards which could contaminate meat are physical (*e.g.* pieces of metal, cardboard, plastic) and chemical hazards (*e.g.* grease used for slaughter equipment and residues after cleaning and disinfection) (Ninios et al. 2014; Codex Alimentarius 2020).

Bacteria are of particular concern in terms of the safety and quality of raw meat (Meat Industry Guide 2017; EFSA & ECDC 2022), as they can multiply in food under the right circumstances, unlike most other hazards. To prevent contamination of meat, a hygienic slaughter process is essential and must be followed by efficient cleaning and disinfection (C&D) procedures (EC 2004; Moura-Alves et al. 2022).

1.2 Microbiological hazards in raw meat

Many pathogenic bacteria are associated with raw meat, *e.g. Campylobacter*, *Salmonella*, Shiga toxin-producing *E. coli* (STEC), *L. monocytogenes* and *Yersinia* (Andersen et al. 1991; Borch et al. 1996; Arguello et al. 2012; Martín et al. 2014; Skarp et al. 2016; Brusa et al. 2022). At European level, campylobacteriosis is the most commonly reported zoonosis in humans, followed by salmonellosis (EFSA & ECDC 2022). However, in Sweden *Salmonella* prevalence is very low in cattle, pigs and poultry (Ågren et al. 2016; National Veterinary Institute 2023), due to the *Salmonella* control programme, which was initiated after a large Salmonella outbreak in 1953 (Wierup et al. 1995). This is reflected in the low number of human domestic cases reported annually (Public Health Agency of Sweden 2022).

1.2.1 *Campylobacter*

Campylobacter can be found in different types of meat, *e.g.* poultry, pork, beef and mutton (Nauta et al. 2009; Walker et al. 2019). *Campylobacter* is the dominant bacterial cause of gastroenteritis worldwide and is associated with consumption of meat products, especially broiler meat (Doorduyn et al. 2010; WHO 2020). Typical symptoms are bloody diarrhoea, abdominal cramps, fever and occasionally vomiting, while certain individuals can experience sequelae such as irritable bowel syndrome (IBS), arthritis and the neurological disorder Guillain-Barré syndrome (Allos 1997; Pope et al. 2007; Peters et al. 2021).

The Swedish poultry industry encountered significant challenges during 2016-2017 due to a sizable *Campylobacter* outbreak (Figure 2), which was traced back to deficient cleaning of chicken transport crates. This facilitated bacterial colonisation of broilers in conjunction with thinning. During the subsequent slaughter process, meat contamination occurred via spread of faecal matter on slaughterhouse surfaces (Hansson et al. 2005; Lofstedt 2019), leading to an outbreak with a five-fold increase in the annual level of reported campylobacteriosis cases. Deficient cleaning of transport crates and the following repercussions in Sweden are documented in a previous study (Hansson et al. 2005). In addition to cross-contamination of meat during production, *Campylobacter* has been detected on food contact surfaces (FCSs) after C&D, from where it contaminated carcasses during the next slaughter shift (Johnsen et al. 2006; Peyrat et al. 2008; Kudirkienė et al. 2011; García-Sánchez et al. 2017).

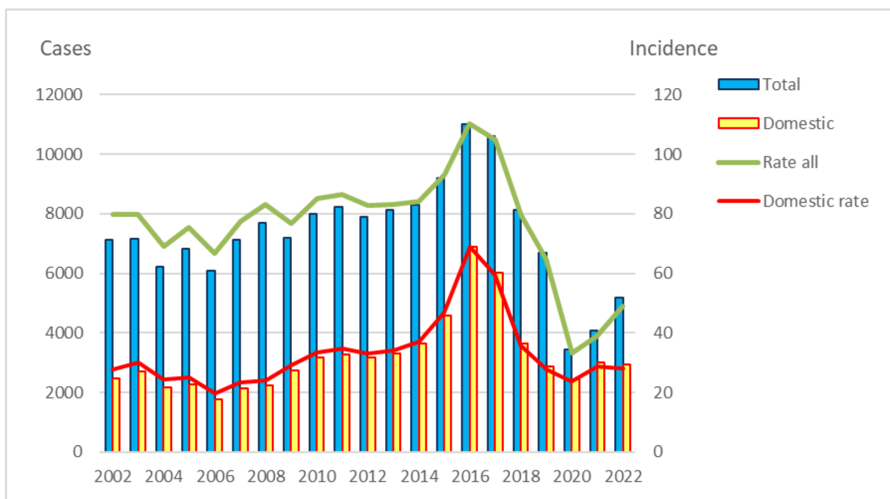


Figure 2. Number of notified human cases of campylobacteriosis and incidence (per 100,000 inhabitants) in Sweden, 2002-2022. Domestic cases are patients who had not travelled outside Sweden within the two weeks before onset of symptoms.

1.2.2 *Listeria monocytogenes*

Listeriosis is the fifth most frequently reported zoonotic disease in humans in Europe and is also of global concern (WHO 2018; EFSA & ECDC 2022). *Listeria monocytogenes* has the highest proportion of hospitalised cases and fatality rates. Furthermore, *Listeria* infections are most commonly reported in the age group over 64 years in the European Union (EU), with a particularly high fatality rate for invasive listeriosis (16.0-22.5%) (EFSA Biohazard Panel 2018; Desai et al. 2019). The invasive form may lead to severe complications such as meningitis and sepsis, particularly among vulnerable groups, and is known to cause abortion (Pagliano et al. 2017).

Meat and meat products are common food sources causing listeriosis outbreaks in Europe, although it is a greater threat in facilities processing ready-to-eat (RTE) meat such as pâte and fermented raw sausages (Hadjicharalambous et al., 2019; EFSA & ECDC 2022). Moreover, *L. monocytogenes* is among the pathogenic bacteria capable of growing in refrigeration temperature (Chan & Wiedmann 2008). Therefore, people with immunocompromised status, the elderly and pregnant women should follow the recommendations provided by the authority, for example they should avoid eating sliced ham that has been stored in refrigeration for more than a week (Swedish Food Agency 2023).

It is essential to control this pathogen at slaughterhouse level to prevent its introduction into meat cutting and processing plants and facilities where RTE products are prepared further along the meat production chain (Codex Alimentarius 2007). Poultry and red meat slaughterhouses can act as hotspots for *L. monocytogenes,* since the environment can easily be contaminated by faecal matter during the slaughter process (Sala et al. 2016; Schäfer et al. 2017). In addition, *L. monocytogenes* has been detected on visually clean hides (Demaître et al. 2021).

Listeria monocytogenes is a typical example of a bacterial species that is capable of producing biofilm, providing protection against factors such as heat, desiccation and the effect of chemical agents used for C&D (Møretrø et al. 2012; Stoller et al. 2019). This poses significant concerns for food business operators (FBOs), as it can lead to outbreaks due to cross-contamination when food products come into contact with inadequately cleaned surfaces. A recent study detected hypervirulent *L. monocytogenes* strains in slaughterhouses (Guidi et al. 2023), which is a particularly important finding as there is an increasing trend in listeriosis cases in Sweden (Figure 3).

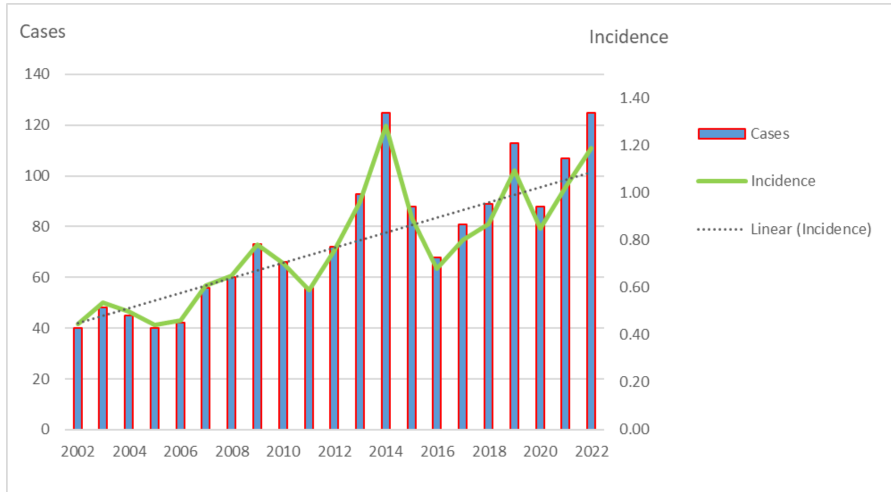


Figure 3. Number of notified human cases of listeriosis and incidence (per 100,000 inhabitants) in Sweden, 2002-2022, with trend curve.

1.2.3 ESBL *Escherichia coli*

Extended-spectrum beta-lactamase-producing bacteria exhibit resistance to conventional penicillins and most cephalosporins (Perez et al. 2007). This resistance is transferrable to other bacteria (Gekenidis et al. 2020; Dorr et al. 2022). Extended-spectrum beta-lactamase-producing bacteria have been found in the gut of both humans and animals (Asir et al. 2015; Tofani et al. 2022). In several European countries, ESBL *E. coli* has also been detected on the carcasses of pigs, sheep, cattle and chickens (Bardoň et al. 2013; Pacholewicz et al. 2015; Biasino et al. 2018; Tsitsos et al. 2022). One of the reasons for presence of ESBL *E. coli* on poultry meat could be treatment of commercial birds with ciprofloxacin to protect them from *E. coli* infections, which is permitted in some countries (Roth et al. 2019). This is cause for concern, since *E. coli* can cross-contaminate meat when food contact surfaces (FCSs) are not properly cleaned and disinfected (Gill & McGinnis 2000). Few studies have investigated the presence of ESBL-producing bacteria on surfaces in food premises, however, one study found that ESBL *E. coli* was more abundant in a sheep slaughterhouse environment than on sheep carcasses (Atlaw et al. 2022). Cross-contamination of broiler carcasses with ESBL *E. coli* during scalding and defeathering has also been observed (Projahn et al. 2019).

Antimicrobial resistance (AMR) is a worldwide threat, with at least 700,000 people estimated to die due to drug-resistant diseases each year (WHO 2019a). The World Health Organization (WHO) has listed critically important antimicrobials that should be reserved for human medicine. These include third- and fourth-generation cephalosporins (WHO 2019b). The resistance of ESBL *E. coli* poses major therapeutic challenges, as it leads to lack of effective treatments for conditions such as urinary tract infections caused by this bacterial species (Hertz et al. 2016). This is evident in the increase in bloodstream infections caused by *E. coli* resistant to third-generation cephalosporins during recent years (WHO 2022).

1.3 Meat spoilage bacteria

Presence of bacteria that cause spoilage of meat is a major concern for FBOs, because it decreases product shelf-life. At societal level, longer shelf-life would lower the environmental footprint of meat production, as spoilage plays a significant role as a driver of food waste. Globally, approximately one-third of all food produced is wasted, which represents about 1.3 billion tons of food per year. It is estimated that in Europe approximately 20% of the meat produced is lost between harvest and consumption (FAO 2011). Raw meat provides suitable conditions for bacterial growth due to its rich nutrient content and sufficient water activity (Adams & Moss 1995; Nylander et al. 2014). Common bacteria causing meat spoilage include Gram-negative bacteria belonging to the order *Enterobacterales* (*e.g. Serratia* spp. and *Proteus* spp.), non-*Enterobacterales* (*e.g. Pseudomonas* spp. and *Acinetobacter* spp.) and Gram-positive bacteria (*e.g. Enterococcus* spp., *Brochotrix thermospacta*, Lactic acid bacteria and *Staphylococcus* spp.) (Doulgeraki et al. 2012; Møretrø & Langsrud 2017; Gong et al. 2019; Lauritsen et al. 2019; Odeyemi et al. 2020; Carvalheira et al. 2021). Spoilage bacteria have been detected on important FCSs such as conveyor belts in slaughterhouses, even after C&D, and in other meat processing premises (Møretrø et al. 2013; Maes et al. 2017; Møretrø & Langsrud 2017; Wang et al. 2018; Maes et al. 2019). Moreover, inadequate C&D of surfaces in slaughterhouses has been found to result in contamination of meat with spoilage bacteria, which emphasises the need for efficient C&D (Samapundo et al. 2019).

1.4 Residential bacteria in slaughterhouses

Bacteria that are removed or inactivated by C&D procedures are referred to as ‘transient’ bacteria. In cases where there are harbourage sites such as cracks in the floor and scratches in cutting boards, bacteria can resist C&D measures and establish themselves within the facility, and are then called ‘residential’ bacteria (Møretrø & Langsrud 2017). *Listeria monocytogenes* is an example of a residential bacterial species, owing to its ability to produce biofilm. Other common residential biofilm-producing bacteria that have been detected in meat processing premises are *Acinetobacter* spp., *Pseudomonas* spp., *Stenotrophomonas* spp., *Serratia* spp., *Citrobacter* spp. and *Staphylococcus* spp., many of which also cause meat spoilage (Xu et al.

2011; Møretrø et al. 2013; Nemeghaire et al. 2014; Adegoke et al. 2017; Cai et al. 2018; Wagner et al. 2020; Zarei et al. 2023). Once residential bacteria such as *L. monocytogenes* and *Acinetobacter* spp. have colonised the processing environment, they pose significant challenges to FBOs, as they are difficult to eliminate (Carvalheira et al. 2021). In cases where bacteria such as *L. monocytogenes* becomes residential, they can persist in meat processing plants for extended periods of time (Ortiz et al. 2010; Fagerlund et al. 2016; Stoller et al. 2019). Moreover, *L. monocytogenes* has been found on FCSs such as conveyor belts and cutting tools in both red meat and poultry slaughterhouses, which increases the likelihood of cross-contamination of the meat (Martín et al. 2014; Gómez et al. 2015; Muhterem-Uyar et al. 2015).

1.5 Cleaning and disinfection as a control measure in slaughterhouses

Cleaning and disinfection is an important control measure to prevent bacterial contamination of meat in slaughterhouses and meat processing premises, which are often located adjacent to or within the slaughterhouse. Different methods for cleaning and/or disinfection should be used at different steps during slaughter and meat processing, which involves C&D of surfaces of equipment and facilities, C&D of transport crates in poultry slaughterhouses and occasionally decontamination of carcasses.

1.5.1 Food legislation and guidelines

The European Union food regulation EC No. 852/2004 on the hygiene of foodstuffs, obliges FBOs to have general hygiene practices (GHP) in place. This is the initial step before implementation of a Hazard Analysis and Critical Control Points (HACCP) plan, to ensure production of safe food (EC 2004). Cleaning and disinfection is part of GHP, and the EU legislation specifies that slaughterhouses should clean and when necessary disinfect facilities and equipment such as crates and containers "in an appropriate manner". However, it does not provide details on the C&D procedure, which gives FBOs the possibility to choose the most appropriate C&D procedure for their facility and production.

The international food standard, "*General principles of food hygiene*" by Codex Alimentarius, contains similar information to the EU legislation, but

also provides information on how to perform C&D, such as the general steps of the C&D procedure (Codex Alimentarius 2020). However, it is very general, since it is not directed specifically at slaughterhouses. In the EU, national guidelines for producers of different meat that have been approved by the national authorities should assist FBOs. For Swedish slaughterhouses slaughtering cattle, lamb and swine, there is a guideline which provides more details about C&D procedures. However, for poultry slaughterhouses and meat processing premises, there are no Swedish national guidelines covering cleaning and disinfection. This places substantial demands on FBOs' knowledge of C&D procedures. The Swedish Food Agency provides advice about C&D procedures that is aimed at food inspectors, but the information can also be useful for FBOs (Swedish Food Agency 2022). However, while legislation and some guidelines are available, they still only provide general information about the C&D procedure. Suggestions on methods, for example chemical products, dilution of the products and contact times, are lacking.

1.5.2 General cleaning and disinfection procedures

Cleaning and disinfection procedures, also referred to as 'sanitation' (Marriott et al. 2018), should cover all spaces in the meat production facility (Lindahl et al. 2009; Thougaard et al. 2023). Cleaning refers to removal of undesired material, generally referred to as 'soil', such as foreign bodies, dust, food residues including microorganisms and allergens (Holah 2014). Cleaning alone can greatly reduce the number of microorganisms in the meat processing environment (Quinn et al., 2011). In slaughterhouses, cleaning should be followed by a disinfection step to inactivate microbes to such an extent that the disinfected surfaces do not contaminate the meat (Lindahl et al. 2009; Codex Alimentarius 2020). The general steps for C&D in slaughterhouses are shown in Figure 4.

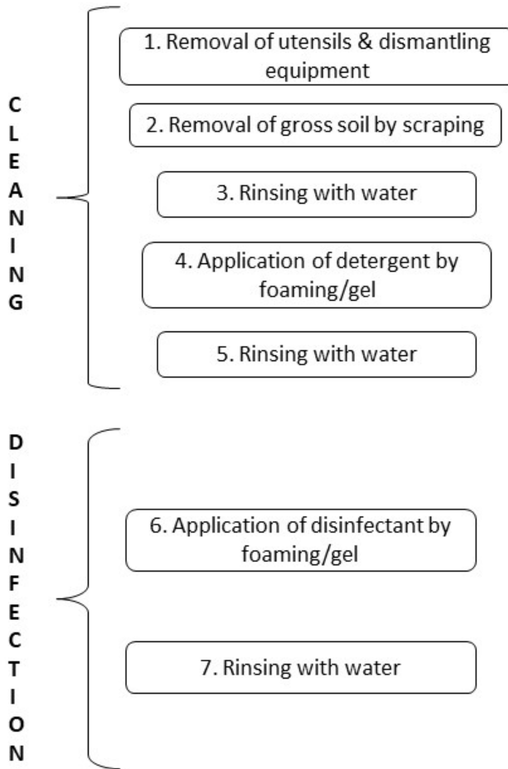


Figure 4. General steps in cleaning and disinfection procedures in slaughterhouses. Source: Modified from Lindahl et al. (2009) and Codex Alimentarius (2020).

The Sinner's circle diagram in Figure 5 describes a satisfactory C&D result arising from interaction between four factors that affect the C&D procedure: mechanical force, chemical process, contact time and water temperature (Wilson et al. 2022). These factors must act together to achieve efficient C&D. If one of these factors is reduced (*e.g.* contact time), another needs to be increased (*e.g.* mechanical force).

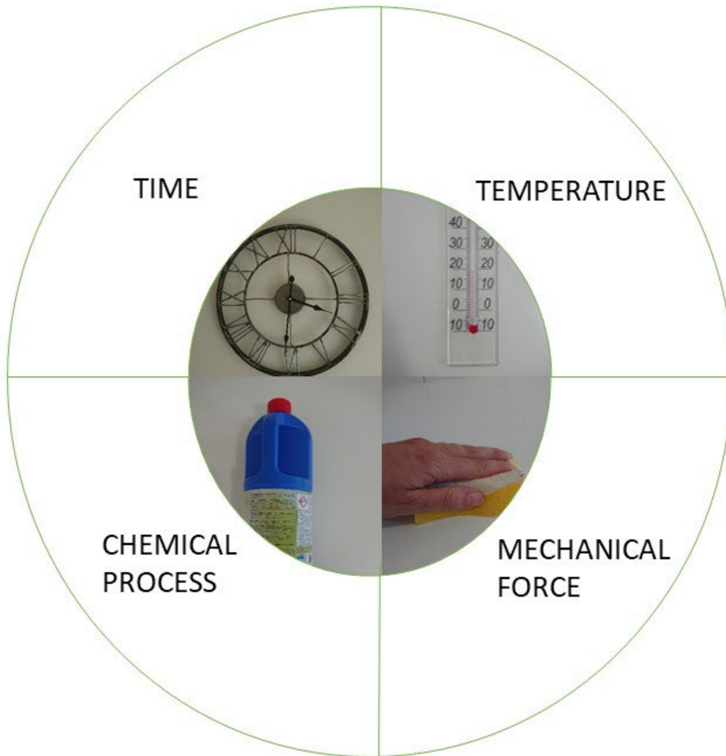


Figure 5. Sinner's circle summarising the four interacting factors in the cleaning and disinfection procedure (photos by author).

1.5.3 Cleaning and disinfection of equipment and facilities

When selecting a C&D method, it is important that it removes the daily accumulation of soil from surfaces (Møretrø & Langsrud 2017). Common detergents used in slaughterhouses and meat processing premises are alkaline compounds with or without chlorine (Khamisse et al. 2012; García-Sánchez et al. 2017; Wang et al. 2018). To remove hard mineral deposits, acids can be applied to surfaces (Stanga 2010; Holah 2014; Marriott et al. 2018). More recently, another type of chemical, enzyme-based detergents, has become more commonly used in the food industry. These should be efficient in removal of biofilm and are less corrosive (Hamza 2017; Nahar et al. 2018). When enzyme-based detergents are combined with alkaline and acidic agents, the C&D efficiency has been shown to increase compared with using

only alkaline or acidic agents (Delhalle et al. 2020). For disinfection, quaternary ammonium compounds (QACs), chlorine-based compounds and acidic agents are commonly used (Olsen et al. 2003; Hutchison et al. 2007; Khamisse et al. 2012; Gantzhorn et al. 2014; García-Sánchez et al. 2017; Wang et al. 2018; Cherifi et al. 2022). Alcohols (*i.e.* ethanol and isopropyl alcohol) can be applied on some surfaces (Holah 2014).

1.5.4 Cleaning and disinfection of chicken transport crates

Transport crates for chickens are potential reservoirs for bacteria such as *Campylobacter* and *Salmonella* (Slader et al. 2002; Hansson et al. 2005; Frosth et al. 2020). When insufficiently cleaned crates are used for transporting chickens from farms to the slaughterhouse, there is risk of spreading *Campylobacter* between the farms in conjunction with thinning (Hansson et al. 2005).

Transport crates are cleaned and disinfected in the slaughterhouse, commonly by using chemicals such as chlorine-based agents with or without alkaline compounds, peracetic acid and QACs (Hinojosa et al. 2018; Morgan et al. 2022). However, even with these chemical treatments, the C&D procedure does not always achieve removal or inactivation of *Campylobacter* from transport crates (Hansson et al. 2005; Peyrat et al. 2008; Atterbury et al. 2020; Perdoncini et al. 2022), which could be due to their difficult-to-clean structure. More recent studies have investigated treatments with conventional chemicals, combined with physical methods (drying by hot forced air), for transport crates, with promising results when drying was used (Dzieciolowski et al. 2022). Another study did not detect any significant decrease in *Campylobacter* after chemical treatment, but did observe an effect of drying (Morgan et al. 2022). However, practical implementation and the cost of drying crates after C&D complicate its use. Another physical method that has been introduced into the food sector (Otto et al. 2011; Hinds et al. 2019) and hospitals (Ramos et al. 2020) in recent years is irradiation with ultraviolet (UV) light, which has shown promising results in disinfection of surfaces (Haughton et al. 2011; Pedrós-Garrido et al. 2018). In particular, a shorter UV wavelength has been shown to have a higher bactericidal effect, *e.g.* it has been found that UV-C treatment with 255-265 nm can achieve a higher level of bacterial inactivation than 275-285 nm (Li et al. 2017; Schöbel et al. 2023). However, there is still a need to evaluate these novel physical disinfection methods on chicken transport crates.

1.5.5 Decontamination of carcasses

When preventive hygiene measures (GHP), including C&D and a hygienic slaughter process, fail to prevent contamination of carcasses with pathogenic bacteria, additional control measures such as carcass disinfection, more correctly called 'decontamination' can be applied (Hawley & Kozlovac 2005; De Busser et al. 2013). For beef, removal of *Salmonella* and STEC is a high priority. For poultry carcasses, many different treatments are available for dealing with the risk of campylobacteriosis (Gichure et al. 2022). A number of different decontamination methods are used in slaughterhouses worldwide and these can be divided into physical methods (*e.g.* water rinsing, steam pasteurisation, irradiation and ultrasound) and chemical methods (*e.g.* organic acids and chlorine application) (Barco et al. 2015; Milios et al. 2017; Antic et al. 2021). These methods could also be combined, for example organic acids with hot water washing (Milios et al. 2017).

In the USA, decontamination is performed on a regular basis by treatment with *e.g.* chlorine or peracetic acids on poultry carcasses (Wideman et al. 2016; Cano et al. 2021; Laranja et al. 2023). However, in the EU approach, decontamination of carcasses should not be considered a substitute for a hygienic slaughter process and treatments used in a slaughterhouse must be approved by the official authority (EFSA Biohazard Panel 2013). Currently, the only substance allowed for decontamination of carcasses in the EU is lactic acid for washing beef carcasses, cuts and trimmings (EC 2013). However, physical methods can achieve greater *Campylobacter* reductions than chemical treatments (Gichure et al. 2022). At poultry slaughter, several physical decontamination treatments are allowed and sometimes used in the EU, including rinsing with potable water (*e.g.* thermal treatment with hot water, steam vacuuming and steam pasteurisation) (Milios et al. 2017). Other authors also include chilling and freezing among the methods for decontamination of carcasses, as these can reduce the amount of bacteria on carcasses (Zweifel & Stephan 2012). Applying a combination of steam and ultrasound is an innovative physical method that has been shown to reduce bacterial numbers on poultry carcasses (Hansen & Larsen 2007; Boysen & Rosenquist 2009; Musavian et al. 2014), but to the author´s knowledge, this treatment has not yet been tested in a large-scale slaughterhouse.

1.6 Monitoring of cleaning and disinfection procedures

After C&D procedures, an environmental sampling plan must be implemented to evaluate C&D efficacy, including microbiological and/or non-microbiological sampling of the meat processing environment (Codex Alimentarius 2020; Agüeria et al. 2021; De Oliveira Mota et al. 2021). It is important that the methods selected by the slaughterhouse are standardised and validated in-house (Moore & Griffith 2007).

1.6.1 Selection of pathogens and hygiene indicators

Under EU legislation, food premises manufacturing RTE foods where *L. monocytogenes* might pose a risk are required to sample both equipment and the processing environment for *L. monocytogenes* analysis (EC 2005; EFSA Biohazard Panel 2018). Furthermore, equipment and environment must be sampled when necessary to ensure compliance with the microbiological criteria on food. For instance, surfaces in poultry slaughterhouses may need to be sampled for *Campylobacter* analysis to assess compliance with the process hygiene criterion for poultry carcasses (EC 2005).

Important microbial hygiene indicators used to assess C&D efficacy in different food industries are total aerobic bacteria, *E. coli*, *Enterobacterales* (bacterial order, which formerly only included the family *Enterobacteriaceae*), yeasts and moulds. The most widely used hygiene indicator in the food industry is total aerobic bacteria, which is also called *e.g.* total aerobic count and total viable count (Griffith 2016; Møretrø & Langsrud 2017; Agüeria et al. 2021). It is an indicator of the general hygiene of the food premises, while *E. coli* is an indicator of faecal contamination (Ninios et al. 2014; Barco et al. 2015). Even though bacteria included in the order *Enterobacterales* can be naturally present in the environment and do not necessarily originate from faeces (Corbellini et al. 2016; Møretrø & Langsrud 2017), their presence can be interpreted as an indicator of faecal contamination in slaughterhouses (Hutchison et al. 2007; Barco et al. 2015; Althaus et al. 2017). Total aerobic bacteria and *Enterobacterales* are also commonly used as indicators of meat spoilage (Borch et al. 1996; Ghollasi-Mood et al. 2016).

1.6.2 Selection of sampling methods and sampling points

European Union legislation requires ISO standard 18593 for surface sampling to be used as a reference for sampling (EC 2005; SIS 2018). This standard describes the use of contact plates (dipslides) and swabbing, but does not mention adenosine triphosphate (ATP)-bioluminescence testing, which is a commonly used non-microbiological monitoring method for assessing C&D efficacy in the food industry (Syguła-Cholewińska et al. 2014). There are advantages and disadvantages with different sampling methods. For instance, ATP-bioluminescence provides results within just a few seconds, enabling immediate corrective actions, while for microbiological methods such as swabbing and dipslides the results take days (Møretrø et al. 2019; Lane et al. 2020; Erkmen 2022). An advantage of swabbing with sponges is the use of physical force, which increases the likelihood of recovering residential bacteria (Møretrø & Langsrud 2017). Dipslides are easy to use, but should only be used on flat surfaces (Griffith 2016). Another important difference between these methods is that ATP-bioluminescence not only measures bacterial cells, but also other cells from organic debris (Møretrø et al. 2019; Lane et al. 2020).

European Union legislation does not specify which areas to sample (EC 2005). However, according to ISO standard 18593, selection of sampling points should be based on historical data trends. The standard lists possible sampling points for FCSs, *e.g.* conveyor belts, slicers, cutting boards and containers, and for non-food contact surfaces (NFCSs), such as drains, floors, hoses, trolley wheels and ceilings.

1.6.3 Interpretation of sampling results

Since the environmental sampling plan should be risk-based and adapted to each specific facility, there are no thresholds for an acceptably clean surface in the legislation or guidelines at EU and national level in Sweden. Each FBO must therefore establish its own thresholds, which means that the values vary between different premises (Griffith 2016; Agüeria et al. 2021; De Oliveira Mota et al. 2021; Erkmen 2022; Abdessater et al. 2023). Collection of quantitative data on the hygiene indicators present on surfaces allows visualisation of trends at different sampling points over time (Møretrø & Langsrud 2017). When deviations from the established thresholds are observed, corrective actions must be implemented (Agüeria et al. 2020).

2. Main aims of the thesis

The overall aim of this thesis was to generate knowledge on the level of contamination in slaughterhouses and meat processing premises and to assess possible improvements for the C&D procedures in slaughterhouses and meat processing premises to prevent spread of foodborne pathogenic and spoilage bacteria. Specific objectives were to:

- Assess the C&D procedures and monitoring activities currently being used in the largest red meat and poultry slaughterhouses with associated meat processing premises in Sweden, and identify the most important challenges related to C&D.

- Determine the removal/inactivation of pathogenic bacteria, including *Campylobacter* spp., *L. monocytogenes* and ESBL *E. coli*, by comparing surfaces of equipment and facilities before and after commonly used C&D procedures in slaughterhouses with associated meat processing premises.

- Determine the reduction in hygiene indicator bacteria, specifically total aerobic bacteria and *Enterobacterales,* by comparing surfaces of equipment and facilities before and after commonly used C&D procedures in slaughterhouses with associated meat processing premises.

- Evaluate the disinfection efficacy of 265-nm UV-C LED light in inactivation of *C. jejuni*, and determine the reduction of total aerobic bacteria and *Enterobacterales* on chicken transport crates in a laboratory study.

- Evaluate the decontamination efficacy of ultrasound-steam and chilling in reducing *Campylobacter* spp. to below the process hygiene criteria (<3.0 log CFU/g neck skin), and determine the resulting reduction in total aerobic bacteria, *Enterobacterales* and *E. coli* on naturally contaminated broiler carcasses in a large-scale slaughterhouse.

- Determine the efficacy of rapid sampling methods, such as dipslides and ATP-bioluminescence, in assessing the cleanliness of surfaces for environmental monitoring purposes, compared with swabbing for total aerobic bacteria.

3. Considerations on materials and methods

This section provides a summary and considerations of the material and methods sections for Paper I-IV and for a descriptive study. The descriptive study was conducted in order to gain prior knowledge to perform the other studies. Detailed descriptions of the procedures performed are presented in the individual papers.

3.1 Selection of slaughterhouses

3.1.1 Descriptive study

To obtain knowledge about the C&D procedures and monitoring activities used in Swedish slaughterhouses, a descriptive study was performed through structured interviews with quality assurance managers. Only slaughterhouses slaughtering poultry, cattle, swine or sheep were included in the study. A list of red meat slaughterhouses was obtained from the Swedish Food Agency and a list of the largest poultry slaughterhouses in Sweden was provided by a representative from the Swedish Meat Poultry Association. The selection criterion for red meat slaughterhouses was that they slaughtered at least 50 animals/day, while the selection criteria for poultry slaughterhouses was that they slaughtered at least 50,000 birds/day. Due to the number of animals slaughtered each day, these red meat and poultry slaughterhouses are subject to regular official controls. The lists obtained included 21 of the largest red meat slaughterhouses and four of the largest poultry slaughterhouses in Sweden.

3.1.2. Cleaning and disinfection (Papers I & II) and decontamination studies (Paper IV)

Two slaughterhouses, one representing poultry slaughter and one the slaughter of cattle and swine, were included in the studies described in Papers I, II and IV. Quality assurance managers at these two slaughterhouses had previously been interviewed in the descriptive study. The red meat slaughterhouse included in Paper I and II, slaughtered approximately 100-120 swine and 25 cattle per day. Inside this slaughter facility, there is an area for cutting, chopping and packaging beef. The poultry slaughterhouse included in Paper I, II and IV, slaughtered approximately 220,000 broilers per day, with line speed around 18,000 birds per hour. Broiler slaughter is within the same facility as the meat processing area, which includes areas for cutting and production of meat preparations and RTE products.

To standardise the sampling procedures in Papers I and II, the same individuals from the research group performed all samplings. All microbiological analysis were initiated within 12 hours of sampling and samples were maintained at a temperature of 2-8°C. The selection criteria were therefore close proximity of the slaughterhouse to the laboratory and an interest from the slaughterhouse in participating in the research.

To enable comparison of C&D efficacy over time within each slaughterhouse in Papers I and II, a large sample size for both slaughterhouses was required. Since there are numerous differences between slaughterhouses due to factors, such as size, design, personnel and routines, it would have been difficult to compare the results if more slaughterhouses had been included. For the same reason, the focus was on the slaughter, cutting, meat preparation and packaging areas, to generate more reliable data for evaluation of C&D efficacy and comparison of different sampling methods.

3.2 Study design and data collection

3.2.1. Descriptive study

The interviews with quality assurance managers were performed using a check-list containing 19 questions developed by the research group, with input from a reference group that included representatives from the Swedish Meat Poultry Association and the Swedish Food Agency. The questions centred on the C&D procedures used by the slaughterhouses, including the main C&D products, and about their monitoring activities, with the focus on surface samplings (Appendix 1). The interviewees were also encouraged to freely elaborate on difficulties or challenges associated with C&D. The interviews were held with one slaughterhouse at a time, via Zoom (Zoom Video Communications, Inc.), and lasted approximately 1.5 hour. The interviews were conducted by the author of this thesis and the responses were recorded in a Word document. Following the interviews, the data were reviewed for consistency and clarity. In instances of missing or unclear information, interviewees were contacted for further clarification. Additional data were also accumulated during the interviews, but are not processed or presented in this thesis.

3.2.2. Cleaning and disinfection studies on equipment and facilities (Papers I & II)

The samplings for Paper I and II were conducted simultaneously over a one-year period, except during winter months (December-March), due to presumably low *Campylobacter* prevalence (National Veterinary Institute 2022). The samplings took place on six occasions in each slaughterhouse, and on each occasion, both before (after the production ended) and after (before the start of the next production shift) C&D procedures. The cleaning staff were not notified about the samplings days, to avoid affecting their performance.

Sampling points on FCSs and NFCSs in both slaughter and processing areas were included and each sampling point was sampled on each sampling occasion. Each sampling point (excluding scald water) was sampled with four sampling methods: swabbing with pre-moistened sponges/swabs with neutralising broth, swabbing with wiping cloth, dipslides and ATP-

bioluminescence (Figure 6). Approximately 45 mL of scald water were also collected.
Microbiological and non-microbiological analyses were then performed as described in Table 1.

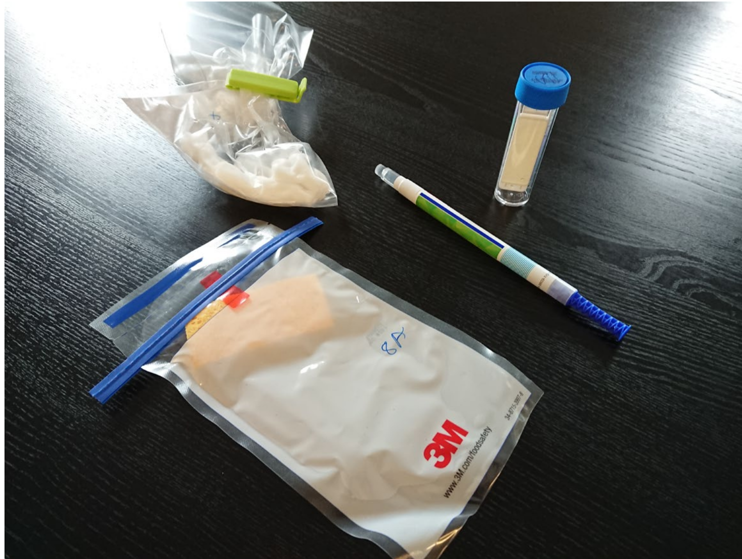


Figure 6. Sampling material: wiping cloth, sponge, dipslide and ATP-bioluminescence swab (photo by author).

Table 1. Hygiene indicators and pathogenic bacteria analysed with each sampling method (scald water excluded)

	Swabbing sponge/swab	**Swabbing wiping cloth**	**Dipslides**	**ATP-bioluminescence**
Total aerobic bacteria/total viable count	X		X	
Enterobacterales	X			
L. monocytogenes	X			
ESBL *E. coli*	X			
***Campylobacter* spp.**		X		
Relative light units				X

Hygiene indicator bacteria (total aerobic bacteria/total viable count, *Enterobacterales*), pathogens (*L. monocytogenes*, ESBL *E. coli*, *Campylobacter* spp.) and ATP-bioluminescence (relative light units, RLU) were analysed (see section 3.3). Scald water samples were analysed for total aerobic bacteria, *Enterobacterales*, *L. monocytogenes*, ESBL *E. coli*, *Campylobacter* spp. and ATP-bioluminescence with ATP tests for water samples.

Since the recommended area for quantification of microorganisms is ≤100 cm^2 (SIS 2018; Erkmen 2022), 100 cm^2 was swabbed at most sampling points. However, the area swabbed at some sampling points was smaller, due to the structure and size of the surface, *e.g.* on small surfaces an area of 25 cm^2 was swabbed. Stainless steel frames (100 cm^2 or 25 cm^2) were used on flat surfaces to delineate an exact area (Figure 7). Even though the ISO standard recommends a larger sampling area (at least 0.1 m^2) for detection of microorganisms if the areas are accessible (SIS 2018), it was decided to swab a smaller area.

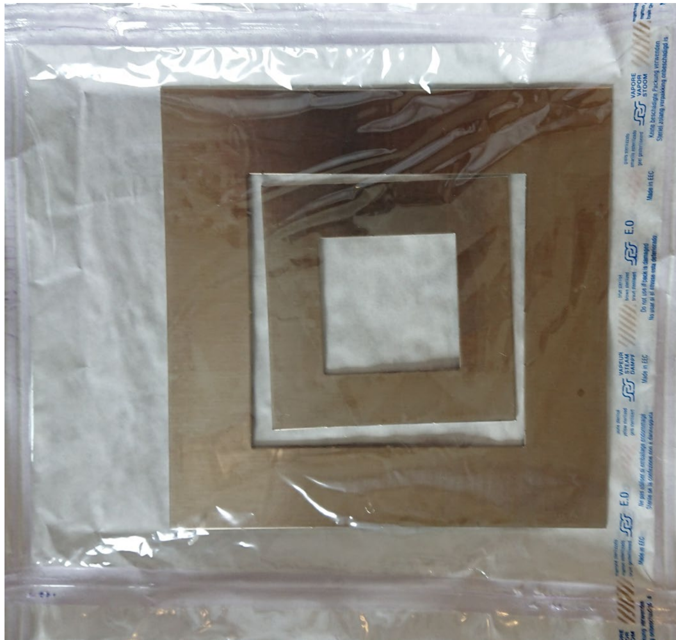


Figure 7. Autoclaved stainless steel frames (100 cm^2 and 25 cm^2) used to delineate an exact sampling area (photo by author).

Data on temperature and relative humidity in the premises were recorded on each sampling occasion and the temperature of the scald water was measured on one sampling occasion in each slaughterhouse, before and after C&D.

To assess the cleanliness of a surface (Paper I), thresholds were selected for each method: 2.5 CFU/cm^2 total aerobic bacteria (swabbing), 1.0 CFU/cm^2 *Enterobacterales* (swabbing), 1.0 CFU/cm^2 total viable count (dipslides) and 1.5 RLU/cm^2 (ATP-bioluminescence). These thresholds were based on previous research, instructions from manufacturers of sampling material and the thresholds used in the slaughterhouses included in the study (Table 1 in Paper I).

3.2.3. UV-C LED light source (Disinfection study, Paper III)

The bacterial reduction by treating transport crates with a novel UV-C light device in Paper III was evaluated in a laboratory setting. The reason for evaluating UV-C light was that the poultry slaughterhouse studied in Papers I, II and IV expressed an interest in installing this device if it proved to be effective and feasible. The equipment consisted of a cabinet with light-emitting diodes (LEDs) (Figure 8).

Figure 8. Light-emitting diode (LED) light cabinet for irradiation of chicken transport crates (photo by Ingrid Hansson).

To analyse bacterial reductions, pieces of a cleaned, well-used chicken transport crate were utilised. To mimic inadequately cleaned transport crates, these pieces were submerged in a mixture of chicken cecum contents and *C. jejuni* broth (Figure 9).

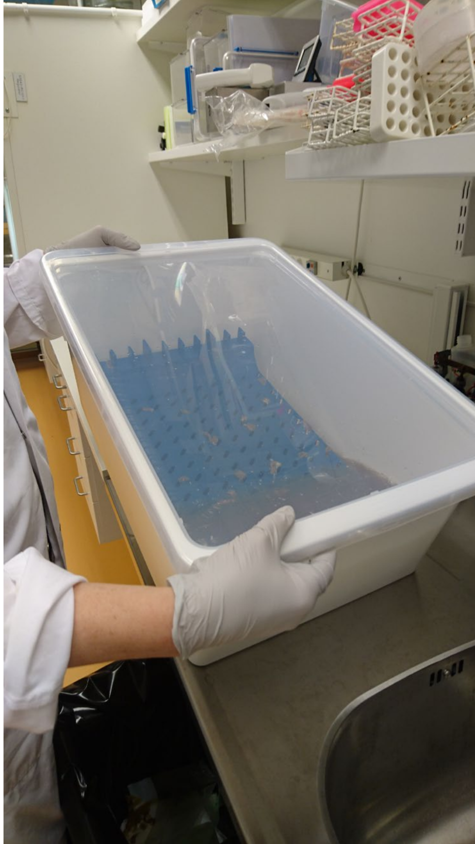


Figure 9. Simulated contamination of a piece of a chicken transport crate (photo by Ingrid Hansson).

One side of the crate was then swabbed with a pre-moistened wiping cloth (with buffered peptone water, BPW) before irradiation with a continuous LED array (wavelength 260-270 nm, emission peak 265 nm) and the other side of the crate was sampled the same way after irradiation. The energy received by the crates depended on the irradiation time, which was set to either 1 min or 3 min, because these treatment times might be feasible if installed in a poultry slaughterhouse.

3.2.4. Ultrasound-steam equipment (Decontamination study, Paper IV)

As a response to the Swedish campylobacteriosis outbreak in 2016-2017, caused by deficiently cleaned chicken transport crates, the poultry slaughterhouse installed decontamination equipment as an additional intervention to reduce *Campylobacter* on chicken carcasses (Figure 10). This equipment consisted of a chamber allowing treatment of whole carcasses while hanging on the shackles on the processing line, just before chilling. The treatment comprises a combination of ultrasound and steam. The ultrasound frequency was fixed at 30-40 kHz and the temperature of the steam was set by the slaughterhouse, based on how it affected the quality of the chicken skin, to either 84-85 °C or 87-88 °C. The chamber contained nozzles applying steam inside and outside the carcasses. The treatment time of each broiler carcass (on average 1.2 to 1.5 s) was determined by the speed of the production line and the length of the treatment chamber, and was within the processing time (1-2 s) recommended by the manufacturer of the equipment.

Figure 10. Chicken carcasses entering and leaving the treatment chamber with ultrasound and steam installed in the poultry slaughterhouse studied in Papers I, II and IV (photo by slaughterhouse staff).

Sampling days were selected based on when *Campylobacter*-positive flocks were presumed to be slaughtered. At the slaughterhouse, random sampling of carcasses within the selected flocks was performed by sampling 10 g of neck skin from one side of the carcasses before entering the ultrasound-steam chamber. The other side of the neck skin was sampled on the same carcasses after they had been treated with ultrasound-steam treatment, sprayed with water and chilled with forced air for approximately 2.5 h. Due to space constraints between the ultrasound-steam chamber and chiller, it was not feasible to sample carcasses immediately after treatment in the ultrasound-steam chamber.

3.3 Microbiological and ATP-bioluminescence analyses

Analyses of total aerobic bacteria, *Enterobacterales* (Papers I, III & IV), *E. coli* (Paper IV), *Campylobacter* spp. (Papers II-IV) and *L. monocytogenes* (Paper II) were performed according to standardised methods (NMKL and ISO), with some modifications. For analysis of ESBL *E. coli*, samples were enriched in 90 mL BPW and then surface-plated on CHROMagar Orientation plates with added cefotaxime (Paper II). Characteristic colonies of *Campylobacter* spp., *L. monocytogenes* (Figure 11) and *E. coli* were re-cultured and identified with matrix-assisted laser desorption ionization-time flight mass spectrometry (MALDI-TOF) (Paper II, IV). *Campylobacter* spp. and *L. monocytogenes* isolates were subjected to whole-genome sequencing (WGS) using Illumina technology and multilocus sequence typing (MLST), core genome MLST (cgMLST) and identification of genes and/or point mutations responsible for AMR (Paper II).

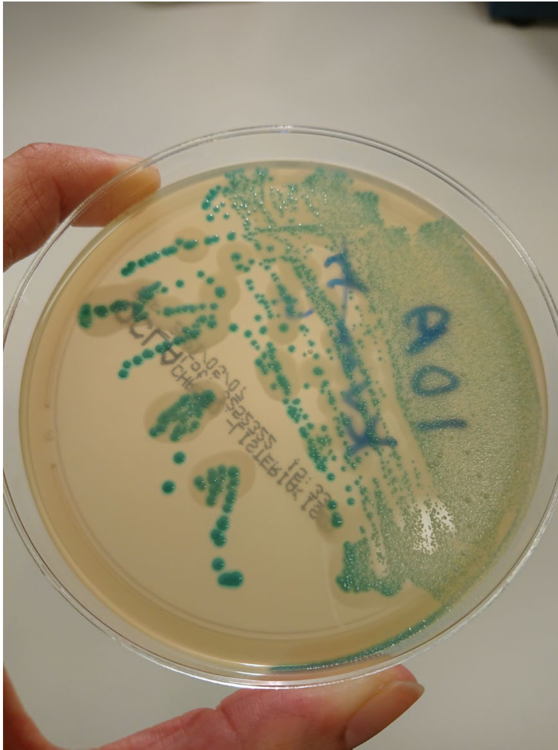


Figure 11. Characteristic *Listeria monocytogenes* colonies surrounded by opaque zones on a chromogenic Listeria agar plate (photo by author).

The microbiological analysis began within 12 h (Papers I & II) and 48 h (Paper IV) after sampling, on samples transported in a chill box to the Biomedical Sciences and Veterinary Public Health laboratory at the Swedish University of Agricultural Sciences, Uppsala, Sweden.

In Paper I, both sides of the dipslides were enumerated after 48 h of incubation and the results were expressed as total viable counts (TVC). To determine the level of cellular material on surfaces, ATP-bioluminescence was measured with a portable ATP-monitoring device in the slaughterhouses within 2 h after sampling (Figure 12). The results were expressed as relative light units (RLU).

Figure 12. ATP-bioluminescence swab and device (photo by author).

Antimicrobial susceptibility testing was performed on *Campylobacter* isolates by measuring antimicrobial minimum inhibitory concentrations (MIC) with broth microdilution, following the manufacturer's instructions and epidemiological cut-offs described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

3.4 Statistical analysis

In Papers I-IV, the results were compiled and analysed using Microsoft Office Excel and R Studio, and bacterial counts (CFU) were $\log_{10}$-transformed. Differences before and after C&D (Paper I) and treatments (Papers III & IV), and between treatment times (Paper III) and temperatures (Paper IV), were deemed significant at $p<0.05$.

In Paper I, all results were transformed to CFU or RLU per 100 cm^2 and were analysed using Anova. Fixed factors were sampling point, occasion, and before or after. *Post-hoc* tests were performed using Tukey's adjustment. Residuals were checked to confirm that they fulfilled the assumption of

normal distribution and equal variances. To assess the diagnostic performance of the dipslide and ATP-bioluminescence methods, swabbing and plating (total aerobic bacteria) was chosen as reference, since it is a widely used and accepted method for surface monitoring (Griffith 2005). The analysis of total aerobic bacteria was of special interest, since none of the participating slaughterhouses uses swabbing in its monitoring activities. To assess the diagnostic performance of dipslides and ATP-bioluminescence, the following indicators were calculated: accuracy (Ac), sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and Cohen's kappa agreement coefficient (κ).

In Papers III and IV, statistical significance was determined by the paired t-test, and the Welch two-sample t-test was conducted to determine significant differences between different treatment temperatures or times for each bacterial group. In Paper IV, simple linear regression was performed to determine whether the initial bacterial amount on the neck skin influenced the level of reduction achieved by the treatment.

4. Main results and discussion

The studies included in this thesis represent evaluations of different C&D measures performed at slaughterhouse level to prevent the occurrence and spread of pathogenic and hygiene indicator bacteria which could cause foodborne disease and meat spoilage (Figure 13).

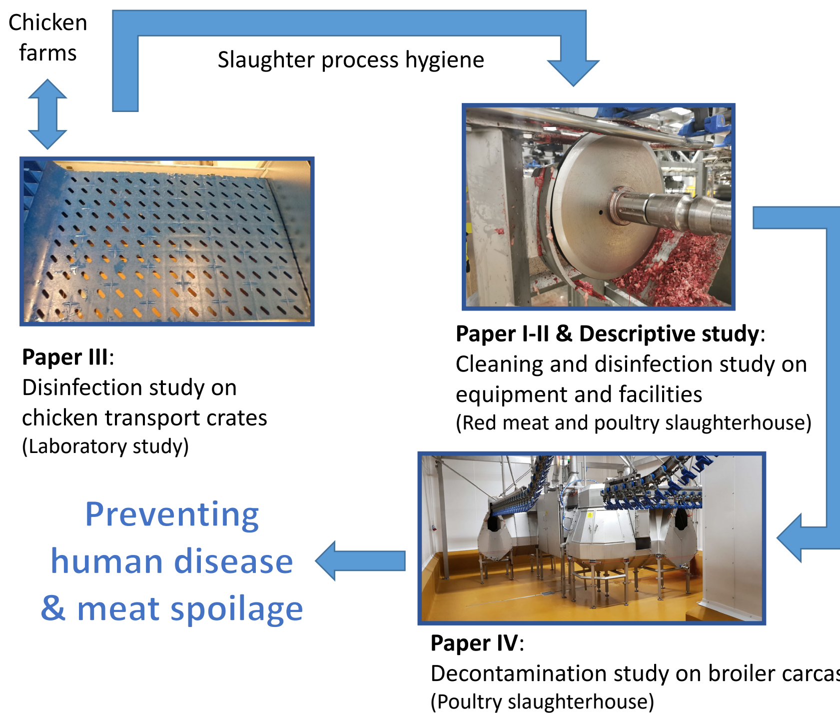


Figure 13. Measures for prevention of spread of pathogenic and hygiene indicator bacteria by cleaning and disinfection at slaughterhouse level evaluated in the studies included in this thesis. The arrows represent bacterial transmission routes (photos by author).

4.1. Descriptive study

A total of 25 slaughterhouses (21 red meat and four poultry) were approached for participation in interviews via email or telephone. Seven slaughterhouses did not answer. Eight slaughterhouses declined to participate, with the most commonly stated reason for not participating being lack of time. Ten slaughterhouses (six red meat and four poultry slaughterhouses) consented to participate in the interviews. The six red meat slaughterhouses accounted for approximately 32% of the annual slaughter volume in Sweden for animals such as cattle, pigs, sheep, goats, and horses, while the four poultry slaughterhouses represented at least 90% of the annual chicken slaughter volume in Sweden. During two of the interviews, the quality assurance managers were accompanied by the cleaning manager. On several occasions during the interviews, the interviewees did not know the answer to all the questions (*e.g.* the C&D agents and concentrations used) and had to supply this complementary information by e-mail after the interviews.

4.1.1 Cleaning and disinfection procedures

All but one slaughterhouse hired an external company for cleaning and disinfection. All slaughterhouses used alkaline detergents with or without chlorine at low concentrations (2-5%), applied with a low-pressure system (Table 2). Chlorine could not be used on all equipment, as it causes corrosion (Stanga 2010). Almost all (90%) slaughterhouses alternated alkaline with acidic agents during cleaning. The contact time for detergents varied between 10 and 30 min, in all slaughterhouses except one, which accepted a lower contact time (5 min) (although this was according to the manufacturer's instructions). The temperature of the water used for cleaning varied from cold (20 °C) to warm (55 °C). The majority (80%) of the slaughterhouses used chlorine-based agents in the disinfection process, applied with low-pressure systems. However, there was greater variation in the agents used for disinfection compared with the detergents, which could be the reason for the larger variation in concentration (0.1-5%) and the larger range of water temperature (10-55°). The low concentration of detergents and disinfectants was expected, as higher concentrations are hazardous to workers (Marriott et al. 2018). Since none of the slaughterhouses used QACs, it is possible that cleaning companies hired by slaughterhouses in Sweden are less prone to use this disinfectant compared with those in other countries, where it is more

widely used in both poultry and red meat slaughterhouses (Hutchison et al. 2007; Peyrat et al. 2008; Khamisse et al. 2012; Holah 2014; García-Sánchez et al. 2017; Wang et al. 2018; Cherifi et al. 2020). The use of sodium hypochlorite instead of QAC might be positive, since biofilm and planktonic cells of *L. monocytogenes* show higher resistance to QACs and since QACs are known to be less efficient against Gram-negative bacteria (Quinn et al. 2011; Chaves et al. 2024).

Table 2. Data from interviews with quality assurance managers at 10 of the largest slaughterhouses in Sweden, slaughtering cattle, swine, sheep, poultry and horses, about their general cleaning and disinfection procedures

Slaughter-house	Species	Operatives	Detergents	Conc	Contact time	Water temp	Application method	Disinfectants	Conc	Contact time	Water temp	Application method
A	Cattle, horse	External	Alkaline with/without chlorine, acids	3-5%	20 min	20°C	Foam, low-pressure	Chlorine based	nd	20 min	20°C	Foam, low-pressure
B	Cattle, swine	External	Alkaline with/without chlorine, acids	5%	15 min	40-45°C	Foam, low-pressure	Chlorine based	0.1%	15 min	40-45°C	Foam, low-pressure
C	Cattle, swine	External	Alkaline with chlorine	4%	15-20 min	nd	Foam, low-pressure	Acids, hydrogen peroxide	4%	nd	nd	Foam, low-pressure
D	Cattle, swine, sheep	External	Alkaline with/without chlorine, acids	3%	5-20 min	50°C	Foam, low-pressure	Chlorine based	3%	5-30 min	50°C	Foam, low-pressure
E	Cattle	External	Alkaline with chlorine, acids	3-5%	10-15 min	50°C	Foam, low-pressure	Chlorine based, acids	3-5%	10-15 min	50°C	Foam, low-pressure
F	Cattle, swine, sheep	External	Alkaline with chlorine, acids	2-5%	15-20 min	<35°C	Foam, low-pressure	Chlorine based, acids	1-3%	nd	10-20°C	Foam, low-pressure
G	Poultry	External	Alkaline with/without chlorine, acids	3-5%	15-20 min	20°C	Foam, low-pressure	Chlorine based	3-5%	15-20 min	15-18°C	Foam, low-pressure
H	Poultry	External	Alkaline with/without chlorine, acids	3%	15-20 min	nd	Foam, low-pressure	Chlorine based	3%	15-20 min	nd	Foam, low-pressure
I	Poultry	External	Alkaline with/without chlorine, acids	3-5%	15-30 min	35°C	Foam, low-pressure	Acids	nd	nd	30-35°C	Foam, low-pressure
J	Poultry	Internal	Alkaline with/without chlorine, acids	4%	15-30 min	55°C	Foam, low-pressure	Chlorine based, acids	1-3%	15-30 min	55°C	Foam, low-pressure

Conc = concentration. nd = Not determined, the slaughterhouse did not provide the information or the respondent did not know.

4.1.2 Monitoring activities

There was large variation in the number of animals slaughtered in each slaughterhouse, which influenced the sampling frequency (Table 3). Only one slaughterhouse used swabbing and plating of total aerobic bacteria. The majority of the slaughterhouses regularly used dipslides (80%) and ATP-bioluminescence (70%) and half of the slaughterhouses used a combination of these two methods. Almost all (90%) slaughterhouses included in the study sampled for *Listeria*. Surfaces mentioned as difficult to clean by at least two slaughterhouse quality assurance managers included cutting tools, organ tables, inside machines, conveyor belts, dehairing scrapers, platforms and singeing machine (used for burning the carcass surface). Conveyor belts, dehairing equipment and cutting tools have previously been shown to be surfaces that are difficult to clean (Rahkio & Korkeala 1997; Hutchison et al. 2007; Piras et al. 2014; Langsrud et al. 2016; Wang et al. 2018). For plastic surfaces such as conveyor belts, a plausible explanation might be the pronounced bacterial adhesion observed on plastic (Veluz et al. 2012).

Half of the slaughterhouses used thresholds provided by the laboratories which analysed the samples or by suppliers or manufacturers of the sampling materials, instead of basing their thresholds on their own risk analysis. When applying a risk-based approach, a high level of microbiological knowledge among quality assurance managers is required. This is a challenge, due to the diverse backgrounds of these individuals. While some of the interviewed had technical or biological expertise, others had only received in-house training, often stemming from their ties to family businesses. If thresholds are derived solely on suggestions from external companies, rather than the FBO's own risk analysis, there may be an increased risk of cross-contamination of meat through *e.g.* accepting a high bacterial level on FCSs after C&D.

Table 3. Data obtained in interviews with quality assurance managers at 10 of the largest slaughterhouses in Sweden, slaughtering cattle, swine, sheep, poultry and horses, about their monitoring activities. Sampling frequencies for each sampling method and the origin of the thresholds used for clean surfaces are shown

Slaughter-house	Species	No. slaughtered animals/day	Swabbing for total aerobic bacteria	Dipslides	ATP-tests	Listeria swabs	Origin thresholds clean surfaces	Difficult to clean surfaces
A	Cattle, horse	50-150	na	once/month	once/month	yes (frequency nd)	Supplier of sampling materials	Hooks, platforms, saws, organ table
B	Cattle, swine	50-150	na	na	once/month	once/year	nd	Saws, cutting machine, conveyor belts
C	Cattle, swine	1,500-2,000	na	once/day	once/day	4 times/year	nd	Inside machines, dehairing scrapers, singeing machine, organ table
D	Cattle, swine, sheep	1,500-2,000	na	once/week	na	once/month	Laboratories	Dehairing scrapers, singeing machine
E	Cattle	50-150	na	2 times/year	once/month	3 times/year	Laboratories	Saws, knives, organ table
F	Cattle, swine, sheep	150-500	na	once/week	na	once/week	nd	Platforms, dehairing scrapers, conveyor belts
G	Poultry	50,000-100,000	na	once/week	na	4 times/year	Supplier of sampling materials	Drills, injectors, knives, pipes, vacuum systems
H	Poultry	100,000-150,000	4 times/year	once/week	once/week	yes (frequency nd)	nd	Inside machines
I	Poultry	50,000-100,000	na	na	once/month	na	Supplier of sampling materials	Cutting facility, transport crates, chilling rooms
J	Poultry	150,000-250,000	na	4 times/week	4 times/week	once/day (when looking for it)	nd	Conveyor belts, displays

na = not applicable, the slaughterhouse did not use that sampling method. nd = not determined, the slaughterhouse did not provide the information or the respondent did not know.

In general, the quality assurance managers reported lack of knowledge and experience exchange among different slaughterhouses in relation to C&D, as evident in the quotes provided below. They also reported difficulties in knowing whether their C&D procedures were the most adequate for maintaining sufficiently clean premises. They expressed a need for more science-based guidelines and recommendations for their C&D procedures and monitoring activities and wanted to know more about their in-house microbiota. The perceived lack of science-based guidelines raises concerns, as it might be an indication of a missing link between the scientific community and the food industry and thus lack of conversion of science-based data into real-life practice.

The main difficulties and challenges related to cleaning and disinfection reported by the quality assurance managers were:

- *Slaughterhouse A:* "It is problematic that laboratories don´t analyse all types of bacteria." "We don´t know how other slaughterhouses clean and disinfect their facilities."
- *Slaughterhouse B:* "We want to know if the cleaning and disinfection products and methods we use are the most appropriate or if there are other more efficient forms available." "We want to map the microbiological flora in our production premises."
- *Slaughterhouse C:* "We would like to verify the efficacy of our cleaning and disinfection procedures."
- *Slaughterhouse D:* "It is important that the cleaning staff is good. "It would be interesting to investigate specific bacteria causing spoilage and how to eliminate them. We would like to avoid building up a house flora, including *Pseudomonas*."
- *Slaughterhouse E:* "Do we take too few samples? "It would be interesting to know how other slaughterhouses clean and disinfect their premises and exchange experiences." "It would be nice to have guidelines based on science." "We would like to get help from experts."
- *Slaughterhouse F:* "We are curious about what chemicals other slaughterhouses use." "Leadership and management are important, and the competence of those who clean. It is hard to find the right management and competence of cleaning staff."
- *Slaughterhouse G:* "It would be interesting to see how they clean and disinfect other slaughterhouses. What cleaning and sampling methods do they use and what results and challenges do they have? It would be nice to get support and ideas on how to do things differently, to share knowledge."

- *Slaughterhouse H:* “When we see a trend in elevated monitoring results, are we doing the right thing?” “What affects the production of biofilm? How much does the surface material influence?”
- *Slaughterhouse I:* “The feathers are squeezed under the conveyor belts and they have to be taken apart regularly in order to clean the belts properly. Hygienic design has been forgotten by the manufacturers, they do not have cleaning in mind, just production.”
- *Slaughterhouse J:* “Are the surfaces clean enough?” “What is present if it is not clean? *Campylobacter*?”

4.1.3 Cleaning and disinfection procedures used by the slaughterhouses (Papers I, II & IV)

The C&D procedures in the red meat slaughterhouse were performed by two operative staff from an external cleaning company. The slaughterhouse relied on the C&D products and methods recommended by the external company, which was alkaline products with/without chlorine altered with acidic chemicals applied with foam at low pressure (Table 3; Slaughterhouse B). The C&D procedures used in the poultry slaughterhouse were very similar to the red meat slaughterhouse, with use of *e.g.* similar chemicals, except that higher water temperature was used and the contact time for chemicals could be longer (Table 3; Slaughterhouse J). A notable distinction was that this slaughterhouse had its own C&D staff, which included 30-40 cleaning operatives.

4.2. Control of pathogenic and hygiene indicator bacteria in slaughterhouses by cleaning and disinfection measures (Papers I-IV)

4.2.1. Efficacy of cleaning and disinfection procedures of equipment and facilities (Papers I & II)

On most sampling occasions, the sampling points were visually clean after C&D, however, soil was sometimes present. Another observation was that most sampling points were wet at the time of sampling especially after C&D, since none of the slaughterhouses used forced ventilation to dry surfaces after C&D. The temperature and relative humidity of the slaughter and processing areas varied greatly during samplings both before and after C&D (Table 4).

Table 4. Observational data on measured temperature (Temp) and relative humidity (RH) at the time of sampling in the slaughter area and processing area in the red meat and poultry slaughterhouses, before and after cleaning and disinfection

	Slaughter area				Processing area			
Slaughter-	Before		After		Before		After	
house	Mean	Range	Mean	Range	Mean	Range	Mean	Range
				Temp (°C)				
Red meat	20.6	18.9-22.4	17.7	8.4-20.7	10.1	6.9-12.5	8.8	6.5-10.8
Poultry	16.6	12.5-22.0	17.4	14.7-20.0	11.3	7.7-14.4	14.5	11.4-17.5
				RH (%)				
Red meat	68.7	35.0-99.0	64.0	50.0-83.0	53.5	18.0-75.0	67.8	51.0-77.0
Poultry	55.3	36.0-76.0	67.7	56.0-80.0	54.8	39-84.7	71.4	39.0-99.9

The temperature of the scald water was measured on one sampling occasion, and was 60.3 °C and 53.7 °C immediately after slaughter, and 44.5 °C and 44.3 °C just before the next slaughter shift started, in the red meat slaughterhouse and poultry slaughterhouse, respectively.

Campylobacter spp.

In Paper II, *C. coli, C. jejuni* and *C. hyointestinalis* were identified before C&D at sampling points in the red meat slaughterhouse, while only *C. jejuni* was identified in the poultry slaughterhouse. Similar occurrence rate of *Campylobacter* spp. was observed in both slaughterhouses (13% and 15.5% in the red meat and poultry slaughterhouse, respectively) (Table 5), which is

interesting as poultry is often the main focus when discussing *Campylobacter*. The reasons could be that chicken is the most common source of campylobacteriosis in humans and that there is lower *Campylobacter* prevalence in fresh meat from pigs than from broilers (EFSA & ECDC 2021). However, the occurrence on surfaces in Paper II was lower than the reported *Campylobacter* prevalence in samples from pigs (49-83%) and cattle (20-78%) in Europe (Meistere et al. 2019; Hansson et al. 2020; Papadopoulos et al., 2020; Idland et al. 2022).

Campylobacter spp. were detected on fewer sampling occasions, but were more widely spread on different surfaces in the poultry slaughterhouse, *i.e.* they were detected on eight sampling points on two sampling occasions in the poultry slaughterhouse compared with five sampling points on five sampling occasions in the red meat slaughterhouse (Table 6). Even though preventive measures on farms to control *Campylobacter* should be in place, such as biosecurity measures, chickens colonised with *Campylobacter* occasionally arrive at the slaughterhouse (Hansson et al. 2005; Perez-Arnedo & Gonzalez-Fandos 2019). From that moment, the responsibility for producing safe meat rests with the slaughterhouse FBO and is achieved through implementation of different control strategies, in which slaughter process hygiene plays an essential role.

The results confirmed that when *Campylobacter*-positive chickens are slaughtered, there is a high risk of contamination of a large proportion of surfaces, as *C. jejuni* isolates of ST257 with identical cgMLST profiles were detected on multiple sampling points in the poultry slaughterhouse on the same sampling occasion. The same *Campylobacter* clones have been shown to survive on different FCSs and NFCSs in another poultry slaughterhouse (Johnsen et al. 2006). In this thesis, all ST257 isolates were of clonal complex CC-257, which has previously been the predominant CC in chicken caecal samples (Wieczorek et al. 2017). Sequence type 257 has also been isolated from water pipes on a broiler farm in Sweden (Ferrari et al. 2019), showing its potential to survive in the environment.

It is worrisome that the spread of *Campylobacter* reached FCSs in the processing area (*i.e.* salt injector needles, conveyor belts and cutting blades) (Figure 14), since the sequence types detected in the poultry slaughterhouse (ST257 and ST19) have caused disease outbreaks related to chicken meat in Sweden in recent years (Public Health Agency of Sweden 2018; Public Health Agency of Sweden & Food Safety Agency 2020).

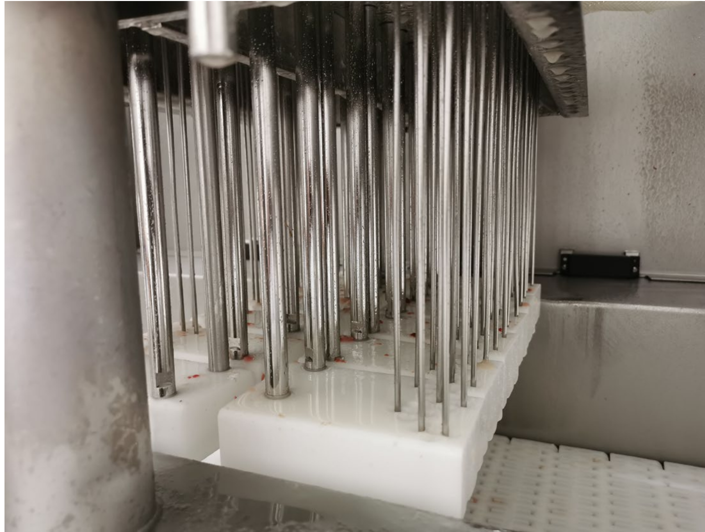


Figure 14. Contaminated salt injector needles (photo by author).

Listeria monocytogenes

In Paper II, *L. monocytogenes* showed higher occurrence in the red meat slaughterhouse (12.5%), where it was also recovered in one sample after C&D, compared with the poultry slaughterhouse (5.0%) (Table 5). *Listeria monocytogenes* was mainly present in the drains in both slaughterhouses (10/12 samples) (Table 6), but was only quantifiable in the red meat slaughterhouse (in two samples before C&D: 3.1 and 1.0 log CFU/100 cm^2) and in one sample after C&D (1.0 log CFU/100 cm^2). *Listeria monocytogenes* was not detected after C&D in the poultry slaughterhouse. Even though drains are NFCSs, *L. monocytogenes* could contaminate nearby FCS during the C&D procedure when splashing occurs with the low-pressure application system (Saini et al. 2012).

In Paper II, *L. monocytogenes* isolates of ST9 with identical cgMLST profiles were identified in a drain and a nearby FCS. *Listeria monocytogenes* was also detected in this drain after C&D, which indicates that it could be a residential strain. The same strain was identified on three separate sampling occasions 15 weeks apart, encompassing nearly one-third of the entire year of sampling. Bacteria detected more than 60 days apart could be considered residential pathogens (Pettengill et al. 2022). However, the possibility of re-

introduction of *L. monocytogenes* by pigs originating from the same farms cannot be excluded.

Most isolates (13/15) recovered from both slaughterhouses were of serogroup IIa (serotype 1/2a and 3a), while two isolates belonged to serogroup IIc (serotype 1/2c and 3c). These serogroups and serotypes are commonly occurring in *L. monocytogenes* outbreaks (Martín et al. 2014; Okpo et al. 2015; Lindblad & Flink, 2017; EFSA Biohazard Panel 2018; Ottoson 2019; M. Ricao, Swedish Food Agency, pers. comm., March 7, 2023). This highlights the importance of controlling this pathogen through C&D measures.

Detection of *L. monocytogenes* on FCSs before C&D indicates a risk of transmission of pathogens (Martín et al. 2014). However, its detection was not surprising, since this bacterial species has previously been detected on FCSs in other poultry and red meat slaughterhouses (Martín et al. 2014; Gómez et al. 2015; Muhterem-Uyar et al. 2015; Demaître et al. 2021; Cherifi et al. 2022; Oswaldi et al. 2022).

Table 5. Occurrence (%) of *Campylobacter* spp. and *Listeria monocytogenes* in the red meat and poultry slaughterhouses before and after cleaning and disinfection (C&D). Ratio of positive samples to total number of samples in brackets

Slaughterhouse	C&D	Red meat	Poultry
***Campylobacter* spp.**	*before*	13.0% (8/62)	15.5% (9/58)
	after	0% (0/0)	0% (0/0)
L. monocytogenes	*before*	12.5% (8/64)	5.0% (3/58)
	after	1.6% (1/64)	0% (0/58)

Table 6. Number of samples in which *Campylobacter* spp., *Listeria monocytogenes* and *Enterobacterales* were detected before and after cleaning and disinfection (C&D), mean before and after C&D, and reduction in total aerobic bacteria (log CFU/100 cm^2) at each sampling point in the two slaughterhouses. The highlighted values of total aerobic bacteria are mean values after C&D which exceeded the selected threshold for clean samples

Slaughter-house	Area		Sampling point	*Campylobacter* spp.		*L. monocytogenes*		*Enterobacterales*		Total aerobic bacteria		
				Before C&D	After C&D	Before C&D	After C&D	Before C&D	After C&D	Mean before C&D	Mean after C&D	Mean reduction
Red meat	Slaughter	**1**	Post-dehairing table pigs					2		4.0 ± 0.6	**4.0 ± 1.7**	0.001 ± 1.1
		2	Scald water					1		4.7 ± 1.3	1.8 ± 1.0	2.8* ± 1.4
		3	Table for cattle organs	2				3		3.0 ± 0.7	1.3 ± 0.9	1.7* ± 1.1
		4	Conveyor belt pig organs	2				4		4.6 ± 0.7	**3.0 ± 1.0**	1.6* ± 2.1
		5	Drain	3		5		6	2	6.0 ± 0.8	**3.6 ± 1.0**	2.4* ± 0.4
		6	Cutting blade cattle/pig carcasses	1				1		2.2 ± 1.5	1.5 ± 1.6	0.7 ± 1.9
	Processing	**7**	Cutting board			1		5		5.2 ± 0.5	2.0 ± 0.9	3.2* ± 0.8
		8	Conveyor belt					2		4.2 ± 1.0	1.7 ± 1.5	2.5* ± 2.5
		9	Conveyor belt					6		4.7 ± 0.5	2.0 ± 0.8	2.7* ± 1.1
		10	Trolley					4		4.2 ± 0.9	1.5 ± 1.1	2.7* ± 1.4
		11	Drain			2	1	5	2	4.8 ± 0.5	**4.6 ± 0.4**	0.1 ± 0.8
Poultry	Slaughter	**12**	Cutting blade bleeding	1				5		4.1 ± 0.9	1.6 ± 1.1	2.5* ± 0.7
		13	Scald water					5		5.4 ± 0.1	2.4 ± 1.5	3.0* ± 1.5
		14	Plucking fingers					5	3	7.1 ± 0.8	**5.5 ± 1.6**	1.6 ± 1.0
		15	Shackle after stunning	1				5	3	5.6 ± 0.9	**5.2 ± 0.8**	0.3 ± 1.4
		Other	Table edible organs			1		nd	nd	nd	nd	nd
		16	Floor lairage	1				5	4	7.1 ± 0.2	**6.5 ± 0.5**	0.6 ± 0.5
	Processing	**17**	Conveyor belt	1				5		3.9 ± 0.7	1.1 ± 0.6	2.8* ± 0.6
		18	Conveyor belt	1				5		3.3 ± 0.6	0.6 ± 0.7	2.7* ± 0.8
		19	Cutting blade thighs	1				1		3.1 ± 0.2	0.8 ± 1.3	2.4* ± 1.1
		20	Salt injector needles	1				5		4.4 ± 0.9	0.7 ± 1.5	3.7* ± 1.0
		21	Drain	2		2		6	2	5.9 ± 0.7	**3.7 ± 1.6**	2.3*± 1.2

nd = not determined. * = significant reduction at $p<0.05$

Enterobacterales

In Paper I, *Enterobacterales* could be enumerated in more samples before C&D in the poultry than in the red meat slaughterhouse (88% and 66%, respectively), but with similar mean count in both slaughterhouses (mean 2.6 ± 0.5 and 2.3 ± 0.5 log CFU/100 cm², respectively). In other studies on a total of 11 pig slaughterhouses, *Enterobacterales* were enumerated to 1.7-6.3 log CFU/cm^2 on FCSs in slaughter and cutting areas during processing (Prendergast et al. 2008; Piras et al. 2014). The level of *Enterobacterales* in Paper I was thus very low compared with that in those studies.

After C&D, *Enterobacterales* could be enumerated from 25% of the samples (mean 2.6 ± 0.8 log CFU/100 cm^2) in the poultry slaughterhouse, compared with 7% (mean 2.4 ± 0.8 log CFU/100 cm^2) in the red meat slaughterhouse. Of the samples taken, 19% from the poultry slaughterhouse and 5% from the red meat slaughterhouses exceeded the cleanliness threshold of 1.0 CFU/cm^2, which is equivalent to 2.0 log CFU/100 cm^2. *Enterobacterales* could not be enumerated on FCSs in the processing areas, while an earlier study detected *Enterobacterales* in 9-19% of the samples from FCSs in meat processing premises after C&D (Tomasevic et al. 2016).

In the red meat slaughterhouse, *Enterobacterales* were only detected in drains after C&D. In addition to drains in the poultry slaughterhouse (Figure 15), *Enterobacterales* were detected on important FCSs such as plucking fingers and shackles (Table 6). *Enterobacterales* were detected in one scald water sample in the red meat slaughterhouse, while it was detected in all five samples (mean 2.5 ± 0.9 log CFU/mL) in the poultry slaughterhouse before C&D. However, *Enterobacterales* were not detected in any of the scald water samples in either of the slaughterhouses after C&D. Much higher *Enterobacterales* levels (4.6-4.9 log CFU/mL) were observed in studies in three other poultry slaughterhouses during processing with similar scald water temperatures (52-54 °C) as in the present study (Goksoy et al. 2004; Whyte et al. 2004).

In general, *Enterobacterales* were detected in a higher number of samples in the poultry slaughterhouse than in the red meat slaughterhouse. This may indicate that faecal contamination was higher in the poultry slaughterhouse (Hutchison et al. 2007; Althaus et al. 2017). However, not all bacterial species of this order originate from faeces, so these bacteria could also have come from *e.g.* the slaughter environment (Janda & Abbott 2021).

Figure 15. Contaminated drain in a slaughterhouse (photo by author).

Total aerobic bacteria

In Paper I, total aerobic bacteria could be enumerated in the majority of samples before and after C&D in both slaughterhouses, which was expected since surfaces are not sterile after C&D (Stanga 2010). The selected threshold (2.5 CFU/cm^2, equal to 2.4 log CFU/100 cm^2) used to assess whether a surface was acceptably clean is commonly used in the literature, relating to both the food industry (Griffith 2005; Lues & Van Tonder 2007; Cunningham et al. 2011; Carrascosa et al. 2012; Ching et al. 2021) and hospitals (Cooper et al. 2007; Sherlock et al. 2009; Luick et al. 2013; Huang et al. 2015). Using this threshold, around half (48-54%) of all sampling points (FCS and NFCS) in the two slaughterhouses were considered sufficiently cleaned and disinfected, and in samples from FCS, a higher number of samples were considered acceptably clean (60-65%). Food contact surfaces in the processing area had the highest amount of acceptably clean samples in both slaughterhouses (Table 6).

In general, surfaces in the processing areas in both slaughterhouses were more properly cleaned and disinfected than surfaces in the slaughter areas, with higher bacterial reductions achieved (2.2 and 2.8 log CFU/100 cm^2 in the red meat and poultry slaughterhouse, respectively) than in slaughter areas (1.3 log CFU/100 cm^2 for both slaughterhouses). This was especially obvious when comparing the same type of sampling points (*e.g.* conveyor belts and

cutting blades). Even though dirty FCSs in the slaughter area, such as post-dehairing table, plucking fingers and shackles, pose a direct risk of cross-contamination, these sampling points were found not to be properly cleaned and disinfected. Similar deficiencies have been described in other slaughterhouses (Rivas et al. 2000; García-Sánchez et al. 2017; Zeng et al. 2021). Moreover, FCSs in the slaughter areas were not always included in the slaughterhouse sampling plans, which is troubling as the FBO is then unaware of the C&D efficacy in these areas.

In general, the conveyor belts in the processing areas in both slaughterhouses were properly cleaned and disinfected, despite the large differences between the materials and structures of these sampling points and the degree of wear and tear. These are positive findings, since other studies have shown that C&D of conveyor belts is often unsuccessful (Gómez et al. 2012; Khamisse et al. 2012; Wang et al. 2018).

Non-food contact surfaces that were found to be dirty after C&D were the lairage floor and drains in the slaughter and processing areas, which were dirty on most sampling occasions in both slaughterhouses. Presence of *Campylobacter* spp., *L. monocytogenes* and *Enterobacterales* was detected in the same drains. Thus while FCSs should be prioritised during C&D as part of the risk-based approach, drains and floors should also be properly cleaned and disinfected to avoid creating an in-house microbiota (Fagerlund et al. 2017; Agüeria et al. 2021). The level of total aerobic bacteria in scald water at the poultry slaughterhouse was lower (0.6-0.8 log CFU/mL) than reported for other poultry slaughterhouses before C&D/during processing (Goksoy et al. 2004; Whyte et al. 2004).

4.2.2. Disinfection study on chicken transport crates (Paper III)

A significant reduction in all target bacteria after UV-C treatment of chicken transport crates was observed for both treatment times (1 and 3 min). On extending the treatment time from 1 to 3 min, a significant increase in reduction in *C. jejuni* was observed. However, no significant increase in reduction was seen for total aerobic bacteria and *Enterobacterales* and, while the bacterial reduction achieved seemed high, there was still a considerable amount of bacteria on the crates after UV-C treatment (Table 7). Thus the aim of inactivating *C. jejuni* from the crates was not achieved.

Table 7. Mean bacteria levels and mean reduction (log CFU/mL) on chicken transport crates before and after irradiation with UV-C light for 1 and 3 min

	Mean before	**Mean after**	**Mean reduction**
	Treatment time 1 min		
Campylobacter jejuni	4.9±0.4	2.9±0.5	2.0* ± 0.5
Total aerobic bacteria	5.7±0.3	4.2±0.2	1.4* ± 0.4
Enterobacterales	4.4±0.4	2.9±0.4	1.5* ± 0.3
	Treatment time 3 min		
Campylobacter jejuni	5.9±0.9	2.8±0.4	3.1* ± 1.0
Total aerobic bacteria	5.3±0.2	3.6±0.6	1.6* ± 0.8
Enterobacterales	4.2±0.4	2.5±0.8	1.8* ± 0.8

* = significant reduction at $p<0.05$

This is not consistent with findings in an earlier study using 265 nm UV-C light, in which the reduction in *Pseudomonas aeruginosa* was almost complete, even on highly contaminated tube lumen surfaces (7 log CFU/mL) (Bak et al. 2010). However, it is impossible to draw any conclusions from comparison of results from different studies on UV-C light treatment, since surfaces, treatment times and bacteria tested differ between studies. For instance, different bacterial species are more or less easy to eliminate (Hinds et al. 2019). It is also common for different units to be used, as observed on comparing the results in this thesis with those reported by Raschle et al. (2019), who investigated the effect of 254 nm UV-C light on slaughterhouse knives and achieved a reduction in total aerobic bacteria after 30 s of irradiation of <1 log CFU/cm^2, while in Paper III the mean reduction was 1.4 log CFU/mL after 1 min of treatment (Table 7). Although difficult, it can be concluded that the bacterial reduction achieved by 265 nm UV-C LED light on transport crates seems promising. However, before installation of this equipment for disinfection of crates in an industrial setting, a longer treatment time should be considered, by *e.g.* building a treatment tunnel through which the crates would pass. Another solution could be to improve the cleaning procedure before the disinfecting step, to reduce the initial bacterial load.

4.2.3. Decontamination study on broiler carcasses (Paper IV)

Treatment of chicken carcasses with ultrasound-steam and chilling gave low bacterial reductions (0.4-0.6 log CFU/g) in routine slaughter in a large-scale slaughterhouse (Table 8). High bacteria levels still remained on the carcasses, with >3 log CFU/g of *C. jejuni, Enterobacterales* and *E. coli* on 12%, 17% and 36% of carcasses, respectively, whereas according to the process hygiene criteria for *Campylobacter* (EC 2005), the amount of *Campylobacter* should not exceed 3.0 log CFU/g neck skin. In 15% of the neck skins samples, the number of total aerobic bacteria was >5 log CFU/g. This shows that the reduction in *C. jejuni* and hygiene indicator bacteria on broiler carcasses was insufficient in the poultry slaughterhouse studied in Paper IV. No significant difference in bacterial reductions was observed on increasing the steam temperature from 84-85 °C to 87-88 °C. According to staff at the slaughterhouse, it was impossible to increase the steam temperature above 88 °C, as this made it difficult to remove the skin from the carcass and for spices to stick to the skin surface during food product preparation. Impaired carcass quality has been observed in other similar treatments on broiler carcasses involving use of high temperatures (Whyte et al. 2003; James et al. 2007). In contrast, when Musavian et al. (2015) investigated the effects of 2 s of ultrasound-steam treatment on contaminated chicken transport crates, they observed a reduction to below the limit of detection from high initial level of total aerobic bacteria similar to that in Paper IV (*i.e.* 5-6 CFU/mL). However, in that study a higher temperature (95 °C) was possible since the treated objects were surfaces, not carcasses.

Table 8. Mean bacteria levels and mean reduction (log CFU/g) on chicken neck skins before and after carcass decontamination in a poultry slaughterhouse

	Mean before	**Mean after**	**Mean reduction**
Campylobacter jejuni	2.5±0.7	2.1±0.8	0.5*±0.8
Total aerobic bacteria	4.9±0.6	4.5±0.6	0.4*±0.7
Enterobacterales	3.1±0.6	2.5±0.5	0.6*±0.6
E. coli	3.3±0.7	2.8±0.6	0.5*±0.6

* = significant reduction at $p<0.05$

Comparing the bacterial reductions observed in the poultry slaughterhouse with those seen in the laboratory study (UV-C light) illustrates the difficulty

in achieving sufficient efficacy in an industrial setting. For example, in a study where ultrasound-steam treatment was tested on pork skin in a laboratory setting, high bacterial reductions (*e.g.* 2.9 log CFU/cm^2 for *E. coli*) were achieved even after a short treatment time (1.5 s) (Morild et al. 2011).

In a large-scale poultry slaughterhouse, it is difficult to achieve sufficient bacterial reduction, especially since the slaughter speed is high. This was observed on comparing the results from the decontamination study in Paper IV with those of other studies investigating ultrasound-steam treatment efficacy in smaller poultry slaughterhouses with slower slaughter speed (Hansen & Larsen 2007; Boysen & Rosenquist 2009; Musavian et al. 2014, 2022). From a food safety perspective, the advantage of installing this decontamination equipment lies in whether the treatment can reduce *Campylobacter* spp. on chicken meat in a way that decreases the number of cases of campylobacteriosis in humans. However, one disadvantage was that the equipment was difficult to clean and disinfect after use. The aspect of difficult-to-clean equipment was also mentioned in one of the interviews in the descriptive study.

The high level of *C. jejuni* and hygiene indicator bacteria, particularly *E. coli,* on poultry carcasses both before and after the decontamination also highlights the importance of high C&D efficacy and hygienic slaughter.

The coefficient of determination R^2 (equal to the squared correlation coefficient) between the initial bacterial amount on neck skins before treatment and the reduction achieved was 0.27, 0.70, 0.60 and 0.60 for *C. jejuni*, *Enterobacterales*, *E. coli* and total aerobic bacteria, respectively, and was significant ($p<0.05$) in all cases (Figure 4 in Paper IV). This indicates that there was a greater bacterial reduction in samples with higher initial bacterial level, which is a known effect (Li et al. 2012).

4.2.4. Assessment of monitoring methods to evaluate surface cleanliness (Paper I)

Swabbing for total aerobic bacteria was used as the reference method when assessing rapid sampling methods (dipslides and ATP-bioluminescence tests). In general, the highest number of acceptably clean samples was observed when using dipslides, while the opposite was seen for ATP-bioluminescence (Figure 16). These differences were most obvious in the red meat slaughterhouse.

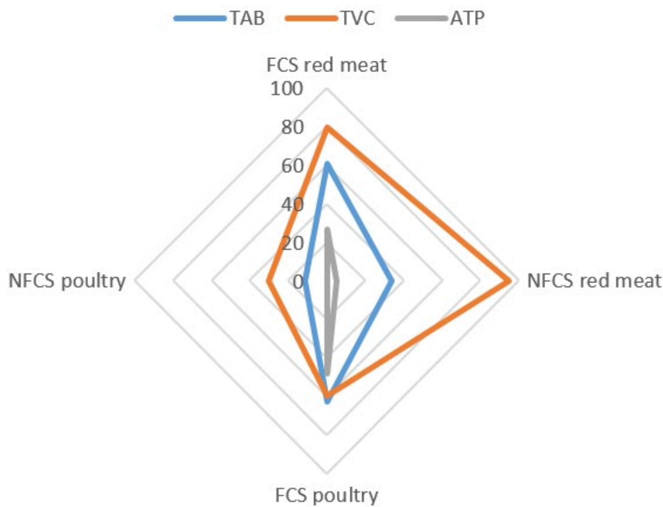


Figure 16. Proportion (%) of acceptably clean food contact surfaces (FCS) and non-food contact surfaces (NFCS) in the red meat and poultry slaughterhouses when using swabbing (total aerobic bacteria, TAB), dipslides (total viable count, TVC) and ATP-bioluminescence (relative light units, RLU).

Dipslide tests

Total viable count could be enumerated in almost all samples before C&D, which was in agreement with swabbing results for total aerobic bacteria. However, total viable count could only be enumerated in 35% and 60% of the dipslides after C&D in the red meat and poultry slaughterhouse, respectively compared with 90% and 73%, respectively, for total aerobic bacteria. All sampling points in the processing areas of both slaughterhouses, except a drain, were considered acceptably clean after C&D (Table 2 in Paper I), according to the selected threshold (1.0 CFU/cm^2, equal to 2.0 log CFU/100 cm^2). A complete reduction in total viable count on all dipslides was observed in the processing areas for some sampling points (conveyor belts and trolley in the red meat slaughterhouse, cutting blade in the poultry slaughterhouse), while for total aerobic bacteria the value was 0.8-2.0 log CFU/100 cm^2. It is problematic that in general, the dipslides detected less bacteria than swabbing (Figure 16), since dipslides are recommended for use in slaughterhouses (Lindahl et al. 2009) and are commonly used in Swedish slaughterhouses, as found in the descriptive study.

ATP-bioluminescence tests

Relative light unit values >0 were obtained for all samples before C&D and almost all samples after C&D. Fewer samples were considered acceptably clean (18% and 35% in the red meat and poultry slaughterhouse, respectively) according to the selected threshold (1.5 RLU/cm^2, equal to 2.2 log RLU/100 cm^2), compared with total aerobic bacteria and total viable count (Figure 16). The only sampling points that were considered acceptably clean according to ATP-bioluminescence on all sampling occasions were the trolley in the red meat slaughterhouse and the salt injector needles in the poultry slaughterhouse (Table 2 in Paper I). Similarly, Ríos-Castillo et al. (2021) observed that ATP-bioluminescence detected more non-successfully cleaned and disinfected surfaces than swabbing when FCSs in supermarkets were sampled. This is not surprising, since ATP-bioluminescence also detects soil residues (Møretrø et al. 2019; Ruiz-Llacsahuanga et al. 2021).

Diagnostic performance of dipslide and ATP-bioluminescence tests

The results from both slaughterhouses showed that the overall sensitivity of the dipslide method was low (Se = 0.52, when using swabbing for total aerobic bacteria as the reference method), indicating a high number of false negative samples. In contrast, the overall specificity for ATP-bioluminescence was low (Sp = 0.45, when swabbing for total aerobic bacteria as the reference method), indicating a high number of false positive samples. Low specificity for ATP, compared with swabbing for total aerobic bacteria as reference, has been observed in another study (Luick et al. 2013).

In addition, both positive and negative predictive values (PPV and NPV) were determined (Table 3 in Paper I). In general, there is a lack of studies assessing the diagnostic performance of different sampling methods, particularly in food industries. Comparison between dipslides and ATP-bioluminescence has been performed in a handful of studies, but only some utilised one or more of the diagnostic tests featured in Paper I (Carrascosa et al. 2012; Luick et al. 2013; Ching et al. 2021). Luick et al. (2013), compared ATP-bioluminescence with total aerobic bacteria after C&D in a health-care setting and observed higher positive predictive value (PPV = 0.90) and lower negative predictive value (NPV = 0.20) than in the present study (PPV 0.54 and NPV 0.89 for the red meat slaughterhouse, PPV 0.71 and NPV 0.93 for the poultry slaughterhouse). Determining the exact reasons for these

discrepancies between the two studies is challenging, given that they were conducted in markedly different environments. However, these differences could indicate that the prevalence according to the reference method (total aerobic bacteria) was higher in Luick et al. (2013) than in Paper I.

The diagnostic indicators are affected by the cut-off value applied. A way to increase the sensitivity of dipslides and the specificity of ATP-bioluminescence tests would be to change the previously selected cut-off values, *i.e.* decrease the threshold for dipslides and increase the threshold for the ATP-bioluminescence method.

4.3. Antimicrobial resistance - AMR (Paper II)

In Paper II, one *C. jejuni* isolate recovered from the poultry slaughterhouse showed phenotypic and genotypic resistance to quinolones (ciprofloxacin or nalidixic acid). Quinolone resistance in *Campylobacter* spp. is not uncommon in poultry and poultry meat in Sweden and other European countries (Torralbo et al. 2015; García-Sánchez et al. 2017; Hansson et al. 2021; Swedres-Svarm 2022). In Paper II, the origin of this resistance was unlikely to be antimicrobial use, as broilers are not treated with quinolones and rarely with other antimicrobials in Sweden (Hansson et al. 2021; T. Dziecziolowski, Swedish Poultry Meat Association, pers. comm., September 19, 2023). Quinolone resistance is worrying, since AMR in animals and humans is linked (EFSA & ECDC 2023). In addition, quinolones are on the list of critically important antimicrobials (WHO, 2019b).

In the red meat slaughterhouse studied, 80% (4/5) of the *C. coli* isolates were phenotypically and genotypically resistant to streptomycin. The species from which the isolates originated is unknown, since both pigs and cattle were slaughtered in the same facility and in Sweden and in other European countries, streptomycin resistance in *C. coli* is high in both pigs (47-70%) and calves (66%) (Swedres-Svarm 2019; EFSA & ECDC 2023). The source of this resistance is unlikely to be treatment of animals with antimicrobials, due to the restrictive antimicrobial use in Sweden. However, the resistance could be due to historical use of streptomycin (Swedres-Svarm 2019; O. Nilsson, National Veterinary Institute, pers. comm., September 21, 2023).

ESBL *E. coli* was not detected in Paper II, which could perhaps be explained by the decreasing prevalence of ESBL *E. coli* in broilers, broiler meat and pig meat, especially in northern Europe, which is most probably due to the

decrease in antimicrobial use in these species (Althaus et al. 2017; Swedres-Svarm 2021; EFSA & ECDC 2023). In Sweden, there has been a significant decrease in ESBL *E. coli* in broiler chickens since 2016, most likely due to reduced presence of such bacteria within the breeding pyramid (Nilsson et al. 2020).

5. Main conclusions

- The most common C&D procedures in the largest slaughterhouses with associated meat processing premises in Sweden included use of alkaline with or without chlorine and acidic agents for cleaning and chlorinated agents for disinfection, applied with a low-pressure system. Dipslides and ATP-bioluminescence were the most commonly used sampling methods. Selection of efficient C&D procedures and implementation of a risk-based approach for monitoring are challenging for quality assurance managers at Swedish slaughterhouses and they usually rely on external companies for selection of thresholds for clean surfaces. Some of the quality assurance managers interviewed expressed desire for more science-based recommendations for C&D and increased knowledge about the in-house microbiota in their facilities.

- In general, commonly used C&D procedures removed/inactivated *Campylobacter* and *L. monocytogenes* from surfaces. However, these pathogens were present on food contact surfaces before C&D and whole-genome sequencing indicated high potential for spread of *C. jejuni* in a poultry slaughterhouse and possible persistence of *L. monocytogenes* in a red meat slaughterhouse. These findings may indicate risk of cross-contamination of meat, followed by foodborne illness, since the pathogen sequence types and serogroups identified are known to cause human disease. Extended-spectrum beta-lactamase *E. coli* was not detected.

- Approximately half of the surfaces were assessed as acceptably clean. Surfaces in processing areas were more properly cleaned and disinfected than surfaces in the slaughter areas of the studied slaughterhouses. Despite C&D, high levels of total aerobic bacteria still remained on critical food contact surfaces, increasing the likelihood of cross-contamination of meat with spoilage bacteria. *Enterobacterales* were mainly present in drains and were detected in more samples in the poultry slaughterhouse than the red meat slaughterhouse.

- Disinfection of chicken transport crates with 265 nm UV-C LED light showed high reductions (1.4-3.1 log CFU/mL) of *C. jejuni,* total aerobic bacteria and *Enterobacterales* in a laboratory study. However, irradiation did not inactivate *C. jejuni* and the remaining bacterial levels of total aerobic bacteria and *Enterobacterales* were still high.

- Decontamination of broiler carcasses with ultrasound-steam and chilling resulted in significant but small reductions (<1.0 log CFU/g reduction) in *C. jejuni,* total aerobic bacteria, *Enterobacterales* and *E. coli* when installed in a large-scale poultry slaughterhouse. The level of *C. jejuni* was still above the process hygiene criterion (3.0 log CFU/g neck skin) in 12% of carcasses.

- Dipslides and ATP-bioluminescence sampling methods, which are commonly used in Swedish slaughterhouses and meat processing premises for monitoring C&D efficacy, showed low accuracy compared with swabbing for total aerobic bacteria. Since swabbing for total aerobic bacteria can be perceived as labour-intensive, a viable option could be to regularly alternate dipslides and ATP-bioluminescence, and periodically swab surfaces for total aerobic bacteria to verify the other methods. Based on the results from swabbing (total aerobic bacteria), thresholds for dipslides and ATP-bioluminescence can be adjusted when necessary.

6. Future perspectives

The results presented in this thesis broaden understanding of the foodborne bacteria present on surfaces within slaughterhouses and meat processing premises, by showing the efficacy of various C&D procedures and monitoring activities in Swedish slaughterhouses with associated meat processing premises. However, certain knowledge gaps remain to be addressed. The ultimate aim of C&D and monitoring is to prevent foodborne illness and prolong the shelf-life of meat products. Some potential areas for further studies and actions are suggested below:

Future studies within this project:

- Investigations on C&D efficacy will continue, with particular focus on residential bacteria, by sampling surfaces in slaughterhouses after C&D in a search for biofilm-producing bacteria. The samples will be analysed by 16S metagenomic sequencing, to determine the bacterial composition on different surfaces over time.

- Selected bacteria from the above study will be used for biofilm production in a laboratory study. The biofilm will be produced on surfaces of different equipment from slaughterhouses and the efficacy of different C&D agents will be evaluated. In addition to conventionally used chemicals, novel products that are claimed to inactivate biofilm efficiently will be tested (*i.e.* enzyme-based detergents).

Dissemination of knowledge to improve practices:

- Results obtained from the studies included in this thesis will be disseminated to slaughterhouses and meat processing premises, to assist them in their choice of C&D procedures and monitoring activities. The results generated are also valid for other food processing plants and can contribute to safer meat products with a longer shelf-life, which will be of benefit to primary producers as consumers demand high-quality food.

Future studies:

- It is a knowledge-demanding task for the food industry to select relevant thresholds for hygiene indicators and there are no guidelines to assist them in this task. The responsibility for producing safe and wholesome food rests with the industry. However, the scientific community should assist in this complicated task by conducting laboratory and industrial trials to determine acceptable levels of different hygiene indicators, which should be based on desired shelf-life and safety of food products. The results from such studies could be used to formulate guidelines for different food businesses, in Europe and globally, seeking to perform their own risk analysis.

References

Abdessater, M., Fayyad, F., Matta, J. & Karam, L. (2023). Assessment of prerequisite programs implementation at food packaging manufacturing companies and hygiene status of food packaging in a developing country: Cross-sectional study. *Heliyon*, 9 (9), e19824. https://doi.org/10.1016/j.heliyon.2023.e19824

Adams, M.R., & Moss, M.O. (1995). Food microbiology. 1. ed. The Royal Society of Chemistry. 35, 166.

Adegoke, A.A., Stenström, T.A. & Okoh, A.I. (2017). Stenotrophomonas maltophilia as an emerging ubiquitous pathogen: looking beyond contemporary antibiotic therapy. *Frontiers in Microbiology*, 8, 2276. https://doi.org/10.3389/fmicb.2017.02276

Ågren, E.C.C., Sternberg Lewerin, S., Wahlström, H., Emanuelson, U. & Frössling, J. (2016). Low prevalence of Salmonella in Swedish dairy herds highlight differences between serotypes. *Preventive Veterinary Medicine*, 125, 38–45. https://doi.org/10.1016/j.prevetmed.2015.12.015

Agüeria, D.A., Libonatti, C. & Civit, D. (2021). Cleaning and disinfection programmes in food establishments: a literature review on verification procedures. *Journal of Applied Microbiology*, 131 (1), 23–35. https://doi.org/10.1111/jam.14962

Allos, B.M. (1997). Association between *Campylobacter* Infection and Guillain-Barré Syndrome. *The Journal of Infectious Diseases*, 176 (2), 125–128. https://doi.org/10.1086/513783

Althaus, D., Zweifel, C. & Stephan, R. (2017). Analysis of a poultry slaughter process: Influence of process stages on the microbiological contamination of broiler carcasses. *Italian Journal of Food Safety*, 6 (4). https://doi.org/10.4081/ijfs.2017.7097

Andersen, J.K., Sørensen, R. & Glensbjerg, M. (1991). Aspects of the epidemiology of Yersinia enterocolitica: a review. *International Journal of Food Microbiology*, 13 (3), 231–237. https://doi.org/10.1016/0168-1605(91)90007-C

Antic, D., Houf, K., Michalopoulou, E. & Blagojevic, B. (2021). Beef abattoir interventions in a risk-based meat safety assurance system. *Meat Science*, 182, 108622. https://doi.org/10.1016/j.meatsci.2021.108622

Arguello, H., Carvajal, A., Collazos, J.A., García-Feliz, C. & Rubio, P. (2012). Prevalence and serovars of Salmonella enterica on pig carcasses, slaughtered pigs and the environment of four Spanish slaughterhouses. *Food Research International*, 45 (2), 905–912. https://doi.org/10.1016/j.foodres.2011.04.017

Asir, J., Nair, S., Devi, S., Prashanth, K., Saranathan, R. & Kanungo, R. (2015). Simultaneous gut colonisation and infection by ESBL-producing

Escherichia coli in hospitalised patients. *Australasian medical journal*, 200–207. https://doi.org/10.4066/AMJ.2015.2358

Atlaw, N.A., Keelara, S., Correa, M., Foster, D., Gebreyes, W., Aidara-Kane, A., Harden, L., Thakur, S. & Fedorka-Cray, P.J. (2022). Evidence of sheep and abattoir environment as important reservoirs of multidrug resistant Salmonella and extended-spectrum beta-lactamase Escherichia coli. *International Journal of Food Microbiology*, 363, 109516. https://doi.org/10.1016/j.ijfoodmicro.2021.109516

Atterbury, R.J., Gigante, A.M., Tinker, D., Howell, M. & Allen, V.M. (2020). An improved cleaning system to reduce microbial contamination of poultry transport crates in the United Kingdom. *Journal of Applied Microbiology*, 128 (6), 1776–1784. https://doi.org/10.1111/jam.14576

Bak, J., Ladefoged, S.D., Tvede, M., Begovic, T. & Gregersen, A. (2010). Disinfection of *Pseudomonas aeruginosa* biofilm contaminated tube lumens with ultraviolet C light emitting diodes. *Biofouling*, 26 (1), 31–38. https://doi.org/10.1080/08927010903191353

Bakhtiary, F., Sayevand, H.R., Remely, M., Hippe, B., Hosseini, H. & Haslberger, A.G. (2016). Evaluation of Bacterial Contamination Sources in Meat Production Line. *Journal of Food Quality*, 39 (6), 750–756. https://doi.org/10.1111/jfq.12243

Barco, L., Belluco, S., Roccato, A. & Ricci, A. (2015). A systematic review of studies on Escherichia coli and Enterobacteriaceae on beef carcasses at the slaughterhouse. *International Journal of Food Microbiology*, 207, 30–39. https://doi.org/10.1016/j.ijfoodmicro.2015.04.027

Bardoň, J., Husičková, V., Chromá, M. & Kolář, M. (2013). Prevalence and characteristics of Escherichia coli strains producing extended-spectrum β-lactamases in slaughtered animals in the Czech Republic. *Journal of Food Protection*, 76 (10), 1773–1777. https://doi.org/10.4315/0362-028X.JFP-13-114

Biasino, W., De Zutter, L., Garcia-Graells, C., Uyttendaele, M., Botteldoorn, N., Gowda, T. & Van Damme, I. (2018). Quantification, distribution and diversity of ESBL/AmpC-producing Escherichia coli on freshly slaughtered pig carcasses. *International Journal of Food Microbiology*, 281, 32–35. https://doi.org/10.1016/j.ijfoodmicro.2018.05.021

Borch, E., Nesbakken, T. & Christensen, H. (1996). Hazard identification in swine slaughter with respect to foodborne bacteria. *International Journal of Food Microbiology*, 30 (1–2), 9–25. https://doi.org/10.1016/0168-1605(96)00988-9

Boubendir, S., Arsenault, J., Quessy, S., Thibodeau, A., Fravalo, P., Thériault, W.P., Fournaise, S. & Gaucher, M.-L. (2021). Salmonella contamination of broiler chicken carcasses at critical steps of the slaughter process and in the environment of two slaughter plants: prevalence, genetic profiles, and association with the Final Carcass Status. *Journal of Food Protection*, 84 (2), 321–332. https://doi.org/10.4315/JFP-20-250

Boysen, L. & Rosenquist, H. (2009). Reduction of thermotolerant Campylobacter species on broiler carcasses following physical decontamination at

slaughter. *Journal of Food Protection*, 72 (3), 497–502. https://doi.org/10.4315/0362-028X-72.3.497
Brusa, V., Restovich, V., Galli, L., Arias, R., Linares, L., Costa, M., Díaz, V.R., Pugin, D. & Leotta, G. (2022). Reduction of Shiga toxin-producing *Escherichia coli* in a beef abattoir. *Food Science and Technology International*, 28 (1), 50–59. https://doi.org/10.1177/1082013221991258
Buncic, S. & Sofos, J. (2012). Interventions to control Salmonella contamination during poultry, cattle and pig slaughter. *Food Research International*, 45 (2), 641–655. https://doi.org/10.1016/j.foodres.2011.10.018
Cai, L., Wang, H., Liang, L., Wang, G., Xu, X. & Wang, H. (2018). Response of formed-biofilm of *Enterobacter cloacae* , *Klebsiella oxytoca* , and *Citrobacter freundii* to chlorite-based disinfectants. *Journal of Food Science*, 83 (5), 1326–1332. https://doi.org/10.1111/1750-3841.14149
Cano, C., Meneses, Y. & Chaves, B.D. (2021). Application of peroxyacetic acid for decontamination of raw poultry products and comparison to other commonly used chemical antimicrobial interventions: a review. *Journal of Food Protection*, 84 (10), 1772–1783. https://doi.org/10.4315/JFP-21-107
Carrascosa, C., Saavedra, P., Millán, R., Jaber, J.R., Pérez, E., Grau, R., Raposo, A., Mauricio, C. & Sanjuán, E. (2012). Monitoring of cleanliness and disinfection in dairies: Comparison of traditional microbiological and ATP bioluminescence methods. *Food Control*, 28 (2), 368–373. https://doi.org/10.1016/j.foodcont.2012.05.001
Carvalheira, A., Silva, J. & Teixeira, P. (2021). Acinetobacter spp. in food and drinking water – A review. *Food Microbiology*, 95, 103675. https://doi.org/10.1016/j.fm.2020.103675
Chan, Y.C. & Wiedmann, M. (2008). Physiology and genetics of Listeria Monocytogenes survival and growth at cold temperatures. *Critical Reviews in Food Science and Nutrition*, 49 (3), 237–253. https://doi.org/10.1080/10408390701856272
Chaves, R.D., Kumazawa, S.H., Khaneghah, A.M., Alvarenga, V.O., Hungaro, H.M. & Sant'Ana, A.S. (2024). Comparing the susceptibility to sanitizers, biofilm-forming ability, and biofilm resistance to quaternary ammonium and chlorine dioxide of 43 Salmonella enterica and Listeria monocytogenes strains. *Food Microbiology*, 117, 104380. https://doi.org/10.1016/j.fm.2023.104380
Cherifi, T., Arsenault, J., Pagotto, F., Quessy, S., Côté, J.-C., Neira, K., Fournaise, S., Bekal, S. & Fravalo, P. (2020). Distribution, diversity and persistence of Listeria monocytogenes in swine slaughterhouses and their association with food and human listeriosis strains. Chang, Y.-F. (ed.) (Chang, Y.-F., ed.) *PLOS ONE*, 15 (8), e0236807. https://doi.org/10.1371/journal.pone.0236807
Cherifi, T., Arsenault, J., Quessy, S. & Fravalo, P. (2022). Co-occurrence of L. monocytogenes with other bacterial genera and bacterial diversity on cleaned conveyor surfaces in a swine slaughterhouse. *Microorganisms*, 10 (3), 613. https://doi.org/10.3390/microorganisms10030613

Ching, C.L., Kamaruddin, A. & Rajangan, C.S. (2021). Assessing the performance of a real-time total adenylate (ATP+ADP+AMP) detection assay for surface hygiene monitoring in food manufacturing plants and commercial kitchens. *Journal of Food Protection*, 84 (6), 973–983. https://doi.org/10.4315/JFP-20-294

Codex Alimentarius (2007). Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in foods. CAC/GL 61 – 2007. https://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/

Codex Alimentarius (2020). General principles of food hygiene. CXC 1-1969. Adopted in 1969. Amended in 1999. Revised in 1997, 2003, 2020. Editorial corrections in 2011. https://www.fao.org/fao-who-codexalimentarius/codex-texts/codes-of-practice/pt/

Cooper, R.A., Griffith, C.J., Malik, R.E., Obee, P. & Looker, N. (2007). Monitoring the effectiveness of cleaning in four British hospitals. *American Journal of Infection Control*, 35 (5), 338–341. https://doi.org/10.1016/j.ajic.2006.07.015

Corbellini, L.G., Júnior, A.B., De Freitas Costa, E., Duarte, A.S.R., Albuquerque, E.R., Kich, J.D., Cardoso, M. & Nauta, M. (2016). Effect of slaughterhouse and day of sample on the probability of a pig carcass being Salmonella-positive according to the Enterobacteriaceae count in the largest Brazilian pork production region. *International Journal of Food Microbiology*, 228, 58–66. https://doi.org/10.1016/j.ijfoodmicro.2016.03.030

Cunningham, A.E., Rajagopal, R., Lauer, J. & Allwood, P. (2011). Assessment of hygienic quality of surfaces in retail food service establishments based on microbial counts and real-time detection of ATP. *Journal of Food Protection*, 74 (4), 686–690. https://doi.org/10.4315/0362-028X.JFP-10-395

De Busser, E.V., De Zutter, L., Dewulf, J., Houf, K. & Maes, D. (2013). Salmonella control in live pigs and at slaughter. *The Veterinary Journal*, 196 (1), 20–27. https://doi.org/10.1016/j.tvjl.2013.01.002

De Oliveira Mota, J., Boué, G., Prévost, H., Maillet, A., Jaffres, E., Maignien, T., Arnich, N., Sanaa, M. & Federighi, M. (2021). Environmental monitoring program to support food microbiological safety and quality in food industries: A scoping review of the research and guidelines. *Food Control*, 130, 108283. https://doi.org/10.1016/j.foodcont.2021.108283

Delhalle, L., Taminiau, B., Fastrez, S., Fall, A., Ballesteros, M., Burteau, S. & Daube, G. (2020). Evaluation of enzymatic cleaning on food processing installations and food products bacterial microflora. *Frontiers in Microbiology*, 11, 1827. https://doi.org/10.3389/fmicb.2020.01827

Demaître, N., Rasschaert, G., De Zutter, L., Geeraerd, A. & De Reu, K. (2021). Genetic Listeria monocytogenes types in the pork processing plant environment: from occasional introduction to plausible persistence in harborage sites. *Pathogens*, 10 (6), 717. https://doi.org/10.3390/pathogens10060717

Desai, A.N., Anyoha, A., Madoff, L.C. & Lassmann, B. (2019). Changing epidemiology of Listeria monocytogenes outbreaks, sporadic cases, and recalls globally: A review of ProMED reports from 1996 to 2018. *International Journal of Infectious Diseases*, 84, 48–53. https://doi.org/10.1016/j.ijid.2019.04.021

Di Bartolo, I., Diez-Valcarce, M., Vasickova, P., Kralik, P., Hernandez, M., Angeloni, G., Ostanello, F., Bouwknegt, M., Rodrìguez-Lázaro, D., Pavlik, I. & Ruggeri, F.M. (2012). Hepatitis E virus in pork production chain in Czech Republic, Italy, and Spain, 2010. *Emerging Infectious Diseases*, 18 (8). https://doi.org/10.3201/eid1808.111783

Doorduyn, Y., Van Den Brandhof, W.E., Van Duynhoven, Y.T.H.P., Breukink, B.J., Wagenaar, J.A. & Van Pelt, W. (2010). Risk factors for indigenous *Campylobacter jejuni* and *Campylobacter coli* infections in The Netherlands: a case-control study. *Epidemiology and Infection*, 138 (10), 1391–1404. https://doi.org/10.1017/S095026881000052X

Dorr, M., Silver, A., Smurlick, D., Arukha, A., Kariyawasam, S., Oladeinde, A., Cook, K. & Denagamage, T. (2022). Transferability of ESBL-encoding IncN and IncI1 plasmids among field strains of different Salmonella serovars and Escherichia coli. *Journal of Global Antimicrobial Resistance*, 30, 88–95. https://doi.org/10.1016/j.jgar.2022.04.015

Doulgeraki, A.I., Ercolini, D., Villani, F. & Nychas, G.-J.E. (2012). Spoilage microbiota associated to the storage of raw meat in different conditions. *International Journal of Food Microbiology*, 157 (2), 130–141. https://doi.org/10.1016/j.ijfoodmicro.2012.05.020

Dzieciolowski, T., Boqvist, S., Rydén, J. & Hansson, I. (2022). Cleaning and disinfection of transport crates for poultry – comparison of four treatments at slaughter plant. *Poultry Science*, 101 (1), 101521. https://doi.org/10.1016/j.psj.2021.101521

EC, (2004). Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. *Official Journal of the European Union, L 139* of 30th of April 2004 (pp. 1). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R0852

EC, (2005). Regulation (EC) No. 2073/2005 of the European Parliament and of the Council of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union, L 338* of 22th of December 2005 (pp. 1-26). https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R2073

EC, (2013). Regulation (EC) No. 101/2013 of the European Parliament and of the Council of 4 February 2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcases. *Official Journal of the European Union, L 34* of 5th of February 2013 (pp. 1-3). https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32013R0101

EFSA Biohazard Panel, (2013). European Food Safety Authority Panel on Biological Hazards. Scientific Opinion on the public health hazards to be

covered by inspection of meat (bovine animals). *EFSA Journal*, *11*:3266, 261 pp. https://doi.org/10.2903/j.efsa.2013.3266
EFSA Biohazard Panel, (2018). European Food Safety Authority Panel on Biological Hazards. Scientific Opinion on the *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. *EFSA Journal*, *16*:5134, 173 pp. https://doi.org/10.2903/j.efsa.2018.5134
EFSA & ECDC, (2021). European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union One Health 2019 Zoonoses Report. *EFSA Journal*, 19(2):6406. https://doi.org/10.2903/j.efsa.2021.6406
EFSA & ECDC, (2022). European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union One Health 2021 Zoonoses Report. *EFSA Journal*, 20(12):7666. https://doi.org/10.2903/j.efsa.2022.7666
EFSA & ECDC, (2023). European Food Safety Authority and European Centre for Disease Prevention and Control, 2023. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2020/2021. *EFSA Journal*, *21*:7867, 232 pp. https://doi.org/10.2903/j.efsa.2023.7867.
Erkmen, O. (2022). Microbiological analysis of foods and food processing environments. Academic Press. (Chapter 7). https:// doi.org/10.1016/c2021-0-01219-0
Fagerlund, A., Langsrud, S., Schirmer, B.C.T., Møretrø, T. & Heir, E. (2016). Genome analysis of Listeria monocytogenes sequence type 8 strains persisting in salmon and poultry processing environments and comparison with related strains. Boneca, I.G. (ed.) (Boneca, I. G., ed.) *PLOS ONE*, 11 (3), e0151117. https://doi.org/10.1371/journal.pone.0151117
Fagerlund, A., Møretrø, T., Heir, E., Briandet, R. & Langsrud, S. (2017). Cleaning and disinfection of biofilms composed of Listeria monocytogenes and background microbiota from meat processing surfaces. Elkins, C.A. (ed.) (Elkins, C. A., ed.) *Applied and Environmental Microbiology*, 83 (17), e01046-17, e01046-17. https://doi.org/10.1128/AEM.01046-17
FAO, (2011). Food and Agriculture Organization of the United Nations. Global food losses and food waste – Extent, causes and prevention. Rome. https://www.fao.org/3/mb060e/mb060e.pdf
Farber, J.M. & Peterkin, P.I. (1991). Listeria monocytogenes, a food-borne pathogen. *Microbiological Reviews*, 55 (3), 476–511. https:/doi: 10.1128/mr.55.3.476-511.1991
Ferrari, S., Frosth, S., Svensson, L., Fernström, L. -L., Skarin, H. & Hansson, I. (2019). Detection of *Campylobacter* spp. in water by dead-end ultrafiltration and application at farm level. *Journal of Applied Microbiology*, 127 (4), 1270–1279. https://doi.org/10.1111/jam.14379
Frosth, S., Karlsson-Lindsjö, O., Niazi, A., Fernström, L.-L. & Hansson, I. (2020). Identification of transmission routes of Campylobacter and on-farm

measures to reduce Campylobacter in chicken. *Pathogens*, 9 (5), 363. https://doi.org/10.3390/pathogens9050363

Gantzhorn, M.R., Pedersen, K., Olsen, J.E. & Thomsen, L.E. (2014). Biocide and antibiotic susceptibility of Salmonella isolates obtained before and after cleaning at six Danish pig slaughterhouses. *International Journal of Food Microbiology*, 181, 53–59. https://doi.org/10.1016/j.ijfoodmicro.2014.04.021

García-Sánchez, L., Melero, B., Jaime, I., Hänninen, M.-L., Rossi, M. & Rovira, J. (2017). Campylobacter jejuni survival in a poultry processing plant environment. *Food Microbiology*, 65, 185–192. https://doi.org/10.1016/j.fm.2017.02.009

Gekenidis, M.-T., Kläui, A., Smalla, K. & Drissner, D. (2020). Transferable extended-spectrum β-lactamase (esbl) plasmids in Enterobacteriaceae from irrigation water. *Microorganisms*, 8 (7), 978. https://doi.org/10.3390/microorganisms8070978

Ghollasi-Mood, F., Mohsenzadeh, M., Housaindokht, M.R. & Varidi, M. (2016). Microbial and chemical spoilage of chicken meat during storage at isothermal and fluctuation temperature under aerobic conditions. *Iranian Journal of Veterinary Science and Technology*, 8 (1). https://doi.org/10.22067/veterinary.v8i1.54563

Gichure, J.N., Kamau Njage, P.M., Wambui, J.M., Dykes, G.A., Buys, E.M. & Coorey, R. (2022). Systematic-review and meta-analysis on effect of decontamination interventions on prevalence and concentration of Campylobacter spp. during primary processing of broiler chickens. *Food Microbiology*, 102, 103923. https://doi.org/10.1016/j.fm.2021.103923

Gill, C.O. & McGinnis, J.C. (2000). Contamination of beef trimmings with Escherichia coli during a carcass breaking process. *Food Research International*, 33 (2), 125–130. https://doi.org/10.1016/S0963-9969(00)00026-0

Goksoy, E.O., Kirkan, S. & Kok, F. (2004). Microbiological quality of broiler carcasses during processing in two slaughterhouses in Turkey. *Poultry Science*, 83 (8), 1427–1432. https://doi.org/10.1093/ps/83.8.1427

Gómez, D., Ariño, A., Carramiñana, J.J., Rota, C. & Yangüela, J. (2012). Sponge versus mini-roller for the surface microbiological control of Listeria monocytogenes, total aerobic mesophiles and Enterobacteriaceae in the meat industry. *Food Control*, 27 (1), 242–247. https://doi.org/10.1016/j.foodcont.2012.03.031

Gómez, D., Iguácel, L., Rota, M., Carramiñana, J., Ariño, A. & Yangüela, J. (2015). Occurrence of Listeria monocytogenes in ready-to-eat meat products and meat processing plants in Spain. *Foods*, 4 (4), 271–282. https://doi.org/10.3390/foods4030271

Gong, Z., Shi, X., Bai, F., He, X., Zhang, H., Li, Y., Wan, Y., Lin, Y., Qiu, Y., Chen, Q., Hu, Q. & Cao, H. (2019). Characterization of a novel diarrheagenic strain of Proteus mirabilis associated with food poisoning in China. *Frontiers in Microbiology*, 10, 2810. https://doi.org/10.3389/fmicb.2019.02810

Griffith, C. (2005). Improving surface sampling and detection of contamination. In: *Handbook of Hygiene Control in the Food Industry*. Elsevier. 588–618. https://doi.org/10.1533/9781845690533.3.588
Griffith, C. (2016). Surface sampling and the detection of contamination. In: *Handbook of Hygiene Control in the Food Industry*. Elsevier. 673–696. https://doi.org/10.1016/B978-0-08-100155-4.00044-3
Guidi, F., Centorotola, G., Chiaverini, A., Iannetti, L., Schirone, M., Visciano, P., Cornacchia, A., Scattolini, S., Pomilio, F., D'Alterio, N. & Torresi, M. (2023). The slaughterhouse as hotspot of CC1 and CC6 Listeria monocytogenes strains with hypervirulent profiles in an integrated poultry chain of Italy. *Microorganisms*, 11 (6), 1543. https://doi.org/10.3390/microorganisms11061543
Hadjicharalambous, C., Grispoldi, L. & Goga, B.C. (2019). Department of Veterinary Medicine, University of Perugia, Perugia, Italy. Quantitative risk assessment of *Listeria monocytogenes* in a traditional RTE product. *EFSA Journal* 2019;17:e170906, 9 pp. https://doi.org/10.2903/j.efsa.2019.e170906
Hamza, T.A. (2017). Bacterial protease enzyme: safe and good alternative for industrial and commercial use. *Int. J. Chem. Biomol. Sci*. 3, 1–10.
Hansen, D. & Larsen, B.S. (2007). Reduction of Campylobacter on chicken carcasses by SonoSteam® treatment. Proceedings of European Congress of Chemical Engineering (ECCE-6) Copenhagen, 16-20 September 2007
Hansson, I., Ederoth, M., Andersson, L., Vagsholm, I. & Olsson Engvall, E. (2005). Transmission of Campylobacter spp. to chickens during transport to slaughter. *Journal of Applied Microbiology*, 99 (5), 1149–1157. https://doi.org/10.1111/j.1365-2672.2005.02689.x
Hansson, I., Ellström, P., Nilsson, O., Chaba, M., Skarin, M., Fernström, L.-L. & Frosth, S. (2021). Differences in genotype and antimicrobial resistance between Campylobacter spp. isolated from organic and conventionally produced chickens in Sweden. *Pathogens*, 10 (12), 1630. https://doi.org/10.3390/pathogens10121630
Hansson, I., Olsson Engvall, E., Ferrari, S., Harbom, B. & Lahti, E. (2020). Detection of *Campylobacter* species in different types of samples from dairy farms. *Veterinary Record*, 186 (18), 605–605. https://doi.org/10.1136/vr.105610
Hansson, I., Pudas, N., Harbom, B. & Engvall, E.O. (2010). Within-flock variations of Campylobacter loads in caeca and on carcasses from broilers. *International Journal of Food Microbiology*, 141 (1–2), 51–55. https://doi.org/10.1016/j.ijfoodmicro.2010.04.019
Hauge, S.J., Johannessen, G.S., Haverkamp, T.H.A., Bjørkøy, S., Llarena, A.K., Spilsberg, B., Leithaug, M., Økland, M., Holthe, J., Røtterud, O.-J., Alvseike, O. & Nagel-Alne, G.E. (2023). Assessment of poultry process hygiene and bacterial dynamics along two broiler slaughter lines in Norway. *Food Control*, 146, 109526. https://doi.org/10.1016/j.foodcont.2022.109526

Haughton, P.N., Lyng, J.G., Cronin, D.A., Morgan, D.J., Fanning, S. & Whyte, P. (2011). Efficacy of UV light treatment for the microbiological decontamination of chicken, associated packaging, and contact surfaces. *Journal of Food Protection*, 74 (4), 565–572. https://doi.org/10.4315/0362-028X.JFP-10-356

Hawley, R.J. & Kozlovac, J.P. (2005). Decontamination. In: Lindler, L.E., Lebeda, F.J., & Korch, G.W. (eds) *Biological Weapons Defense*. Humana Press. 333–348. https://doi.org/10.1385/1-59259-764-5:333

Hertz, F.B., Schønning, K., Rasmussen, S.C., Littauer, P., Knudsen, J.D., Løbner-Olesen, A. & Frimodt-Møller, N. (2016). Epidemiological factors associated with ESBL- and non ESBL-producing *E. coli* causing urinary tract infection in general practice. *Infectious Diseases*, 48 (3), 241–245. https://doi.org/10.3109/23744235.2015.1103895

Hinds, L.M., O'Donnell, C.P., Akhter, M. & Tiwari, B.K. (2019). Principles and mechanisms of ultraviolet light emitting diode technology for food industry applications. *Innovative Food Science & Emerging Technologies*, 56, 102153. https://doi.org/10.1016/j.ifset.2019.04.006

Hinojosa, C., Caldwell, D., Byrd, J., Droleskey, R., Lee, J., Stayer, P., Resendiz, E., Garcia, J., Klein, S., Caldwell, D., Pineda, M. & Farnell, M. (2018). Use of foaming disinfectants and cleaners to reduce aerobic bacteria and Salmonella on poultry transport coops. *Animals*, 8 (11), 195. https://doi.org/10.3390/ani8110195

Holah, J.T. (2014). Cleaning and disinfection practices in food processing. In: *Hygiene in Food Processing*. Elsevier. 259–304. https://doi.org/10.1533/9780857098634.3.259

Huang, Y.-S., Chen, Y.-C., Chen, M.-L., Cheng, A., Hung, I.-C., Wang, J.-T., Sheng, W.-H. & Chang, S.-C. (2015). Comparing visual inspection, aerobic colony counts, and adenosine triphosphate bioluminescence assay for evaluating surface cleanliness at a medical center. *American Journal of Infection Control*, 43 (8), 882–886. https://doi.org/10.1016/j.ajic.2015.03.027

Hussain, M. & Dawson, C. (2013). Economic impact of food safety outbreaks on food businesses. *Foods*, 2 (4), 585–589. https://doi.org/10.3390/foods2040585

Hutchison, M.L., Thomas, D.J.I., Small, A.H., Buncic, S. & Howell, M. (2007). Implementation of compulsory Hazard Analysis Critical Control Point system and its effect on concentrations of carcass and environmental surface bacterial indicators in United Kingdom red meat slaughterhouses. *Journal of Food Protection*, 70 (7), 1633–1639. https://doi.org/10.4315/0362-028X-70.7.1633

Idland, L., Granquist, E.G., Aspholm, M. & Lindbäck, T. (2022). The prevalence of Campylobacter spp., Listeria monocytogenes and Shiga toxin-producing Escherichia coli in Norwegian dairy cattle farms: A comparison between free stall and tie stall housing systems. *Journal of Applied Microbiology*, 132 (5), 3959–3972. https://doi.org/10.1111/jam.15512

Ivbule, M., Miklaševičs, E., Čupāne, L., Bērziņa, L., Bālinš, A. & Valdovska, A. (2017). Presence of methicillin-resistant Staphylococcus aureus in slaughterhouse environment, pigs, carcasses, and workers. *Journal of Veterinary Research*, 61 (3), 267–277. https://doi.org/10.1515/jvetres-2017-0037

James, C., James, S.J., Hannay, N., Purnell, G., Barbedo-Pinto, C., Yaman, H., Araujo, M., Gonzalez, M.L., Calvo, J., Howell, M. & Corry, J.E.L. (2007). Decontamination of poultry carcasses using steam or hot water in combination with rapid cooling, chilling or freezing of carcass surfaces. *International Journal of Food Microbiology*, 114 (2), 195–203. https://doi.org/10.1016/j.ijfoodmicro.2006.09.019

Janda, J.M. & Abbott, S.L. (2021). The changing face of the family *Enterobacteriaceae* (Order: " *Enterobacterales* "): New members, taxonomic issues, geographic expansion, and new diseases and disease syndromes. *Clinical Microbiology Reviews*, 34 (2), e00174-20. https://doi.org/10.1128/CMR.00174-20

Johnsen, G., Kruse, H. & Hofshagen, M. (2006). Genotyping of Campylobacter jejuni from broiler carcasses and slaughterhouse environment by amplified fragment length polymorphism. *Poultry Science*, 85 (12), 2278–2284. https://doi.org/10.1093/ps/85.12.2278

Khamisse, E., Firmesse, O., Christieans, S., Chassaing, D. & Carpentier, B. (2012). Impact of cleaning and disinfection on the non-culturable and culturable bacterial loads of food-contact surfaces at a beef processing plant. *International Journal of Food Microbiology*, 158 (2), 163–168. https://doi.org/10.1016/j.ijfoodmicro.2012.07.014

Kudirkienė, E., Bunevičienė, J., Brøndsted, L., Ingmer, H., Olsen, J.E. & Malakauskas, M. (2011). Evidence of broiler meat contamination with post-disinfection strains of Campylobacter jejuni from slaughterhouse. *International Journal of Food Microbiology*, 145, S116–S120. https://doi.org/10.1016/j.ijfoodmicro.2010.06.024

Lane, K., McLandsborough, L.A., Autio, W.R. & Kinchla, A.J. (2020). Efficacy of ATP monitoring for measuring organic matter on postharvest food contact surfaces. *Journal of Food Protection*, 83 (10), 1829–1837. https://doi.org/10.4315/0362-028X.JFP-19-443

Langsrud, S., Moen, B., Møretrø, T., Løype, M. & Heir, E. (2016). Microbial dynamics in mixed culture biofilms of bacteria surviving sanitation of conveyor belts in salmon-processing plants. *Journal of Applied Microbiology*, 120 (2), 366–378. https://doi.org/10.1111/jam.13013

Laranja, D.C., Cacciatore, F.A., Malheiros, P.D.S. & Tondo, E.C. (2023). Application of peracetic acid by spray or immersion in chicken carcasses to reduce cross-contamination in the slaughter process. *Journal of Food Safety*, 43 (1), e13019. https://doi.org/10.1111/jfs.13019

Lauritsen, C.V., Kjeldgaard, J., Ingmer, H., Bisgaard, M. & Christensen, H. (2019). Microbiota encompassing putative spoilage bacteria in retail packaged broiler meat and commercial broiler abattoir. *International Journal of*

Food Microbiology, 300, 14–21. https://doi.org/10.1016/j.ijfoodmicro.2019.04.003

Li, Y., Shimizu, T., Zimmermann, J.L. & Morfill, G.E. (2012). Cold atmospheric plasma for surface disinfection. *Plasma Processes and Polymers*, 9 (6), 585–589. https://doi.org/10.1002/ppap.201100090

Li, Y., Wang, M., Guan, D., Lv, H., Zhao, J., Yu, X., Yang, X. & Wu, C. (2017). A study on the decontaminated efficiency of ultraviolet device on the indoor airborne bacteria. *Procedia Engineering*, 205, 1376–1380. https://doi.org/10.1016/j.proeng.2017.10.281

Lindblad, M. & Flink, C. (2017). *Listeria monocytogenes* in cold smoked or smoked fish and sliced cured meats- Coordinated control project 2016. Report 31. Swedish Food Agency. https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2017/listeria-i-kallrok-och-gravad-fisk-samt-charkvaror-livsmedelsverkets-rapportserie-nr-31-2017.pdf

Lindblad, M., Hansson, I., Vågsholm, I. & Lindqvist, R. (2006). Postchill Campylobacter prevalence on broiler carcasses in relation to slaughter group colonization level and chilling system. *Journal of Food Protection*, 69 (3), 495–499. https://doi.org/10.4315/0362-028X-69.3.495

Lindahl, C., Benfalk, C., Kisekka-Ndawula, P. & Uppgård, E. (2009). Industry guidelines for small scale slaughter and cutting of cattle, pigs and lambs. Report: Institute of Agriculture and Environmental Technology (JTI) report Agriculture & Industry 385. https://www.livsmedelsverket.se/globalassets/foretag-regler-kontroll/branschriktlinjer/slakt---smaskalig-slakt-och-styckning-av-storboskap-gris-och-lamm.pdf

Lofstedt, R. (2019). The management and communication of a food risk controversy: the Swedish campylobacter case. *Journal of Risk Research*, 22 (6), 803–816. https://doi.org/10.1080/13669877.2019.1608287

Lues, J.F.R. & Van Tonder, I. (2007). The occurrence of indicator bacteria on hands and aprons of food handlers in the delicatessen sections of a retail group. *Food Control*, 18 (4), 326–332. https://doi.org/10.1016/j.foodcont.2005.10.010

Luick, L., Thompson, P.A., Loock, M.H., Vetter, S.L., Cook, J. & Guerrero, D.M. (2013). Diagnostic assessment of different environmental cleaning monitoring methods. *American Journal of Infection Control*, 41 (8), 751–752. https://doi.org/10.1016/j.ajic.2012.09.019

Maes, S., Heyndrickx, M., Vackier, T., Steenackers, H., Verplaetse, A. & Reu, K.D. (2019). Identification and spoilage potential of the remaining dominant microbiota on food contact surfaces after cleaning and disinfection in different food industries. *Journal of Food Protection*, 82 (2), 262–275. https://doi.org/10.4315/0362-028X.JFP-18-226

Maes, S., Huu, S.N., Heyndrickx, M., Weyenberg, S. van, Steenackers, H., Verplaetse, A., Vackier, T., Sampers, I., Raes, K. & Reu, K.D. (2017). Evaluation of two surface sampling methods for microbiological and chemical analyses to assess the presence of biofilms in food companies.

Journal of Food Protection, 80 (12), 2022–2028. https://doi.org/10.4315/0362-028X.JFP-17-210

Manage, D.P., Lauzon, J., Jones, C.M., Ward, P.J., Pilarski, L.M., Pilarski, P.M. & McMullen, L.M. (2019). Detection of pathogenic Escherichia coli on potentially contaminated beef carcasses using cassette PCR and conventional PCR. *BMC Microbiology*, 19 (1), 175. https://doi.org/10.1186/s12866-019-1541-4

Marriott, N.G., Schilling, M.W. & Gravani, R.B. (2018). Principles of Sanitation. 6 ed. Springer International Publishing, 158, 160.

Martín, B., Perich, A., Gómez, D., Yangüela, J., Rodríguez, A., Garriga, M. & Aymerich, T. (2014). Diversity and distribution of Listeria monocytogenes in meat processing plants. *Food Microbiology*, 44, 119–127. https://doi.org/10.1016/j.fm.2014.05.014

Meat Industry Guide, (2017). Microbiological criteria. Food Standards Scotland. (Chapter 13). https://www.foodstandards.gov.scot/downloads/MIG_-_Chapter_13.pdf

Meistere, I., Ķibilds, J., Eglīte, L., Alksne, L., Avsejenko, J., Cibrovska, A., Makarova, S., Streikiša, M., Grantiņa-Ieviņa, L. & Bērziņš, A. (2019). Campylobacter species prevalence, characterisation of antimicrobial resistance and analysis of whole-genome sequence of isolates from livestock and humans, Latvia, 2008 to 2016. *Eurosurveillance*, 24 (31). https://doi.org/10.2807/1560-7917.ES.2019.24.31.1800357

Milios, K., Drosinos, E.H. & Zoiopoulos, P.E. (2017). Carcass decontamination methods in slaughterhouses: a review. *Journal of the Hellenic Veterinary Medical Society*, 65 (2), 65. https://doi.org/10.12681/jhvms.15517

Moore, G. & Griffith, C. (2007). Problems associated with traditional hygiene swabbing: the need for in-house standardization. *Journal of Applied Microbiology*, 103 (4), 1090–1103. https://doi.org/10.1111/j.1365-2672.2007.03330.x

Møretrø, T., Heir, E., Nesse, L.L., Vestby, L.K. & Langsrud, S. (2012). Control of Salmonella in food related environments by chemical disinfection. *Food Research International*, 45 (2), 532–544. https://doi.org/10.1016/j.foodres.2011.02.002

Møretrø, T. & Langsrud, S. (2017). Residential bacteria on surfaces in the food industry and their implications for food safety and quality: residential bacteria in food industry. *Comprehensive Reviews in Food Science and Food Safety*, 16 (5), 1022–1041. https://doi.org/10.1111/1541-4337.12283

Møretrø, T., Langsrud, S. & Heir, E. (2013). Bacteria on meat abattoir process surfaces after sanitation: characterisation of survival properties of *Listeria monocytogenes* and the commensal bacterial flora. *Advances in Microbiology*, 03 (03), 255–264. https://doi.org/10.4236/aim.2013.33037

Møretrø, T., Normann, M.A., Sæbø, H.R. & Langsrud, S. (2019). Evaluation of ATP bioluminescence-based methods for hygienic assessment in fish industry. *Journal of Applied Microbiology*, 127 (1), 186–195. https://doi.org/10.1111/jam.14292

Morgan, R.B., Sierra–Arguello, Y.M., Perdoncini, G., Borges, K.A., Furian, T.Q., Gomes, M.J.P., Lima, D., Salle, C.T.P., Moraes, H.L.S. & Nascimento, V.P. (2022). Comparison of transport crates contamination with Campylobacter spp. before and after the cleaning and disinfection procedure in broiler slaughterhouses. *Poultry Science*, 101 (7), 101909. https://doi.org/10.1016/j.psj.2022.101909

Morild, R.K., Christiansen, P., Sørensen, A.H., Nonboe, U. & Aabo, S. (2011). Inactivation of pathogens on pork by steam-ultrasound treatment. *Journal of Food Protection*, 74 (5), 769–775. https://doi.org/10.4315/0362-028X.JFP-10-338

Moura-Alves, M., Carvalho, M., Baggio Ribeiro, D.H., Barbosa, J., Silveira, L., Pista, Â., Pinto, H.P., Saraiva, C., Teixeira, P. & Esteves, A. (2022). Hygiene indicators and Salmonellae on surfaces of swine carcasses from two slaughterhouses in northern Portugal. *Journal of Food Protection*, 85 (11), 1566–1575. https://doi.org/10.4315/JFP-21-312

Muhterem-Uyar, M., Dalmasso, M., Bolocan, A.S., Hernandez, M., Kapetanakou, A.E., Kuchta, T., Manios, S.G., Melero, B., Minarovičová, J., Nicolau, A.I., Rovira, J., Skandamis, P.N., Jordan, K., Rodríguez-Lázaro, D., Stessl, B. & Wagner, M. (2015). Environmental sampling for Listeria monocytogenes control in food processing facilities reveals three contamination scenarios. *Food Control*, 51, 94–107. https://doi.org/10.1016/j.foodcont.2014.10.042

Musavian, H.S., Butt, T.M., Larsen, A.B. & Krebs, N. (2015). Combined steam-ultrasound treatment of 2 seconds achieves significant high aerobic count and Enterobacteriaceae reduction on naturally contaminated food boxes, crates, conveyor belts, and meat knives. *Journal of Food Protection*, 78 (2), 430–435. https://doi.org/10.4315/0362-028X.JFP-14-155

Musavian, H.S., Butt, T.M., Ormond, A., Keeble, D. & Krebs, N.H. (2022). Evaluation of steam-ultrasound decontamination on naturally contaminated broilers through the analysis of Campylobacter, total viable count, and Enterobacteriaceae. *Journal of Food Protection*, 85 (2), 196–202. https://doi.org/10.4315/JFP-21-223

Musavian, H.S., Krebs, N.H., Nonboe, U., Corry, J.E.L. & Purnell, G. (2014). Combined steam and ultrasound treatment of broilers at slaughter: A promising intervention to significantly reduce numbers of naturally occurring campylobacters on carcasses. *International Journal of Food Microbiology*, 176, 23–28. https://doi.org/10.1016/j.ijfoodmicro.2014.02.001

Nahar, S., Mizan, F.R., Ha, A.J. & Ha, S.-D. (2018). Advances and future prospects of enzyme-based biofilm prevention approaches in the food industry. *Comprehensive Reviews in Food Science and Food Safety,* 17(6), 1484–1502. https://doi:10.1111/1541-4337.12382

Nakamura, A., Takahashi, H., Koike, F., Kuda, T. & Kobayashi, M. (2023). Transition of microbial contamination on the surface of carcass during the cattle slaughter process. *Food Microbiology*, 112, 104245. https://doi.org/10.1016/j.fm.2023.104245

National Advisory Committee on Microbiological Criteria for Foods, (2022). Adopted 22 April 2021, Washington, D.C. 2018–2020. Use of water in animal production, slaughter, and processing. *Journal of Food Protection,* 85(12), 1756–1778. https://doi.org/10.4315/JFP-22-144

National Veterinary Institute, (2022). Surveillance of infectious diseases in animals and humans in Sweden. SVA:s report series 89, 1654-7098. https://www.sva.se/media/yhnflvxo/salmonellosis.pdf

Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., Van Der Logt, P., Fazil, A., Christensen, B., Katsma, E., Borck, B. & Havelaar, A. (2009). A comparison of risk assessments on Campylobacter in broiler meat. *International Journal of Food Microbiology*, 129 (2), 107–123. https://doi.org/10.1016/j.ijfoodmicro.2008.12.001

Nemeghaire, S., Argudín, M.A., Feßler, A.T., Hauschild, T., Schwarz, S. & Butaye, P. (2014). The ecological importance of the Staphylococcus sciuri species group as a reservoir for resistance and virulence genes. *Veterinary Microbiology*, 171 (3–4), 342–356. https://doi.org/10.1016/j.vetmic.2014.02.005

Nilsson, O., Börjesson, S., Landén, A., Greko, C. & Bengtsson, B. (2020). Decreased detection of ESBL- or pAmpC-producing Escherichia coli in broiler breeders imported into Sweden. *Acta Veterinaria Scandinavica*, 62 (1), 33. https://doi.org/10.1186/s13028-020-00532-4

Ninios, T., Lundén, J., Korkeala, H., & Fredriksson-Ahomaa, M. (2014). Meat inspection and control in the slaughterhouse. 1. ed. Wiley Blackwell, Chapter 19.

Nylander, A., Jonsson, L., Marklinder, L. & Nydahl, M. (2014). Food Science. Studentlitteratur. 2 ed., 223.

Odeyemi, O.A., Alegbeleye, O.O., Strateva, M. & Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety*, 19(2):311–331. https://doi:10.1111/1541-4337.12526

Okpo, E., Leith, J., Smith-Palmer, A., Bell, J., Parks, D., Browning, F., Byers, L., Corrigan, H., Webster, D., Karcher, A.M., Murray, A. & Storey, T. (2015). An outbreak of an unusual strain of Listeria monocytogenes infection in North-East Scotland. *Journal of Infection and Public Health*, 8 (6), 612–618. https://doi.org/10.1016/j.jiph.2015.05.009

Olsen, J.E., Brown, D.J., Madsen, M. & Bisgaard, M. (2003). Cross-contamination with Salmonella on a broiler slaughterhouse line demonstrated by use of epidemiological markers. *Journal of Applied Microbiology*, 94 (5), 826–835. https://doi.org/10.1046/j.1365-2672.2003.01911.x

Ortiz, S., López, V., Villatoro, D., López, P., Dávila, J.C. & Martínez-Suárez, J.V. (2010). A 3-Year Surveillance of the Genetic Diversity and Persistence of Listeria monocytogenes in an Iberian Pig Slaughterhouse and Processing Plant. *Foodborne Pathogens and Disease*, 7 (10), 1177–1184. https://doi.org/10.1089/fpd.2010.0535

Oswaldi, V., Lüth, S., Dzierzon, J., Meemken, D., Schwarz, S., Feßler, A.T., Félix, B. & Langforth, S. (2022). Distribution and characteristics of Listeria spp. in pigs and pork production chains in Germany. *Microorganisms*, 10 (3), 512. https://doi.org/10.3390/microorganisms10030512

Otto, C., Zahn, S., Rost, F., Zahn, P., Jaros, D. & Rohm, H. (2011). Physical methods for cleaning and disinfection of surfaces. *Food Engineering Reviews*, 3 (3–4), 171–188. https://doi.org/10.1007/s12393-011-9038-4

Ottoson, J. (2019). Occurrence and growth of *Listeria monocytogenes* in food. Risk assessment report. Swedish Food Agency. https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2017/2017-nr-09-del-2-forekomst-och-tillvaxt-av-listeria-monocytogenes-i-livsmedel-riskvarderingsrapport.pdf

Pacholewicz, E., Liakopoulos, A., Swart, A., Gortemaker, B., Dierikx, C., Havelaar, A. & Schmitt, H. (2015). Reduction of extended-spectrum-β-lactamase- and AmpC-β-lactamase-producing Escherichia coli through processing in two broiler chicken slaughterhouses. *International Journal of Food Microbiology*, 215, 57–63. https://doi.org/10.1016/j.ijfoodmicro.2015.08.010

Pagliano, P., Arslan, F. & Ascione, T. (2017). Epidemiology and treatment of the commonest form of listeriosis: meningitis and bacteraemia. *Infezioni in Medicina*. 25(3), 210–216. https://www.infezmed.it/media/journal/Vol_25_3_2017_2.pdf

Papadopoulos, D., Petridou, E., Filioussis, G., Papadopoulos, T., Papageorgiou, K., Chatzistilianou, M., Kritas, S. (2020). Prevalence and antibiotic resistance of Campylobacter coli and Campylobacter jejuni in Greek swine farms. *Am. J. Microbiol. Immunol.* 5 (6). https://doi:10.28933/ajmi-2020-02-2605

Pedrós-Garrido, S., Condón-Abanto, S., Clemente, I., Beltrán, J.A., Lyng, J.G., Bolton, D., Brunton, N. & Whyte, P. (2018). Efficacy of ultraviolet light (UV-C) and pulsed light (PL) for the microbiological decontamination of raw salmon (Salmo salar) and food contact surface materials. *Innovative Food Science & Emerging Technologies*, 50, 124–131. https://doi.org/10.1016/j.ifset.2018.10.001

Perdoncini, G., Sierra Arguello, Y.M., Moreira Lima, L., Quedi Furian, T., Apellanis Borges, K., Beatriz Rodrigues, L., Ruschel Dos Santos, L., Borsoi, A., Werlang Isolan, L., Pereira Gomes, M.J., Pippi Salle, C.T., De Souza Moraes, H.L. & Pinheiro Do Nascimento, V. (2022). Detection and quantification of *Campylobacter* in poultry slaughterhouses using conventional microbiological technique, most probable number, and real-time PCR. *Foodborne Pathogens and Disease*, 19 (2), 143–150. https://doi.org/10.1089/fpd.2021.0071

Perez, F., Endimiani, A., Hujer, K. & Bonomo, R. (2007). The continuing challenge of ESBLs. *Current Opinion in Pharmacology*, 7 (5), 459–469. https://doi.org/10.1016/j.coph.2007.08.003

Perez-Arnedo, I. & Gonzalez-Fandos, E. (2019). Prevalence of Campylobacter spp. in poultry in three Spanish farms, a slaughterhouse and a further processing plant. *Foods*, 8 (3), 111. https://doi.org/10.3390/foods8030111

Peruzy, M.F., Houf, K., Joossens, M., Yu, Z., Proroga, Y.T.R. & Murru, N. (2021). Evaluation of microbial contamination of different pork carcass areas through culture-dependent and independent methods in small-scale slaughterhouses. *International Journal of Food Microbiology*, 336, 108902. https://doi.org/10.1016/j.ijfoodmicro.2020.108902

Peters, S., Pascoe, B., Wu, Z., Bayliss, S.C., Zeng, X., Edwinson, A., Veerabadhran-Gurunathan, S., Jawahir, S., Calland, J.K., Mourkas, E., Patel, R., Wiens, T., Decuir, M., Boxrud, D., Smith, K., Parker, C.T., Farrugia, G., Zhang, Q., Sheppard, S.K. & Grover, M. (2021). Campylobacter jejuni genotypes are associated with post-infection irritable bowel syndrome in humans. *Communications Biology*, 4 (1), 1015. https://doi.org/10.1038/s42003-021-02554-8

Pettengill, J.B., Rand, H., Wang, S.S., Kautter, D., Pightling, A. & Wang, Y. (2022). Transient and resident pathogens: Intra-facility genetic diversity of Listeria monocytogenes and Salmonella from food production environments. Spanu, C. (ed.) (Spanu, C., ed.) *PLOS ONE*, 17 (9), e0268470. https://doi.org/10.1371/journal.pone.0268470

Peyrat, M., Soumet, C., Maris, P. & Sanders, P. (2008). Recovery of Campylobacter jejuni from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination. *International Journal of Food Microbiology*, 124 (2), 188–194. https://doi.org/10.1016/j.ijfoodmicro.2008.03.030

Piras, F., Fois, F., Mazza, R., Putzolu, M., Delogu, M.L., Lochi, P.G., Pani, S.P. & Mazzette, R. (2014). Salmonella prevalence and microbiological contamination of pig carcasses and slaughterhouse environment. *Italian Journal of Food Safety*, 3 (4). https://doi.org/10.4081/ijfs.2014.4581

Pope, J.E., Krizova, A., Garg, A.X., Thiessen-Philbrook, H. & Ouimet, J.M. (2007). Campylobacter reactive arthritis: a systematic review. *Seminars in Arthritis and Rheumatism*, 37 (1), 48–55. https://doi.org/10.1016/j.semarthrit.2006.12.006

Prendergast, D.M., Duggan, S.J., Fanning, S., Cormican, M., Gonzales-Barron, U., Butler, F. & Duffy, G. (2008). Prevalence and numbers of *Salmonella* spp. and Enterobacteriaceae on pork cuts in abattoirs in the Republic of Ireland. *Journal of Applied Microbiology*, 105 (4), 1209–1219. https://doi.org/10.1111/j.1365-2672.2008.03854.x

Projahn, M., Von Tippelskirch, P., Semmler, T., Guenther, S., Alter, T. & Roesler, U. (2019). Contamination of chicken meat with extended-spectrum beta-lactamase producing- Klebsiella pneumoniae and Escherichia coli during scalding and defeathering of broiler carcasses. *Food Microbiology*, 77, 185–191. https://doi.org/10.1016/j.fm.2018.09.010

Public Health Agency of Sweden, (2018). Report: Epidemiological typing of *Campylobacter* isolates collected week 11 2018. https://www.folkhalsomyndigheten.se/contentassets/bc4b2a860df24cec84568829414f6cd6/epidemiologisk-typning-campylobacterisolat-insamlade-vecka-11-2018-rev-16-juli-2018.pdf

Public Health Agency of Sweden and Food Safety Agency, (2020). Report: *Campylobacter* from the store to the clinic. Comparisons. https://www.folkhalsomyndigheten.se/contentassets/219eded9d0d740469be4e42bd6131fb6/campylobacter-butik-klinik-jamforelser-2019.pdf

Public Health Agency of Sweden, (2022). *Campylobacter* infections, disease statistics. https://www.folkhalsomyndigheten.se/folkhalsorapportering-statistik/statistik-a-o/sjukdomsstatistik/campylobacterinfektion/?p=94294&tab=tab-report

Quinn, P.J., Markey, B.K., Leonard, F.C., FitzPatrick, E.S., Fanning, E.S., Hartigan, P.J. (2011). Veterinary Microbiology and Microbial Disease. 2. ed. Wiley-Blackwell. Chapter 94.

Rahkio, T.M. & Korkeala, H.J. (1997). Use of Hygicult-tpc® in slaughterhouse hygiene control. *Acta Veterinaria Scandinavica*, 38 (4), 331–338. https://doi.org/10.1186/BF03548479

Ramos, C.C.R., Roque, J.L.A., Sarmiento, D.B., Suarez, L.E.G., Sunio, J.T.P., Tabungar, K.I.B., Tengco, G.S.C., Rio, P.C. & Hilario, A.L. (2020). Use of ultraviolet-C in environmental sterilization in. *International Journal of Health Sciences*, 14 (6)

Raschle, S., Zweifel, C., Zurfluh, K. & Stephan, R. (2019). Decontamination of knives used in a slaughterhouse by a commercial non-thermal UV-C treatment. *Italian Journal of Food Safety*, 8 (2). https://doi.org/10.4081/ijfs.2019.8107

Reich, F., Atanassova, V., Haunhorst, E. & Klein, G. (2008). The effects of Campylobacter numbers in caeca on the contamination of broiler carcasses with Campylobacter. *International Journal of Food Microbiology*, 127 (1–2), 116–120. https://doi.org/10.1016/j.ijfoodmicro.2008.06.018

Ríos-Castillo, A.G., Ripolles-Avila, C. & Rodríguez-Jerez, J.J. (2021). Evaluation of bacterial population using multiple sampling methods and the identification of bacteria detected on supermarket food contact surfaces. *Food Control*, 119, 107471. https://doi.org/10.1016/j.foodcont.2020.107471

Rivas, T., Vizcaíno, J.A. & Herrera, F.J. (2000). Microbial contamination of carcasses and equipment from an iberian pig slaughterhouse. *Journal of Food Protection*, 63 (12), 1670–1675. https://doi.org/10.4315/0362-028X-63.12.1670

Roth, N., Käsbohrer, A., Mayrhofer, S., Zitz, U., Hofacre, C. & Domig, K.J. (2019). The application of antibiotics in broiler production and the resulting antibiotic resistance in Escherichia coli: A global overview. *Poultry Science*, 98 (4), 1791–1804. https://doi.org/10.3382/ps/pey539

Ruiz-Llacsahuanga, B., Hamilton, A., Zaches, R., Hanrahan, I. & Critzer, F. (2021). Utility of rapid tests to assess the prevalence of indicator organisms (Aerobic plate count, Enterobacteriaceae, coliforms, Escherichia coli, and Listeria spp.) in apple packinghouses. *International Journal of Food Microbiology*, 337, 108949. https://doi.org/10.1016/j.ijfoodmicro.2020.108949

Saini, J.K., Marsden, J.L., Fung, Y.C.D. & Crozier-Dodson, B.A. (2012). Evaluation of potential for translocation of *Listeria monocytogenes* from floor drains to food contact surfaces in the surrounding environment using *Listeria innocua* as a surrogate. *Advances in Microbiology*, 02 (04), 565–570. https://doi.org/10.4236/aim.2012.24073

Sala, C., Morar, A., Tîrziu, E., Nichita, I., Imre, M. & Imre, K. (2016). Environmental occurrence and antibiotic susceptibility profile of Listeria monocytogenes at a slaughterhouse raw processing plant in Romania. *Journal of Food Protection*, 79 (10), 1794–1797. https://doi.org/10.4315/0362-028X.JFP-16-052

Samapundo, S., de Baenst, I., Aerts, M., Cnockaert, M., Devlieghere, F. & Van Damme, P. (2019). Tracking the sources of psychrotrophic bacteria contaminating chicken cuts during processing. *Food Microbiology*, 81, 40–50. https://doi.org/10.1016/j.fm.2018.06.003

Schäfer, D.F., Steffens, J., Barbosa, J., Zeni, J., Paroul, N., Valduga, E., Junges, A., Backes, G.T. & Cansian, R.L. (2017). Monitoring of contamination sources of Listeria monocytogenes in a poultry slaughterhouse. *LWT*, 86, 393–398. https://doi.org/10.1016/j.lwt.2017.08.024

Schöbel, H., Diem, G., Kiechl, J., Chistè, D., Bertacchi, G., Mayr, A., Wilflingseder, D., Lass-Flörl, C. & Posch, W. (2023). Antimicrobial efficacy and inactivation kinetics of a novel LED-based UV-irradiation technology. *Journal of Hospital Infection*, 135, 11–17. https://doi.org/10.1016/j.jhin.2022.12.023

Seliwiorstow, T., Baré, J., Berkvens, D., Van Damme, I., Uyttendaele, M. & De Zutter, L. (2016). Identification of risk factors for Campylobacter contamination levels on broiler carcasses during the slaughter process. *International Journal of Food Microbiology*, 226, 26–32. https://doi.org/10.1016/j.ijfoodmicro.2016.03.010

Sherlock, O., O'Connell, N., Creamer, E. & Humphreys, H. (2009). Is it really clean? An evaluation of the efficacy of four methods for determining hospital cleanliness. *Journal of Hospital Infection*, 72 (2), 140–146. https://doi.org/10.1016/j.jhin.2009.02.013

SIS, (2018). Swedish Standards Institute. SS-EN ISO 18593:2018. Microbiology of the food chain- Horizontal methods for surface sampling (ISO 18593:2018). https://www.sis.se/en/produkter/mathematics-natural-sciences/microbiology/food-microbiology/ss-en-iso-185932018/

Skarp, C.P.A., Hänninen, M.-L. & Rautelin, H.I.K. (2016). Campylobacteriosis: the role of poultry meat. *Clinical Microbiology and Infection*, 22 (2), 103–109. https://doi.org/10.1016/j.cmi.2015.11.019

Slader, J., Domingue, G., Jørgensen, F., McAlpine, K., Owen, R.J., Bolton, F.J. & Humphrey, T.J. (2002). Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. *Applied and Environmental Microbiology*, 68 (2), 713–719. https://doi.org/10.1128/AEM.68.2.713-719.2002

Spescha, C., Stephan, R. & Zweifel, C. (2006). Microbiological contamination of pig carcasses at different stages of slaughter in two european union–

approved abattoirs. *Journal of Food Protection*, 69 (11), 2568–2575. https://doi.org/10.4315/0362-028X-69.11.2568

Stanga, M. (2010). Sanitation: Cleaning and disinfection in the food industry. Wiley-VCH. 484, 514.

Stoller, A., Stevens, M., Stephan, R. & Guldimann, C. (2019). Characteristics of Listeria Monocytogenes strains persisting in a meat processing facility over a 4-year period. *Pathogens*, 8 (1), 32. https://doi.org/10.3390/pathogens8010032

Swedish Food Agency, (2022). Swedish Food Agency provides guidance on how the requirements in the legislation can be achieved. Cleaning. https://kontrollwiki.livsmedelsverket.se/artikel/346/rengoring

Swedish Food Agency, (2023). Advice for risk groups - how to avoid becoming infected with listeria. https://www.livsmedelsverket.se/om-oss/press/nyheter/pressmeddelanden/rad-till-riskgrupper-sa-undviker-du-att-smittas-av-listeria [2023-10-15]

Swedres-Swarm, (2019). Report: Consumption of antibiotics and occurrence of antibiotic resistance in Sweden. Public Health Agency of Sweden and National Veterinary Institute. https://www.folkhalsomyndigheten.se/publikationer-och-material/publikationsarkiv/s/swedres-svarm-2019/

Swedres-Swarm, (2021). Report: Consumption of antibiotics and occurrence of antibiotic resistance in Sweden. Public Health Agency of Sweden and National Veterinary Institute. https://www.folkhalsomyndigheten.se/publikationer-och-material/publikationsarkiv/s/swedres-svarm-2021/

Swedres-Swarm, (2022). Report: Sales of antibiotics and occurrence of antibiotic resistance in Sweden. Public Health Agency of Sweden and National Veterinary Institute. https://www.sva.se/media/ticcp2zu/swedres-svarm-2022-edit-230808.pdf

Syguła-Cholewińska, J., Lech, T., Szostak-Kot, J., Błyskal, B. & Sawoszczuk, T. (2014). ATP bioluminescence method in surface hygiene monitoring. Department of Microbiology, Cracow University of Economics, Krakow, 31-510.

Tam, C. (2003). Campylobacter coli—an important foodborne pathogen. *Journal of Infection*, 47 (1), 28–32. https://doi.org/10.1016/S0163-4453(03)00042-2

Thougaard, H., Møller Madsen, R., Just Munch, J., & Kamuk A. (2023). Basic microbiology with food applications. 3 ed. Studentlitteratur. 352.

Todd, E.C.D., Michaels, B.S., Greig, J.D., Smith, D., Holah, J. & Bartleson, C.A. (2010). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 7. Barriers to reduce contamination of food by workers. *Journal of Food Protection*, 73 (8), 1552–1565. https://doi.org/10.4315/0362-028X-73.8.1552

Tofani, S., Albini, E., Blasi, F., Cucco, L., Lovito, C., Maresca, C., Pesciaroli, M., Orsini, S., Scoccia, E., Pezzotti, G., Magistrali, C.F. & Massacci, F.R. (2022). Assessing the load, virulence and antibiotic-resistant traits of

ESBL/Ampc E. coli from broilers raised on conventional, antibiotic-free, and organic farms. *Antibiotics*, 11 (11), 1484. https://doi.org/10.3390/antibiotics11111484
Tomasevic, I., Kuzmanović, J., Anđelković, A., Saračević, M., Stojanović, M.M. & Djekic, I. (2016). The effects of mandatory HACCP implementation on microbiological indicators of process hygiene in meat processing and retail establishments in Serbia. *Meat Science*, 114, 54–57. https://doi.org/10.1016/j.meatsci.2015.12.008
Torralbo, A., Borge, C., García-Bocanegra, I., Méric, G., Perea, A. & Carbonero, A. (2015). Higher resistance of Campylobacter coli compared to Campylobacter jejuni at chicken slaughterhouse. *Comparative Immunology, Microbiology and Infectious Diseases*, 39, 47–52. https://doi.org/10.1016/j.cimid.2015.02.003
Tsitsos, A., Economou, V., Chouliara, E., Ambrosiadis, I. & Arsenos, G. (2022). A comparative study on microbiological and chemical characteristics of small ruminant carcasses from abattoirs in Greece. *Foods*, 11 (15), 2370. https://doi.org/10.3390/foods11152370
Veluz, G.A., Pitchiah, S. & Alvarado, C.Z. (2012). Attachment of Salmonella serovars and Listeria monocytogenes to stainless steel and plastic conveyor belts. *Poultry Science*, 91 (8), 2004–2010. https://doi.org/10.3382/ps.2011-01689
Wagner, E.M., Pracser, N., Thalguter, S., Fischel, K., Rammer, N., Pospíšilová, L., Alispahic, M., Wagner, M. & Rychli, K. (2020). Identification of biofilm hotspots in a meat processing environment: Detection of spoilage bacteria in multi-species biofilms. *International Journal of Food Microbiology*, 328, 108668. https://doi.org/10.1016/j.ijfoodmicro.2020.108668
Walker, L.J., Wallace, R.L., Smith, J.J., Graham, T., Saputra, T., Symes, S., Stylianopoulos, A., Polkinghorne, B.G., Kirk, M.D. & Glass, K. (2019). Prevalence of Campylobacter coli and Campylobacter jejuni in retail chicken, beef, lamb, and pork products in three Australian states. *Journal of Food Protection*, 82 (12), 2126–2134. https://doi.org/10.4315/0362-028X.JFP-19-146
Wang, H., He, A. & Yang, X. (2018). Dynamics of microflora on conveyor belts in a beef fabrication facility during sanitation. *Food Control*, 85, 42–47. https://doi.org/10.1016/j.foodcont.2017.09.017
Wheatley, P., Giotis, E.S. & McKevitt, A.I. (2014). Effects of slaughtering operations on carcass contamination in an Irish pork production plant. *Irish Veterinary Journal*, 67 (1), 1. https://doi.org/10.1186/2046-0481-67-1
WHO, (2018). World Health Organization. Fact sheet, Listeriosis. https://www.who.int/news-room/fact-sheets/detail/listeriosis [2023-10-15]
WHO, (2019a). World Health Organization. New report calls for urgent action to avert antimicrobial resistance crisis. https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis [2023-10-15]

WHO, (2019b). World Health Organization. Critically important antimicrobials for human medicine, 6th revision 2018. Geneva. https://www.who.int/publications/i/item/9789241515528

WHO, (2020). World Health Organization. Fact sheet, *Campylobacter*. https://www.who.int/news-room/fact-sheets/detail/campylobacter [2023-10-15]

WHO, (2022). World Health Organization. Global antimicrobial resistance and use surveillance system (GLASS) report 2022. Geneva. https://iris.who.int/bitstream/handle/10665/364996/9789240062702-eng.pdf?sequence=1

Whyte, P., McGill, K. & Collins, J.D. (2003). An assessment of steam pasteurization and hot water immersion treatments for the microbiological decontamination of broiler carcasses. *Food Microbiology*, 20 (1), 111–117. https://doi.org/10.1016/S0740-0020(02)00084-9

Whyte, P., McGill, K., Monahan, C. & Collins, J.D. (2004). The effect of sampling time on the levels of micro-organisms recovered from broiler carcasses in a commercial slaughter plant. *Food Microbiology*, 21 (1), 59–65. https://doi.org/10.1016/S0740-0020(03)00040-6

Wideman, N., Bailey, M., Bilgili, S.F., Thippareddi, H., Wang, L., Bratcher, C., Sanchez-Plata, M. & Singh, M. (2016). Evaluating best practices for Campylobacter and Salmonella reduction in poultry processing plants. *Poultry Science*, 95 (2), 306–315. https://doi.org/10.3382/ps/pev328

Wieczorek, K., Denis, E., Lachtara, B. & Osek, J. (2017). Distribution of Campylobacter jejuni multilocus sequence types isolated from chickens in Poland. *Poultry Science*, 96 (3), 703–709. https://doi.org/10.3382/ps/pew343

Wierup, M., Engström, B., Engvall, A. & Wahlström, H. (1995). Control of Salmonella enteritidis in Sweden. *International Journal of Food Microbiology*, 25 (3), 219–226. https://doi.org/10.1016/0168-1605(94)00090-S

Wilson, D.I., Christie, G., Fryer, P.J., Hall, I.M., Landel, J.R. & Whitehead, K.A. (2022). Lessons to learn from roadmapping in cleaning and decontamination. *Food and Bioproducts Processing*, 135, 156–164. https://doi.org/10.1016/j.fbp.2022.07.011

Xu, H., Lee, H.-Y. & Ahn, J. (2011). Characteristics of biofilm formation by selected foodborne pathogens: biofilm formation by foodborne pathogens. *Journal of Food Safety*, 31 (1), 91–97. https://doi.org/10.1111/j.1745-4565.2010.00271.x

Zarei, M., Rahimi, S., Fazlara, A. & Anvari, S.E. (2023). High biofilm-forming Pseudomonas strains isolated from poultry slaughterhouse surfaces: Their importance in the persistence of Salmonella enteritidis in slaughterhouses. *International Journal of Food Microbiology*, 390, 110126. https://doi.org/10.1016/j.ijfoodmicro.2023.110126

Zeng, H., De Reu, K., Gabriël, S., Mattheus, W., De Zutter, L. & Rasschaert, G. (2021). Salmonella prevalence and persistence in industrialized poultry

slaughterhouses. *Poultry Science*, 100 (4), 100991. https://doi.org/10.1016/j.psj.2021.01.014

Zweifel, C., Capek, M. & Stephan, R. (2014). Microbiological contamination of cattle carcasses at different stages of slaughter in two abattoirs. *Meat Science*, 98 (2), 198–202. https://doi.org/10.1016/j.meatsci.2014.05.029

Zweifel, C. & Stephan, R. (2012). Microbial decontamination of poultry carcasses 1 1Adapted from Food Control 21, M. Loretz, R. Stephan and C. Zweifel, 'Antimicrobial activity of decontamination treatments for poultry carcasses: a literature survey', 791–804, copyright (2010), with permission from Elsevier. In: *Microbial Decontamination in the Food Industry*. Elsevier. 60–95. https://doi.org/10.1533/9780857095756.1.60

Popular science summary

Proper cleaning and disinfection in slaughterhouses and meat processing premises is a prerequisite for production of safe, high-quality meat. Cleaning and disinfection should be performed in a way that minimizes the occurrence of bacteria which cause human disease and food spoilage. All slaughterhouses and meat processing premises must be cleaned and disinfected daily, which can be a challenge in these often wet environments with many pieces of equipment. The high speed of the slaughter process also increases the likelihood of contamination of surfaces with bacteria. Although many cleaning and disinfection procedures are available, bacteria that can cause disease and food spoilage may still be present on surfaces that come into direct contact with meat after cleaning and disinfection. This poses a risk of these bacteria contaminating meat, affecting both the safety of consumers and the shelf-life of the meat. Some bacteria, such as *Listeria monocytogenes* can form biofilm, which consists of populations of bacteria enclosed in a network that adheres to surfaces and to other bacteria. This biofilm protects bacteria from external forces and makes them difficult to remove with cleaning and disinfection procedures. Listeriosis in humans is a severe disease with a high risk of hospitalisation and death, especially in vulnerable groups such as the elderly, newborns and people with suppressed immune status. It can also cause spontaneous abortion. Another example of how inadequate cleaning and disinfection at slaughterhouses can affect human health is the high spike in human cases of campylobacteriosis in Sweden in 2016-2017, which was caused by insufficiently cleaned transport crates for chickens.

The aim of this thesis was to obtain better knowledge of how the occurrence and spread of bacteria in slaughterhouses and meat processing premises can be reduced, and to provide advice on this issue, by evaluating

the efficacy of various cleaning and disinfection procedures on equipment, the slaughterhouse environment and carcasses. In addition to detection and measuring the amount of bacteria present in different areas, comparison of different sampling methods through analyses for residual bacteria and organic material was used to evaluate whether surfaces were sufficiently clean. Swedish slaughterhouses were also interviewed regarding their cleaning and disinfection routines and the sampling methods they use to assess surface cleanliness.

The results showed that the cleaning and disinfection procedures used in slaughterhouses were sufficient regarding removal of pathogenic bacteria such as *L. monocytogenes* and *Campylobacter*. However, not all surfaces were sufficiently cleaned and disinfected, as high levels of hygiene indicator bacteria were detected even on surfaces that were visibly clean. It was also observed that surfaces in the processing area were more properly cleaned and disinfected than those in the slaughter area. A cause of concern was that *L. monocytogenes* and *Campylobacter* were detected on surfaces that were in direct contact with meat before cleaning and disinfection. When bacteria are present on such surfaces, there is a high risk of the meat being contaminated with these bacteria during processing and further along in the production chain, causing disease in the consumer. Extended-spectrum beta-lactamase (ESBL)-producing *E. coli*, which is known to cause antibiotic resistant infections in humans was not detected in any of the slaughterhouses studied. The results also showed that rapid tests (dipslides, ATP-bioluminescence) for evaluating surface cleanliness, which are commonly used by Swedish slaughterhouses and meat processing premises, but also by others such as food businesses and hospitals for humans and animals, varied in reliability.

Interviews with quality assurance managers at slaughterhouses revealed that they did not know whether the cleaning and disinfection methods they used were the most appropriate for their facilities. They had not performed a risk analysis, which is necessary when deciding on a threshold for assessing whether a surface is sufficiently clean. Instead, they relied on suggestions from external companies. The quality assurance managers also reported a lack of science-based guidelines to assist them in deciding which cleaning and disinfection procedures to use.

Evaluation of new technologies, such as 265 nm UV-C LED irradiation of transport crates for chickens and a combination of ultrasound and steam

followed by chilling for broiler carcasses, revealed significant bacterial reductions. However, these techniques require a certain time of action, which is difficult to combine with high slaughter speed.

In conclusion, this thesis showed that current cleaning and disinfection procedures in slaughterhouses and meat processing premises do not always reduce bacteria to an acceptable level.

Populärvetenskaplig sammanfattning

Korrekt rengöring och desinfektion på slakterier och styckningsanläggningar är en förutsättning för produktion av säkra livsmedel av hög kvalitet. Det bör göras på ett sätt som minimerar förekomst av sjukdomsframkallande och produktförstörande bakterier. Alla slakterier och styckningsanläggningar måste rengöras och desinficeras dagligen, vilket kan vara en utmaning i dessa ofta våta miljöer med mycket utrustning. Det höga tempot under slaktprocessen ökar också risken för förorening av köttet med bakterier. Även om många rengörings- och desinfektionsmetoder finns tillgängliga så avlägsnar de inte alla bakterier från ytor. Det kan förekomma både sjukdomsframkallande och livsmedelsförstörande bakterier på ytor som kommer i kontakt med köttet efter rengöring och desinfektion. Detta innebär en risk för att dessa bakterier kontaminerar köttet, vilket påverkar såväl om maten är säker att äta samt försämrar hållbarheten. Vissa bakterier såsom *Listeria monocytogenes* kan bilda så kallad biofilm, vilket är flera lager av bakterier i ett nätverk som har fäst sig till en yta och till varandra. Biofilm skyddar bakterierna och är svåra att avlägsna med rengöring och desinfektions processer. För de som drabbas av sjukdomen listerios krävs ofta behandling på sjukhus och dödligheten är hög, särskilt hos riskgrupper, såsom äldre, nyfödda och andra med försvagat immunförsvar. Den kan också orsaka missfall hos gravida kvinnor. Ett annat exempel på hur bristande rengöring av utrustning på slakterier har påverkat människors hälsa var det kraftigt förhöjda antalet fall av sjukdomen campylobacterios i Sverige 2016-2017, orsakat av dåligt rengjorda transportlådor för kyckling.

Syftet med avhandlingen var att få ökad kunskap och därmed kunna ge råd om hur förekomst och spridning av bakterier på slakterier och styckningsanläggningar kan minskas genom att utvärdera effekten av olika rengörings- och desinfektionsprocesser på utrustning, i slakterimiljön och på slaktkroppar. I studien ingick en jämförelse mellan olika provtagningsmetoder som används för att få en uppfattning om en yta är tillräckligt ren eller inte, genom påvisande av kvarvarande bakterier och

organiskt material. Förutom att påvisa och räkna antalet bakterier, så intervjuades personal på svenska slakterier och styckningsanläggningar gällande deras rutiner för rengöring och desinfektion. I intervjuerna framkom vilka provtagningsmetoder som användes för att utvärdera rengöringseffekten på ytor. Dessa metoder jämfördes sedan i en separat studie.

Resultaten visade att rengörings- och desinfektionsmetoderna för ytor var tillräckliga gällande avlägsnande av sjukdomsframkallande bakterier såsom *L. monocytogenes* och *Campylobacter*. Däremot var inte alla ytor tillräckligt rena, då antalet bakterier var över de gränsvärden som används för bedömning av god hygien, även på ytor som såg rena ut. På båda slakterierna uppmärksammades också att ytorna i styckningsdelen var mer ordentligt rengjorda än i slakteridelen. De var dock oroande att de sjukdomsframkallande bakterierna *L. monocytogenes* och *Campylobacter* återfanns på produktkontaktytor innan rengöring. När bakterier förekommer på sådana ytor, finns en hög risk för förorening av köttet med dessa bakterier, som i ett senare skede kan orsaka sjukdom hos konsumenten. Inga extended-spectrum beta-lactamase (ESBL) producerande *E. coli*, vilka kan orsaka antibiotikaresistenta infektioner hos människor, hittades på ytor.

Studier utfördes av snabbtester för rengöringskontroll såsom tryckplattor och ATP-bioluminescence, vilka är vanligt förekommande på svenska slakterier och styckningsanläggningar, andra livsmedelsföretag och sjukhus för både människor och djur. Resultaten visade dock att dessa metoder var av varierande tillförlitlighet.

Intervjuerna med kvalitetscheferna på slakterierna med tillhörande styckningsanläggningar avslöjade att slakterierna inte visste ifall de rengörings- och desinfektionsmetoder de använder är de mest adekvata. De använde sig inte av riskanalys, som är ett redskap som bör användas när man sätter gränsvärden för hur mycket bakterier och organiskt material som kan accepteras på en rengjord yta. Istället använde de gränsvärden som föreslagits av externa företag. Kvalitetscheferna uttryckte också avsaknad av vetenskapsbaserade riktlinjer som skulle kunna användas för att välja den mest lämpliga rengörings- och desinfektions metoden, som t.ex. vilka kemiska produkter och appliceringsmetoder samt lämpliga gränsvärden för tillräckligt rengjorda ytor.

I avhandlingen ingick även utvärderingar av nya desinfektionstekniker såsom UV-C LED strålning för desinfektion av transportlådor som används för att transportera kycklingar från gården till slakteriet. Dessutom undersöktes om en kombination av ultraljud-vattenånga samt kylning av kycklingslaktkroppar signifikant kunde minska antalet bakterier. Dessa

tekniker visade på signifikanta reduktioner av bakterier, de kräver dock en viss verkningstid vilket kan vara svårt att kombinera med ett högt tempo vid slakt.

Sammanfattningsvis visade avhandlingen att nuvarande rengörings- och desinfektionsprocesser på slakterier och styckningsanläggningar inte alltid minskar mängden bakterier till en acceptabel nivå.

Acknowledgements

The work presented in this thesis was carried out at the Department of Biomedical Sciences and Veterinary Public Health (BVF) at the Swedish University of Agricultural Sciences (SLU) in Uppsala. This project was funded by the **Swedish Farmers' Foundation for Agricultural Research** and the **Royal Swedish Agricultural Academy**. It was also supported by the **Elsa and Ivar Sandberg Foundation of Sweden**.

I have had the honour to have knowledgeable and supportive supervisors.

To my main supervisor, **Ingrid Hansson**, thank you sharing your expertise and hands-on knowledge about bacteria. I also very much appreciate you helping me in the lab and with the late night samplings in the slaughterhouses.

To my co-supervisor, **Sofia Boqvist**, thank you for being available during busy times, being positive and giving me very useful advice.

To my co-supervisor and head of department, **Ivar Vågsholm**, thank you for making this PhD possible and for helping me to see the bigger picture.

To my co-supervisor, **Sara Frosth**, thank you for helping with the sequencing part of the work and for seeing things from a different angle.

Thank you to my co-authors from the Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima), **Trond Møretrø** and **Solveig Langsrud**, for sharing your expertise within the field of environmental bacteria in the food industry and answering all my questions.

Thanks to my co-author and colleague **Emma Bergenkvist** for driving me to slaughterhouses and for being a helpful companion in the lab.

To **Lise-Lotte Fernström, Moa Skarin, Helena Ljung** and **Marina Falk,** thank you for helping me in the lab and answering my complicated questions. Special thanks to **Lise-Lotte** for your creative solutions and instant feedback to my endless questions.

To the **slaughterhouse staff**, thank you for great collaborations. Special thanks to the poultry slaughterhouse for helping me with logistics and sampling during long nights.

Thank you to the former head of department, **Bengt Guss,** and the present head of department, **Eva Tydén,** for always being supportive and giving me helpful advice during this journey.

To **Susanna Sternberg Lewerin,** thank you for explaining the epidemiological aspects of this work and for the peptalks.

Thanks to **Hedvig Gröndal** for giving me advice about how to handle data from the interview study.

Thanks to **Claudia von Brömssen** and **Jesper Rydén** from the Centre for Statistics for being available and kindly answering all my questions.

To the members of the National Network for Teachers in Food Microbiology, **Anna, Jenny, Marie-Louise, Roger, Stina-Mina, Su-Lin** and **Ingela**, thank you for all the inspiring words and for reassuring me that everything will be fine. You are a real safety net!

Thank you to all my colleagues at BVF for making it fun to go to work. Thank you to my corridor neighbours, **Behdad** and **Krista,** for nice corridor and lunch talks. Special thanks to my food safety colleagues **Jakub, Karin, Emma** and **Olga** for being so kind and building me up when I doubted myself. I could not have done without you!

Thank you to my **family** for always believing in me. Special thanks to my mother **Aila** for her commitment and for the cute front page illustration.

Thank you to all my **friends** for being there for me! Special thanks to **Jacaranda**, for saying the right things at the right time.

Finally, thank you **Daniel**, for being by my side and being so loving and understanding. I am so grateful to have you in my life!

Appendix I

Interview questions for quality assurance managers at slaughterhouses

1. What is your educational background?
2. What animal species are slaughtered in your facility?
3. How many animals are slaughtered each day?
4. Who performs cleaning and disinfection in the slaughterhouse/meat processing premises? (external/internal cleaning staff)

<u>Detergents:</u>

5. Which agents are used?
6. At what concentration are they diluted in water?
7. How long is the contact time, *i.e.* how long are the products left to act before they are rinsed away?
8. What is the temperature of the water used for cleaning?
9. How are the agents applied on surfaces?

<u>Disinfectants:</u>

10. Which agents are used?
11. At what concentration are they diluted in water?
12. How long is the contact time, *i.e.* how long are the products left to act before they are rinsed away?
13. What is the temperature of the water used for cleaning?
14. How are the agents applied on surfaces?

<u>Monitoring activities:</u>

15. Which sampling methods are used to evaluate cleaning and disinfection efficacy?
16. With what frequency are these sampling methods performed?
17. How did you select the microbial and non-microbial threshold/s for clean surfaces?
18. Which surfaces do you think are the most difficult to clean?
19. What difficulties and challenges have you experienced in relation to cleaning and disinfection?

I

Journal of Food Protection 86 (2023) 100155

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Assessment of ATP-Bioluminescence and Dipslide Sampling to Determine the Efficacy of Slaughterhouse Cleaning and Disinfection Compared with Total Aerobic and *Enterobacterales* Counts

Madeleine Moazzami [1,*], Emma Bergenkvist [1], Sofia Boqvist [1], Sara Frosth [1], Solveig Langsrud [2], Trond Møretrø [2], Ivar Vågsholm [1], Ingrid Hansson [1]

[1] *Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden*
[2] *Norwegian Institute of Food, Fishery and Aquaculture Research, N 1430 s, Norway*

ARTICLE INFO

Keywords:
ATP
Dipslide
Enterobacterales
Indicator bacteria
Meat processing
Total aerobic bacteria

ABSTRACT

Inadequate cleaning and disinfection (C&D) in slaughterhouses can cause bacterial contamination of meat, resulting in foodborne disease and reduced meat quality. Different methods for monitoring the efficacy of C&D procedures are available, but few studies have assessed their reliability. This study examined C&D efficacy in slaughterhouses and evaluated the diagnostic performance of methods for measuring surface hygiene.

One red meat and one poultry slaughterhouse in Sweden were each visited on six occasions before and six occasions after C&D. Sampling points were sampled with: swabbing and plating for total aerobic bacteria (TAB) and *Enterobacterales* (EB); dipslides for total viable count; and ATP-bioluminescence tests. To evaluate the diagnostic performance of the dipslide and ATP-bioluminescence methods, the results were compared with (TAB) as a reference.

In total, 626 samples were collected. For the majority of samples, TAB was lower after than before C&D and EB were mainly detected before C&D, indicating C&D efficacy. Greater reductions in mean TAB were observed in processing areas (2.2 and 2.8 log CFU/100 cm^2 in red meat and poultry slaughterhouse, respectively) than in slaughter areas (1.3 log CFU/100 cm^2 in both slaughterhouses). Approximately half of all samples were assessed as non acceptably clean (52% for red meat and 46% for poultry slaughterhouse) according to previously published thresholds. Critical food contact surfaces that were insufficiently cleaned and disinfected were plucking fingers, shackles, and a post-dehairing table. Cleaning and disinfection of drains and floors were inadequate.

The ATP-bioluminescence method showed low specificity compared with the reference (TAB) in both the red meat (0.30) and poultry slaughterhouses (0.64). The sensitivity of dipslides was low (0.26) in the red meat slaughterhouse compared with TAB. A combination of ATP-bioluminescence and dipslides could provide more accurate estimates of C&D efficacy.

Food legislation within the European Union (EU) requires cleaning and disinfection (C&D) of surfaces in direct contact with food products (food contact surfaces, FCS) and non food contact surfaces (NFCS), including processing equipment on food premises (EC, 2004; Ninios et al., 2014). Sterilization is not achieved by C&D, so low microbial load can be expected on surfaces after C&D (Stanga, 2010). A threshold for satisfactory microbial hygiene on FCS or NFCS after C&D is not defined in EU legislation, meaning that food business operators (FBO) must decide their thresholds based on hazard analysis and critical control points (HACCPs), and good hygiene practices (GHPs) (Codex Alimentarius, 2020). A low microbial load minimizes the risk of cross-contamination, spread, and multiplication of pathogenic and food spoilage bacteria, that could have detrimental effects on public health and decrease the shelf-life of food. The slaughterhouse environment can easily be contaminated with organic debris such as fecal mat-

Abbreviations: C&D, Cleaning and disinfection; EB, *Enterobacterales*; TAB, Total aerobic bacteria.

* Corresponding author.
E-mail address: madeleine.moazzami@slu.se (M. Moazzami).

https://doi.org/10.1016/j.jfp.2023.100155
Received 15 June 2023; Accepted 28 August 2023
Available online 1 September 2023
0362-028X/© 2023 The Author(s). Published by Elsevier Inc. on behalf of International Association for Food Protection.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

ter and other body fluids. Therefore, C&D is an important hurdle in the control of foodborne pathogens and spoilage bacteria.

The active components in detergents used for C&D in red meat and poultry slaughterhouses are commonly alkaline compounds with or without chlorine (i.e., sodium hypochlorite, sodium hydroxide, potassium hydroxide), which are recommended to be used alternately with acidic compounds (i.e., peracetic acid, phosphoric acid, sulfuric acid). The active components in disinfectants are very similar to those in detergents (i.e., sodium hypochlorite, sodium hydroxide, alternated with peracetic acid, acetic acid, hydrogen peroxide). Instead of using alkaline or acidic agents as disinfectants, quaternary ammonium compounds are commonly used (García-Sánchez et al., 2017; Hutchison et al., 2007; Khamisse et al., 2012; Wang et al., 2018). When disinfectants are used in food production premises, the antimicrobial efficacy can be affected by various environmental factors such as temperature, humidity, surface materials, and residues of organic substances. However, standardized laboratory tests on the antimicrobial effect of disinfectants are usually performed in controlled conditions using suspension tests, and on surfaces that are easy to clean such as stainless steel, which is flat and rather resistant to scratching (SS-EN 14349:2012; SS-EN 1276:2019). Different types of surface materials are used in meat processing premises, and scratches and cracks that develop after a period of use can become harborage sites for bacteria (Fagerlund et al., 2017). These resident bacteria become part of the in-house microbiota, which is not removed by common C&D procedures and adds to continuous cross-contamination of food, thereby posing a threat to food safety and quality (Fagerlund et al., 2016; García-Sánchez et al., 2017). Moreover, pathogenic bacteria may persist on surfaces due to insufficient C&D, leading to outbreaks of foodborne diseases. For example, an outbreak with five-fold higher annual levels of campylobacteriosis occurred in Sweden in 2016–2017 (Lofstedt, 2019), caused by inadequate cleaning of chicken transport crates. *Listeria monocytogenes* is also well known to cause outbreaks due to its ability to form biofilm, survive in food production environments, and resist C&D procedures (Fagerlund et al., 2017; Stephan et al., 2015). Other important pathogens of concern in slaughter and carcass processing are *Salmonella enterica* and Shiga toxin-producing *E. coli* (STEC). *Salmonella enterica* has been recovered from cleaned and disinfected surfaces in swine and poultry slaughterhouses (Arguello et al., 2012; Marin et al., 2022), while STEC has been detected on surfaces in cattle slaughterhouses after C&D (Brusa et al., 2021; Tutenel et al., 2003).

A concern for slaughterhouse FBOs is the risk of cross-contamination of products by meat spoilage bacteria, i.e., *Pseudomonas* spp., *Acinetobacter* spp., *Stenotrophomonas* spp., and bacteria belonging to the order *Enterobacterales* (EB, a bacterial order which formerly only included family *Enterobacteriaceae*). Spoilage bacteria have been detected on surfaces such as conveyor belts and cutting tools after C&D (Maes et al., 2017, 2019; Møretrø et al., 2013; Møretrø & Langsrud 2017; Wang et al., 2018). There are indications that the microbial population of the slaughterhouse environment affect the microbial load on carcasses more than the indigenous microbiota of the slaughtered animal, and spoilage bacteria on meat have been traced back to contaminated surfaces in the slaughterhouse due to inadequate C&D (Peruzy et al., 2021; Samapundo et al., 2019).

Total aerobic bacteria (TAB) and EB can be used as indicators of the hygiene status in meat processing plants, while EB can be used as an indicator of fecal contamination in slaughterhouses (Althaus et al., 2017; Hutchison et al., 2007). A study in the UK found that almost one-third of 94 red meat slaughterhouses failed to meet the specified measured TAB threshold for acceptably clean surfaces (2 log CFU/cm^2) (Hutchison et al., 2007). Different methods for monitoring the efficacy of C&D procedures are available, including ATP-bioluminescence, contact plates (dipslides), and swabbing and plating of sponge/swab samples (Maes et al., 2017; Moore & Griffith, 2002; Møretrø et al., 2019). Methods such as ATP-bioluminescence and dipslides are easy to use compared with swabbing and plating. The EU standard (SS-EN ISO 18593:2018) for surface sampling does not specify sampling frequency and sampling points, which are therefore selected based on risk-based principles. Additionally, the standard mentioned above only describes two sampling methods, swabbing and dipslides, and not the ATP-bioluminescence method, which is widely used by the industry.

Several studies have investigated bacterial contamination on carcasses in slaughterhouses (Hansson et al., 2010; Hauge et al., 2023; Lindblad et al., 2006; Moazzami et al., 2021; Peruzy et al., 2021). However, there still seems to be a gap in knowledge concerning different hygiene indicator bacteria on different environmental surfaces and equipment. Additionally, there is a lack of published studies comparing different hygiene monitoring methods in industrial settings. Therefore, the aims of the present study were to determine the efficacy of C&D, and to evaluate the diagnostic performance of methods for assessing surface hygiene in slaughter areas and adjacent meat processing areas.

Materials and Methods

Study design. Two Swedish slaughterhouses were included in the study: a small/medium-scale red meat slaughterhouse, slaughtering approximately 100–120 swine and 25 cattle per day, and a large-scale poultry slaughterhouse, slaughtering approximately 220,000 broilers per day, both with adjacent areas processing raw meat (including cutting, meat preparation, and packaging facilities). Both slaughterhouses use a rotation of alkaline and acidic chemicals for C&D, and a low-pressure water pump (approximate pressure 28–35 bar) for their application during C&D (Fig. 1). Neither of the slaughterhouses uses forced ventilation to dry surfaces after C&D. Each slaughterhouse was visited on six occasions before C&D, and on six occasions after C&D. All visits before C&D were made after the end of the last working shift, which was immediately before C&D (Monday afternoon/evening), and all the visits after C&D were made before the start of the morning shift (Tuesday morning). Sampling was carried out from October 2020 to October 2021.

Identification of sampling points. The quality assurance staff at the slaughterhouses were involved in the selection of sampling points. When practically possible, surfaces known to be difficult to clean and/or critical due to potential cross-contamination of the meat were selected as sampling points. Both FCS and NFCS, including scald water, were selected for sampling, with 11 sampling points in the red meat slaughterhouse (6 in the slaughter area, 5 in the processing area) and 10 sampling points in the poultry slaughterhouse (5 in the slaughter area, 5 in the processing area). In the red meat slaughterhouse, cattle and swine were slaughtered in the same slaughter area, while only beef was handled in the processing area. All sampling points were sampled on each sampling occasion, before and after C&D procedures.

Sampling procedure and sample analysis. On each sampling occasion, each sampling point, except scald water, was sampled with three different methods: swabbing with sponge (Hydra-Sponge 1.5*3 inches Sponge w/10 mL Letheen broth, 3M Health Care, St. Paul, USA)/swab (Swab-sampler with 10 mL D/E Neutralizing broth, 3M Health Care, St. Paul, USA), dipslide (Envirocheck® Contact TVC, Merck KGaA, Darmstadt, Germany), and ATP-bioluminescence tests (Surface ATP/Water-Free ATP, Clean-Trace™ Test, 3M Health Care, St. Paul, USA), on surfaces adjacent to each other (Fig. 2). Sampling was performed aseptically. When possible on flat surfaces, sterilized stainless steel frames were used to delineate an exact sampling area. For practical reasons, different size frames were used for swabbing with sponge/swab (100 cm^2) and ATP-bioluminescence (25 cm^2). For sampling points with smaller areas, 25 cm^2 were swabbed with sponge/swab. Five plucking fingers, one shackle, and five salt injector needles were sampled on each occasion, and the total sampling area

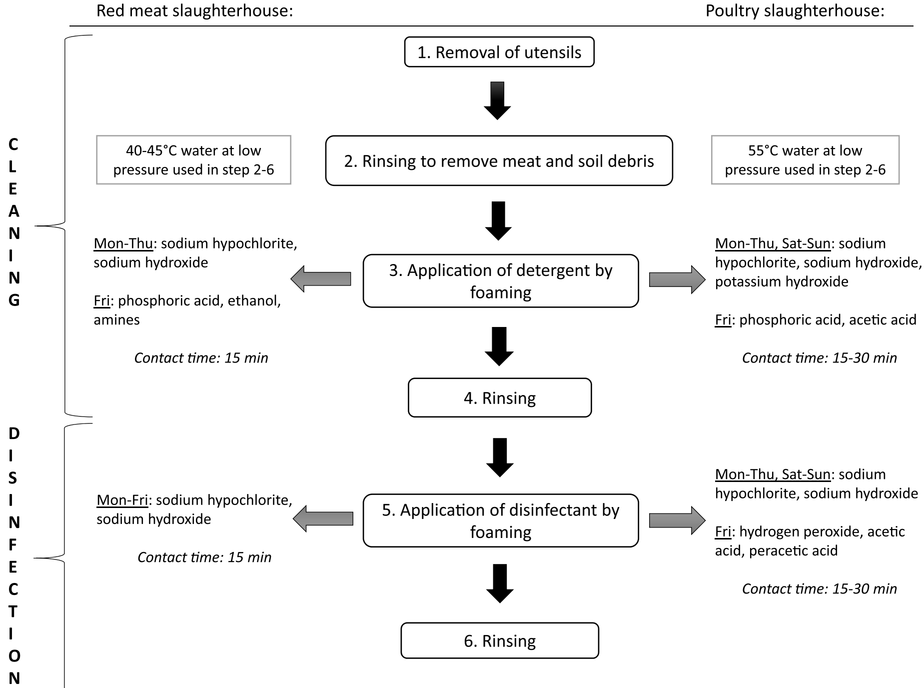


Figure 1. Flow chart of the general cleaning and disinfection procedures at the two slaughterhouses included in this study.

was estimated to be 100 cm^2, 25 cm^2, and 25 cm^2, respectively). The area of the dipslides was always 19 cm^2. The same individuals performed all samplings during the study. The temperature of the scald water was measured on one sampling occasion in each slaughterhouse.

After each sampling, the sponges, swabs, dipslides, and scald water were transported in an insulated box with refrigerant gel packs to the Biomedical Sciences and Veterinary Public Health laboratory at the Swedish University of Agricultural Sciences, Uppsala, Sweden. The temperature was checked upon arrival. Only samples with temperature 2–8°C were accepted for analysis, which began within 12 h after sampling.

Swabbing and plating. The prehydrated sponges (7.6 by 4 cm) were used for swabbing and enumeration of TAB and EB (Fig. 2A). For practical reasons, cutting blades and salt injector needles were sampled using a prehydrated swab sampler (Fig. 2B). Swabbing was performed using firm and even pressure, with overlapping horizontal and vertical strokes. Approximately 45 mL of scald water was collected in a sterile plastic bottle from the upper part of the scald water tank, before C&D (directly after slaughter finished) and after C&D (immediately before the next slaughter shift started).

In the laboratory, sponges were homogenized for 120 s at 240 rpm (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, New Jersey, USA), while swabs and scald water were vortexed for approximately 10 s. From each sponge/swab/scald water sample, a 10-fold serial dilution in 0.1% (v/v) peptone water (Dilucups, LabRobot Products AB, Stenungsund, Sweden) was prepared. TAB were enumerated according to NMKL 86 (5th Ed. 2013). From the dilution series prepared for each sample, 1.0 mL aliquots of each dilution were plated on an aerobic count plate (3M Petrifilm™, 3M Health Care, St. Paul, USA) and left to solidify. Plates were then incubated at 30 ± 1 °C for 72 ± 3 h. Bacterial counts were preferably performed on plates with 25–250 colonies and expressed as log CFU/100 cm^2. The detection limit was 1.0 log CFU/area sampled, or for scald water 1.0 log CFU/mL.

Analysis of EB was performed according to NMKL 144 (3rd Ed. 2005). The previously prepared 10-fold dilutions were used to estimate EB counts in the samples. From each dilution, 1.0 mL was plated on an *Enterobacteriacae* count plate (3M Petrifilm™, 3M Health Care, St. Paul, USA) and left to solidify. Plates were then incubated at 37 ± 1 °C for 24 ± 2 h. Bacterial counts were preferably performed on plates with 15–150 colonies, and the number of EB was expressed as log CFU/100 cm^2. The detection limit was 1.0 log CFU/area sampled, or for scald water 1.0 log CFU/mL.

Dipslide method. The dipslide test for total viable count (TVC) was pressed firmly and evenly onto the surface to be sampled, and then turned over and the second side was pressed in the same way next to the first sampling site (Fig. 2C). After transport to the laboratory, the dipslides were incubated in upright position at 37 ± 1 °C and checked for growth after 48 ± 4 h. Colonies on both sides of the dipslide (19 cm^2) were counted, and the TVC was expressed as log CFU/100 cm^2.

ATP-bioluminescence method. To determine the level of cellular material on surfaces, adenosine triphosphate (ATP)-bioluminescence was used. The ATP level in scald water was measured with Water-Free ATP tests. Before use, Surface ATP and Water-Free ATP tests were kept in foil pouches to protect the ATP reagent from light and stored at 2–8°C. Approximately 24 h before sampling, they were moved to room temperature (around 20°C) according to the manufacturer's instruc-

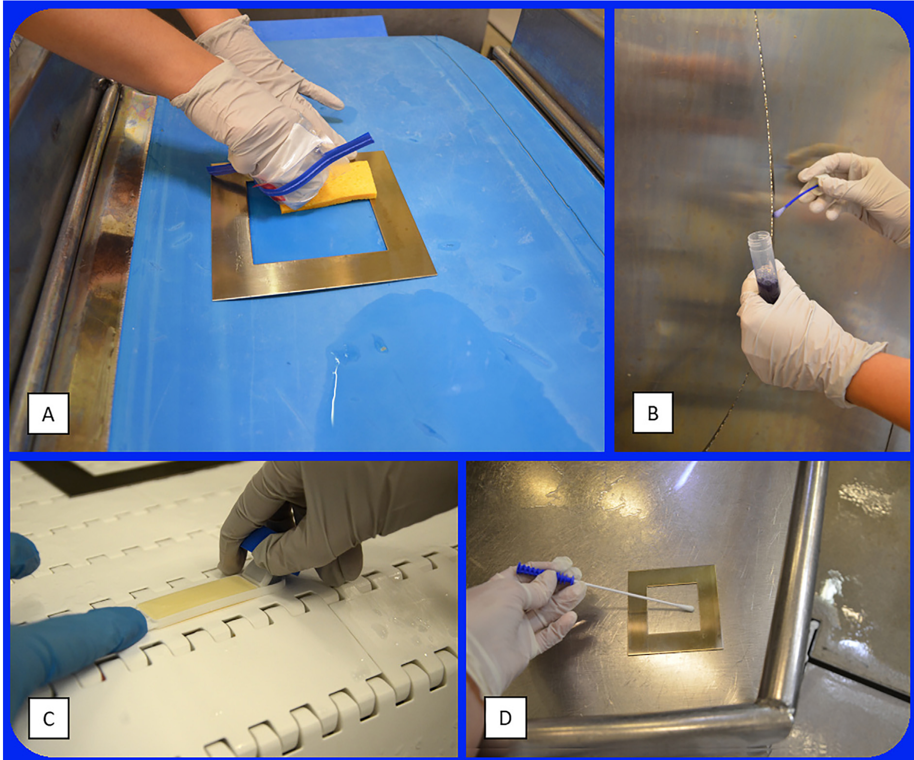


Figure 2. A) Swabbing conveyor belt with prehydrated sponge, B) swabbing cutting blade with prehydrated swab, C) pressing dipslide on conveyor belt and, D) swabbing post-dehairing table with ATP-swab.

Table 1
Selected thresholds for clean surfaces regarding total aerobic bacteria (TAB), total viable count (TVC), ATP-bioluminescence (relative light units, RLU), and *Enterobacterales* (EB)

	Selected threshold/ cm^2	Source
TAB - swabbing & plating	2.5 CFU	Ching et al. (2021); Griffith, 2005; Ninios et al. (2014)
TVC- dipslides	1.0 CFU	Eurofins Food & Feed Testing Sweden AB (2021), based on Swedish Food Agency (1998)
ATP - bioluminescence	1.5 RLU	Technical bulletin 3M^{TM} Clean-Trace ^{TM} Hygiene Monitoring and Management System (2019)
EB - swabbing & plating	1.0 CFU	Gómez et al. (2012); Statutory Instruments (2002). The meat (Hazard Analysis Critical Control Point) (England) Regulations 2002

tions. Each surface was swabbed with firm and even pressure, using overlapping horizontal and vertical strokes, and at the same time, the swab was rotated over its own axis (Fig. 2D). Water ATP swabs used to measure the ATP levels in scald water were immersed completely under the liquid surface and shaken gently to remove possible air bubbles. ATP levels were measured within 2 h of sampling by placing the swabs into the ATP monitoring device (Clean-Trace LM1, 3M Health Care, St. Paul, USA). Results were recorded as relative light units (RLU) within the system device range of 0–6.0 log RLU. Surface ATP test results were expressed as log RLU/100 cm^2 and Water-Free ATP test results as log RLU/145 µL ± 15 µL (the volume of liquid tested).

Thresholds for clean surfaces. To assess whether the estimated number of bacteria or organic residues remaining on a surface after C&D was acceptable, thresholds were selected for each sampling method/bacterial group (Table 1). Due to lack of thresholds for clean surfaces in current legislation, selected values were first chosen from instructions from producers of the sampling materials used in the study (3M Science Applied to Life, 2019; Eurofins Food & Feed Testing Sweden AB, 2021). If such instructions were lacking, thresh-

olds from the literature and in proposed legislation were used (Ching et al., 2021; Gómez et al., 2012; Griffith, 2005; Ninios et al., 2014; Statutory Instrument, 2002; Swedish Food Agency, 1998). The thresholds used routinely in the two slaughterhouses were also considered.

Statistical analysis. To evaluate the reduction in bacterial and ATP levels before and after C&D, R Studio software (RStudio version 1.2.1335) was used. To enable comparison of the methods, the results were transformed into CFU/RLU per 100 cm^2. The values were $\log_{10}$-transformed and modeled using Anova. The factors sampling point, occasion, and before/after were fixed factors in the model. Post hoc tests were performed to determine significantly different mean levels of bacteria before and after C&D for different sampling points, using Tukey's adjustment. Residuals were checked to confirm that they fulfilled the assumption of normal distribution and equal variances. Differences between mean values before and after C&D were deemed significant at $P < 0.05$.

To evaluate the diagnostic performance of the dipslide and ATP-bioluminescence methods, swabbing and plating for cultivation of TAB was used as a reference method. Results were only included in the comparison of methods when all three sampling methods were successfully conducted at the same sampling point on the same sampling occasion. The sensitivity and specificity calculations were according to Bonita et al. (2006).

Values were considered *true positives* when results from the reference method and the dipslide/ATP-bioluminescence methods indicated non acceptable level of cleanliness. Values were considered *true negatives* when results from the reference method and the dipslide/ATP-bioluminescence methods were considered to indicate acceptable cleanliness. Values were considered *false positives* when acceptably clean according to the reference method but non acceptable according to the dipslide/ATP-bioluminescence methods. Finally, values were considered *false negatives* when not acceptably clean according to the reference method, but acceptable according to the dipslide/ATP-bioluminescence methods. Indicators of diagnostic performance (accuracy, Ac; sensitivity, Se; specificity, Sp; positive predictive value, PPV; negative predictive value, NPV; Cohen's kappa agreement coefficient, κ) were calculated using the statistical software MedCalc Software Ltd (Ostend, Belgium). For Cohen's kappa coefficient, a value of $\kappa > 0.9$ indicates almost perfect agreement, values between 0.8 and 0.9 indicate strong agreement, from 0.6 to 0.79 moderate agreement, from 0.4 to 0.59 weak agreement, from 0.21 to 0.39 minimal agreement, and from 0 to 0.2 no agreement (McHugh, 2012).

Results

In total, 626 samples were collected before ($n = 313$) and after ($n = 313$) C&D procedures (these samples included scald water, the results for which are presented separately). All sampling points could not be sampled on all occasions, (e.g., when the cleaning staff started to clean earlier than planned). Each sampling point was sampled on 4–6 occasions before and 4–6 occasions after C&D. All samples had a temperature of 2–8°C on arriving at the laboratory and were accepted for analysis. In both slaughterhouses, most surfaces were visually clean after C&D, but traces of feces, feathers, meat, fat, etc. were observed on some surfaces. The majority of the surfaces were wet at the time of sampling, especially after C&D.

Total aerobic bacteria. In the red meat slaughterhouse, TAB could be enumerated from 98% (57/58) of the samples taken before C&D and 90% (52/58) of the samples taken after C&D. For the majority (90%) of the samples after C&D, the bacterial numbers were lower than in the corresponding samples before C&D. However, 10% (6/58) of the samples had TAB levels 0.1–2 log higher after C&D, representing samples from the post-dehairing table, the drain in the processing area, and the soft conveyor belt (3/6, 2/6, 1/6 samples, respectively). The greatest mean reduction (>3.0 log CFU/100 cm^2) was recorded for the cutting board. Overall, for 52% (30/58) of the samples taken after C&D from the red meat slaughterhouse, the values were above the selected threshold for clean samples (2.5 CFU/cm^2, equal to 2.4 log CFU/100 cm^2) (Table 1). For FCS, the values were above this threshold for 40% (14/35) of the samples. The highest mean values after C&D were observed for the drains, the post-dehairing table, and the conveyor belt for pig organs, with the highest individual bacterial count in one sample from the post-dehairing table (6.9 log CFU/100 cm^2). The table for cattle organs had the lowest mean value after C&D (Table 2).

In the poultry slaughterhouse, TAB could be enumerated from all samples (48/48) taken before C&D and 73% (35/48) of samples taken after C&D. For the majority (92%) of the samples, the bacterial numbers were lower after C&D than in the corresponding samples before C&D, but for 8% (4/48) of the samples, the values were 0.1–1.3 log higher after C&D. These samples were from the shackles and lairage floor (3/5 and 1/5 samples, respectively). The strongest C&D effect was seen for the salt injector needles (>3.0 log reduction). Overall, 46% (22/48) of the samples from the poultry slaughterhouse were non acceptably clean (>2.4 log CFU/100 cm^2). All samples of the plucking fingers and shackles were non acceptably clean after C&D. For FCS, 35% (13/37) were assessed as non acceptably clean. The highest mean values after C&D were observed for the plucking fingers, shackles, lairage floor, and drain, where one sample from the lairage floor had the highest individual bacterial count (7.0 log CFU/100 cm^2). The lowest mean values after C&D were observed for conveyor belts, cutting blade, and salt injector needles (Table 2).

In terms of C&D efficacy at the two slaughterhouses, the reduction in mean TAB at the red meat slaughterhouse was 1.3 and 2.2 log CFU/100 cm^2 in the slaughter and processing area, respectively. The corresponding reductions in the poultry slaughterhouse were 1.3 and 2.8 log CFU/100 cm^2, respectively. Moreover, the conveyor belts in the poultry slaughterhouse had lower bacterial numbers after C&D than the conveyor belts in the red meat slaughterhouse. A greater reduction after C&D was observed in the processing areas, except the drains, and the processing areas had more acceptably clean samples compared with the slaughter areas in both slaughterhouses. In the red meat slaughterhouse, the conveyor belts located in the processing area had a higher number of acceptably clean samples and greater TAB reductions than the conveyor belt located in the slaughter area. In the poultry slaughterhouse, TAB could be enumerated after C&D on the cutting blade in the slaughter area in 4/5 samples, but on the cutting blade in the processing area in only 2/6 samples. Furthermore, the mean TAB count after C&D was higher for the cutting blade in the slaughter area than for the cutting blade in the processing area (1.6 and 0.8 log CFU/100 cm^2, respectively), even though these sampling points were very similar and the same C&D procedure was used. The drains (in both slaughter and processing areas) and lairage floor were non acceptably clean on most sampling occasions in both slaughterhouses. The TAB reduction seen for two drains, one in the red meat slaughterhouse (sampling point 5) and one in the poultry slaughterhouse (sampling point 21), was 2.4 log and 2.3 log, respectively, which were among the greatest reductions observed in this study. However, since the mean TAB values before C&D were very high (5.9–6.0 log CFU/100 cm^2), high bacterial levels still remained after the C&D procedure (Table 2).

Total viable count-Dipslide. In the red meat slaughterhouse, TVC could be enumerated from 96% (52/54) of the dipslides before C&D and from 35% (19/54) of the dipslides after C&D. A total reduction in TVC on all dipslides was observed for the conveyor belts and the trolley in the processing area. In 7% (4/54) of the dipslides, the values were 0.2–1.3 log higher after C&D than on the corresponding dipslides before C&D, representing dipslides from the post-dehairing table and the cutting blade for carcasses (3/5, and 1/5 dipslides respectively). In the slaughter area, the post-dehairing table, the drain, and the cutting blade were considered nonacceptably clean (4/5, 1/5, and

Table 2
Mean log CFU/100 cm^2 of total aerobic bacteria (determined by swabbing and plating), log CFU/100 cm^2 of total viable count (on dipslides) and log RLU/100 cm^2 of ATP-bioluminescence before and after cleaning and disinfection (C&D), mean reduction after C&D and percentage of acceptably clean samples according to selected thresholds. Scald water bacterial count was measured as log CFU/mL for TAB and TVC, and as RLU/145 µL for ATP-bioluminescence

				Total aerobic bacteria				Total viable count, dipslides				ATP-bioluminescence			
Slaughterhouse	Area		Sampling point	Mean before C&D	Mean after C&D	Mean reduction	% acceptable samples	Mean before C&D	Mean after C&D	Mean reduction	% acceptable samples	Mean before C&D	Mean after C&D	Mean reduction	% acceptable samples
Red meat	**Slaughter**	**1**	Post-dehairing table pigs, stainless steel (upper part)[b]	4.0 ± 0.6	4.0 ± 1.7	0.001 ± 1.1	17	2.9 ± 0.5	2.3 ± 1.3	0.6 ± 1.3	20	3.9 ± 0.3	2.4 ± 0.2	1.5* ± 0.4	20
		2	Scald water[b]	4.7 ± 1.3	1.8 ± 1.0	2.8* ± 1.4	[a]	[a]	[a]	[a]	[a]	3.8 ± 0.5	2.1 ± 0.3	1.8* ± 0.8	[a]
		3	Table for cattle organs, stainless steel (upper part)[c]	3.0 ± 0.7	1.3 ± 0.9	1.7* ± 1.1	100	2.2 ± 1.3	1.0 ± 0.9	1.3* ± 1.3	100	3.7 ± 0.5	2.3 ± 0.2	1.4* ± 0.6	20
		4	Conveyor belt pig organs, soft plastic (upper part)[c]	4.6 ± 0.7	3.0 ± 1.0	1.6* ± 2.1	17	2.9 ± 0.6	0.6 ± 0.9	2.3* ± 1.1	100	4.4 ± 0.4	2.9 ± 0.3	1.6* ± 0.5	0
		5	Drain, stainless steel (inside and outside)[c]	6.0 ± 0.8	3.6 ± 1.0	2.4* ± 0.4	17	3.4 ± 0.5	0.8 ± 1.1	2.7* ± 1.4	80	4.9 ± 0.4	3.6 ± 0.5	1.3* ± 0.6	0
		6	Cutting blade cattle/pig carcasses, stainless steel[b]	2.2 ± 1.5	1.5 ± 1.6	0.7 ± 1.9	80	2.4 ± 1.4	1.6 ± 1.0	0.8* ± 1.7	60	4.9 ± 0.5	3.9 ± 0.7	1.0* ± 0.9	0
	Processing	**7**	Cutting board, plastic (upper part)[b]	5.2 ± 0.5	2.0 ± 0.9	3.2* ± 0.8	50	2.9 ± 0.4	0.4 ± 0.6	2.5* ± 0.7	100	5.5 ± 0.5	3.0 ± 0.3	2.5* ± 0.5	0
		8	Conveyor belt, soft plastic (upper part)[b]	4.2 ± 1.0	1.7 ± 1.5	2.5* ± 2.5	67	1.9 ± 0.3	0.0 ± 0.0	1.9* ± 0.3	100	5.0 ± 0.5	2.7 ± 0.5	2.3* ± 0.9	0
		9	Conveyor belt, hard plastic (upper part)[b]	4.7 ± 0.5	2.0 ± 0.8	2.7* ± 1.1	83	2.6 ± 0.4	0.0 ± 0.0	2.6* ± 0.4	100	5.5 ± 0.4	2.4 ± 0.3	3.1* ± 0.6	40
		10	Trolley, stainless steel (bottom)[b]	4.2 ± 0.9	1.5 ± 1.1	2.7* ± 1.4	67	2.4 ± 0.5	0.0 ± 0.0	2.4* ± 0.5	100	5.4 ± 1.2	1.8 ± 0.4	3.6* ± 1.0	100
		11	Drain, stainless steel (inside and outside)[c]	4.8 ± 0.5	4.6 ± 0.4	0.1 ± 0.8	0	2.7 ± 1.0	0.5 ± 0.7	2.1* ± 0.6	100	4.2 ± 0.7	3.5 ± 0.2	0.7* ± 0.8	0
Poultry	**Slaughter**	**12**	Cutting blade bleeding, stainless steel[b]	4.1 ± 0.9	1.6 ± 1.1	2.5* ± 0.7	80	3.8 ± 0.2	2.9 ± 0.6	0.9* ± 0.6	0	3.3 ± 0.3	3.0 ± 0.8	0.4 ± 1.0	0
		13	Scald water[b]	5.4 ± 0.1	2.4 ± 1.5	3.0* ± 1.5	[a]	[a]	[a]	[a]	[a]	5.1 ± 0.5	2.6 ± 1.0	2.5* ± 1.2	[a]
		14	Plucking fingers, rubber[b]	7.1 ± 0.8	5.5 ± 1.6	1.6 ± 1.0	0	3.6 ± 0.2	2.8 ± 0.6	0.8* ± 0.6	0	5.2 ± 1.2	4.7 ± 1.0	0.5 ± 1.0	0
		15	Shackle after stunning, stainless steel[b]	5.6 ± 0.9	5.2 ± 0.8	0.3 ± 1.4	0	3.5 ± 0.4	2.7 ± 1.5	0.9* ± 1.5	20	3.6 ± 0.9	3.3 ± 0.6	0.3 ± 1.1	0
		16	Floor lairage, concrete[c]	7.1 ± 0.2	6.5 ± 0.5	0.6 ± 0.5	0	3.9 ± 0.1	3.7 ± 0.2	0.2 ± 0.2	0	4.3 ± 0.3	3.8 ± 0.6	0.5 ± 0.7	0
	Processing	**17**	Conveyor belt, soft plastic (upper part)[b]	3.9 ± 0.7	1.1 ± 0.6	2.8* ± 0.6	100	2.9 ± 0.3	0.3 ± 0.6	2.6* ± 0.7	100	4.6 ± 0.3	2.0 ± 0.3	2.6* ± 0.5	75
		18	Conveyor belt, hard plastic (upper part)[b]	3.3 ± 0.6	0.6 ± 0.7	2.7* ± 0.8	100	2.3 ± 1.1	0.2 ± 0.4	2.1* ± 1.5	100	4.7 ± 0.2	1.9 ± 0.3	2.8* ± 0.4	80
		19	Cutting blade thighs, stainless steel[b]	3.1 ± 0.2	0.8 ± 1.3	2.4* ± 1.1	83	3.5 ± 0.2	0.0 ± 0.0	3.5* ± 0.2	100	4.9 ± 0.5	1.7 ± 0.3	3.2* ± 0.5	80
		20	Salt injector needles, stainless steel[b d]	4.4 ± 0.9	0.7 ± 1.5	3.7* ± 1.0	80	3.3 ± 0.3	0.8 ± 0.9	2.6* ± 0.6	100	3.7 ± 0.3	1.2 ± 0.8	2.5* ± 1.1	100
		21	Drain, stainless steel (inside and outside)[c]	5.9 ± 0.7	3.7 ± 1.6	2.3* ± 1.2	33	3.6 ± 0.1	1.4 ± 1.1	2.2* ± 1.0	60	4.2 ± 0.5	3.4 ± 0.5	0.8 ± 0.4	0

[a]Not applicable. [b]Food contact surface. [c]Non food contact surface. [d]Hard plastic under needles was sampled for dipslides. ± Standard deviation. *Significant reduction at $P < 0.05$.

2/5 dipslides, respectively) (Table 2). All sampling points in the processing area were considered acceptably clean after C&D, according to the selected threshold (1.0 CFU/cm^2, equal to 2.0 log CFU/100 cm^2) (Table 1).

In the poultry slaughterhouse, TVC could be enumerated from 98% (44/45) of the dipslides before C&D and from 60% (27/45) of the dipslides after C&D. A complete reduction in TVC was observed on the cutting blade for thighs on all dipslides. In 4% (2/45) of the dipslides, which were from the shackles and the hard conveyor belt, the values were 0.5 log and 1.0 log higher respectively after C&D than on the corresponding dipslides before C&D. The lairage floor had the highest mean values after C&D and the lowest reduction. Almost half of the samples (47%, 21/45) were considered non acceptably clean after C&D. The cutting blade for bleeding, plucking fingers, shackles, and lairage floor had the fewest acceptably clean samples and showed limited effect of C&D (< 1.0 log reductions) (Table 2).

ATP-bioluminescence method. In the red meat slaughterhouse, RLU values > 0 were obtained for all samples before C&D (50/50) and after C&D (50/50). Only one sample (drain in the processing area) had a higher RLU value (0.2 log higher) after C&D than in the corresponding sample before C&D. The greatest mean RLU reductions (> 3.0 log) were observed for the hard conveyor belt and the trolley, both located in the processing area. The highest individual value after C&D was obtained for one sample from the cutting blade for carcasses (4.8 log RLU/100 cm^2). The majority (82%) of the samples were considered non acceptably clean according to the selected threshold (1.5 RLU/cm^2, equal to 2.2 log RLU/100 cm^2) (Table 1). Higher mean RLU values were observed in the processing than in the slaughter area before C&D, but the reduction was greater in the processing area, resulting in similar RLU levels after C&D in both areas. The trolley was the only sampling point that was acceptably clean on all sampling occasions (Table 2).

In the poultry slaughterhouse, RLU values > 0 were obtained for all samples before C&D (43/43) and for 98% (42/43) of the samples after C&D. In 9% (4/43) of the samples, the values were 0.2–1.5 log higher after C&D than in the corresponding samples before C&D. More than half of the samples (65%, 28/43) were considered non acceptably clean after C&D. The highest mean RLU values after C&D were observed for the lairage floor and plucking fingers, with the latter having the highest individual value in one sample (5.6 log RLU/100 cm^2). The only sampling point which was considered acceptably clean on all sampling occasions was the salt injector needles. Other sampling points with low mean RLU values after C&D were the hard conveyor belt and the cutting blade for thighs in the processing area (< 2 log RLU/100 cm^2) (Table 2).

Diagnostic performance of dipslide and ATP-bioluminescence methods. In general, method accuracy, measured as agreement of the dipslide and ATP-bioluminescence results with the reference method (TAB), was higher for the poultry slaughterhouse than for the red meat slaughterhouse. In the red meat slaughterhouse, sensitivity was very low (Se = 0.26) for the dipslide method, with 17 dipslides from six different sampling points assessed as acceptable according to the dipslide method, while the reference method assessed the level of cleanliness at those points as non acceptable, indicating a high number of false negatives. The ATP-bioluminescence method showed low specificity in the red meat slaughterhouse (Sp = 0.30), in which 19 samples from seven different sampling points were assessed as non acceptable according to the ATP-bioluminescence results while the reference method assessed the level of cleanliness as acceptable. Cohen's kappa (κ) values indicated minimal level of agreement with the reference method for both the dipslide and ATP-bioluminescence methods in the red meat slaughterhouse. In the poultry slaughterhouse, the κ values indicated that the dipslide method had moderate agreement and the ATP-bioluminescence had weak agreement with the reference method (Table 3).

Enterobacterales. In the red meat slaughterhouse, EB could be enumerated from 66% (38/58) of the samples before C&D, with a mean count of 2.3 ± 0.5 log CFU/100 cm^2. After C&D, EB could only be enumerated from 7% (4/58) of the samples, all from the drains (1.6, 2.2, 2.2, 3.4 log CFU/100 cm^2). The selected threshold for clean samples (1.0 CFU/cm^2, equal to 2.0 log CFU/100 cm^2) (Table 1) was exceeded in 5% (3/58) of the samples.

In the poultry slaughterhouse, EB could be enumerated from 88% (42/48) of the samples before C&D, with a mean count of 2.6 ± 0.5 log CFU/100 cm^2. After C&D, EB could be enumerated from 25% (12/48) of the samples, representing plucking fingers, shackles, lairage floor, and drain (3/5, 3/5, 4/5, 2/6 samples, respectively). The selected threshold for clean samples was exceeded in 19% (9/48) of the samples. The highest EB values (> 3.0 log CFU/100 cm^2) were observed in three samples (shackles and lairage floor).

Scald water. Before C&D, all scald water samples were visibly dirty and had a strong smell. The mean TAB values before C&D were similar, and the reductions in TAB and RLU were significant in both slaughterhouses (Table 2).

In the red meat slaughterhouse, four scald water samples were analyzed before C&D and four samples after C&D. In all samples (8/8), TAB and RLU values were above the detection limit, before and after C&D. After C&D, two samples had TAB values > 2.0 log CFU/mL and one sample had a RLU value > 2.0 log RLU/145 μL. *Enterobacterales* were detected in one of the samples before C&D, but not detected in any of the samples after C&D. The temperature of scald water was measured on one sampling occasion and was 60.3°C directly after slaughter/before C&D and 44.5°C after C&D.

In the poultry slaughterhouse, five scald water samples were analyzed before C&D and five samples after C&D. Total aerobic bacteria could be enumerated from all samples before C&D, and from four samples after C&D. RLU values > 0 were obtained for all samples (before and after C&D, 10/10), and three samples after C&D had RLU values > 3.0 log RLU/145 μL. EB could be enumerated from all samples before C&D (mean 2.5 ± 0.9 log CFU/mL), but not from any of the samples after C&D. The temperature of scald water was measured on one sampling occasion and was 53.7°C directly after slaughter/before C&D and 44.3°C after C&D.

Discussion

Most surfaces sampled in this study were visibly clean after C&D. However, this did not mean that bacteria were absent, which is in agreement with previous findings (Khamisse et al., 2012). Moreover, visible dirt was observed after C&D on some sampling points such as plucking fingers and shackles, the uneven surfaces of which appear difficult to clean. These sampling points were considered non acceptably clean and EB were detected in the majority of the samples after C&D. This is consistent with previous findings that cleaned shackles and plucking fingers are among the most contaminated surfaces in poultry slaughterhouses (García-Sánchez et al., 2017; Zeng et al., 2021). In the poultry slaughterhouse examined in the present study, there was no standard operating procedure (SOP) for cleaning and disinfecting the shackles. They were close to other equipment that was cleaned and disinfected, and thus were only cleaned unintentionally *in situ* (without removal from the overhead conveyor system). Cleaning of shackles *in situ* has previously been observed in another slaughterhouse (Samapundo et al., 2019). This could explain the inadequate cleaning of the shackles, which were dirtier after C&D than before on more than half of the sampling occasions. The plucking fingers were included in the SOP, but the quality assurance staff reported difficulties in cleaning this type of irregular rubber surface. Moreover, the slaughterhouse did not include sampling of plucking fingers and shackles in its hygiene monitoring protocol, because these surfaces belong to the slaughter area, which is considered a "dirty" area of the slaughterhouse. In gen-

Table 3
Diagnostic accuracy of the dipslide and ATP-bioluminescence methods compared with swabbing and plating for total aerobic bacteria (TAB) as the reference method. Values in brackets indicate 95% confidence interval

Slaughterhouse	Red meat		Poultry	
	TVC-dipslide	ATP-bioluminescence	TVC-dipslide	ATP-bioluminescence
Total No. of samples	50	50	43	43
No. of nonacceptably clean samples	7	41	21	28
No. of nonacceptably clean samples with TAB	23		21	
Accuracy	0.64 (0.49–0.77)	0.60 (0.45–0.74)	0.81 (0.67–0.92)	0.79 (0.64–0.90)
Sensitivity	0.26 (0.13–0.47)	0.96 (0.79–0.99)	0.81 (0.60–0.92)	0.95 (0.77–0.99)
Specificity	0.96 (0.82–0.99)	0.30 (0.16–0.49)	0.82 (0.62–0.93)	0.64 (0.43–0.80)
Positive predictive value	0.86 (0.49–0.97)	0.54 (0.39–0.68)	0.81 (0.60–0.92)	0.71 (0.53–0.85)
Negative predictive value	0.60 (0.46–0.74)	0.89 (0.57–0.98)	0.82 (0.62–0.93)	0.93 (0.70–0.99)
Cohen´s kappa agreement coefficient	0.24 (0–0.52)	0.24 (0–0.50)	0.63 (0.51–0.75)	0.58 (0.34–0.82)

eral, it was considered less important to clean the "dirty" area of the slaughterhouse thoroughly and it was not included in the sampling protocol. This goes against the hurdle concept, since ignoring contamination of "dirty" areas presumes a sufficient reduction procedure for carcasses before entering the clean side. Overall, the results showed that the efficacy of C&D was better in the processing area than in the slaughter area. This was observed e.g., when comparing the results for the cutting blade for bleeding in the "dirty" slaughter area of the poultry slaughterhouse with those for the cutting blade for thighs in the "clean" processing area. Another example of an inadequately cleaned surface was the lairage floor, presumably because of limited time between the slaughter shifts at the poultry slaughterhouse, caused by a high number of birds slaughtered each day, which left little time for C&D procedures (2–4 h). There was only time for rinsing feces and foaming with detergent before new birds arrived at the lairage.

Visible dirt was observed after C&D on the post-dehairing table in the red meat slaughterhouse. In half of the samples after C&D, this surface had very high TAB values. High TAB count (3.8 log CFU/cm^2) has also been observed on post-dehairing tables in other studies (Rivas et al., 2000). It is a major concern when such a FCS is insufficiently cleaned, since it creates the risk of cross-contamination of meat (Okpo et al., 2015; Samapundo et al., 2019). The red meat slaughterhouse included in this study had problems with high TAB values on pig carcasses, which were believed to be caused by the insufficiently cleaned post-dehairing table.

High TAB values were found for the drains and the conveyor belt for pig organs after C&D in the red meat slaughterhouse. The conveyor belt looked worn and displayed large scratches, which could harbor bacteria. However, these surfaces were NFCS and were not as critical for food safety as FCS. However, if NFCS such as drains still contain a high amount of bacteria after C&D, a resident house microbiota could be created. A particular *L. monocytogenes* strain has been found to persist for many years in a drain in a Norwegian food processing plant (Fagerlund et al., 2016). Remaining resident bacteria could be transferred from NFCS to FCS when rinsing the drains if contaminated aerosols land on nearby FCS such as conveyor belts (Saini et al., 2012).

Both conveyor belts located in the processing area of the poultry slaughterhouse (sampling point 17 was smooth with lumps, sampling point 18 was modular) were successfully cleaned. These surfaces had similar TAB reductions to those reported for clean conveyor belts in another study (Gómez et al., 2012), although it is unclear whether the slaughterhouse examined in that study was for poultry or red meat. The TAB reductions for the conveyor belts in the poultry slaughterhouse in the present study were higher than those observed in a beef processing plant (Wang et al., 2018), which is surprising since that study examined manual scrubbing and drying of surfaces, which should improve the C&D procedure, compared to the present study where in general, manual scrubbing was not used. In a beef processing plant in another study, C&D of a conveyor belt in a cutting room did not lead to a significant reduction in CFU, and large amounts of bacteria were still present even after rigorous C&D (Khamisse et al., 2012). In the present study, the level of cleanliness of the two conveyor belts in the poultry slaughterhouse was deemed acceptable based on the ATP-bioluminescence results, which were similar to those in another study performed in a poultry slaughterhouse (Rodrigues et al., 2018). In the present study, EB could not be detected on conveyor belts after C&D, which is in agreement with the findings by Wang et al. (2018). Possible reasons are that the cleaning staff prioritized cleaning conveyor belts and that these particular FCS mostly had intact surfaces without scratches. It should also be mentioned that, especially in the processing area of the poultry slaughterhouse, the cleaning staff were aware of the time and location of the sampling procedure, which could have influenced the results.

Enterobacterales could be enumerated in more samples before C&D in the poultry slaughterhouse than in the red meat slaughterhouse. This may indicate that the poultry slaughtering process causes a higher level of fecal contamination of surfaces than the slaughter of cattle and swine. Greater amounts of *E. coli*, EB, and TAB on poultry meat compared with pork and beef have been observed in a previous study (Ghafir et al., 2008). This is most likely due to intestinal rupture during slaughter occurring more often in poultry slaughter. Moreover, more water is used when slaughtering poultry compared with slaughtering cattle and pigs, which facilitates the spread of bacteria (Adams & Moss, 1995; Ninios et al., 2014). However, the TAB reductions in the present study were higher in the processing area of the poultry than in the red meat slaughterhouse (2.8 vs. 2.2 log CFU/100 cm^2), indicating a stronger C&D effect. The slaughterhouses used similar C&D products and procedures, except that the poultry slaughterhouse used 10–15°C higher water temperature for the C&D procedure. Other factors that could also have influenced the results included the type of meat, surface, material, and wear and tear.

To enable evaluation of the performance of the dipslide and ATP-bioluminescence methods in comparison with swabbing and plating (TAB), acceptable thresholds had to be selected for each sampling method to decide whether a surface could be considered acceptably clean. Conventional swabbing and plating was chosen as reference, because it is a widely accepted bacteriological method and can be used to swab places difficult to reach (Griffith, 2016). It was not possible to compare ATP-bioluminescence with EB, since the latter is more specific. It is important to emphasize that there are no standardized thresholds for when a surface is sufficiently clean at the European or national level (Sweden). Even the European standard, which should be applied in sampling and analysis (SS-EN ISO 18593:2018), does not mention thresholds or specify how to interpret the results. Thus, each FBO selects thresholds based on trends measured over time and different FBOs use different thresholds. In this study, the same thresholds for evaluating cleanliness were used for both slaughterhouses, and for both FCS and NFCS. However, a FBO may decide to accept a greater amount of bacteria/organic debris on NFCS and use different

thresholds on different surfaces. Based on the results of the present study, lowering the threshold for the dipslide method and increasing the threshold for the ATP-bioluminescence method should possibly be considered for the red meat slaughterhouse. In this study, two assessment outcomes were used (acceptably and non acceptably clean), but some slaughterhouses also use marginal ranges with values in between the acceptable and non acceptable values. It should be noted that the greater sampling area in swabbing and plating could also have impacted the microbial concentration compared with the other methods studied.

The indicators of diagnostic performance used here have previously been used in other studies comparing different methods for monitoring cleaning and disinfection in food premises (Carrascosa et al., 2012; Ching et al., 2021) and in health care settings (Luick et al., 2013). In the present study, the dipslide method showed lower sensitivity (Se = 0.26) and agreement (κ = 0.24) in the red meat slaughterhouse than in the poultry slaughterhouse (Se = 0.81, κ = 0.63). Carrascosa et al. (2012) observed similar agreement between dipslide and TAB (contact plates) (κ = 0.59) in dairies as seen in the poultry slaughterhouse in the present study. They also found that the dipslide method detected fewer unacceptably clean surfaces than ATP-bioluminescence. The lower sensitivity and agreement in the red meat slaughterhouse could be due to the large difference between the slaughter process for cattle/swine and poultry. The system in the poultry slaughterhouse was mainly automatic, where hanging rotating blades cut the meat, while the system in the cattle/swine slaughterhouse was manual, using, i.e., cutting boards, which resulted in more cuts/cracks in which bacteria could hide. The dipslides did not reach those areas, which could be the reason for the high number of false negatives for that method compared with swabbing and plating. This suggests that dipslides may not be scientifically appropriate for drawing conclusions pertaining to the efficacy of C&D and for determining appropriate microbiological hygiene on cutting boards in cattle and swine slaughterhouses. Other limitations with dipslides are that it is difficult to ensure that the entire agar is pressed on the surface, risking lower detachment of bacteria from the surface, and the lack of mechanical pressure compared with swabbing, which may lead to less bacteria being sampled from the surface. Furthermore, the dipslide method is semi-quantitative, because it is difficult to quantify exactly the number of bacterial colonies when large numbers of bacteria are present, due to non dilution of the sample (Griffith, 2005). However, dipslides are relatively cheap, easy to use, and can be incubated by the FBO.

Low specificity was observed for the ATP-bioluminescence method in both slaughterhouses (0.30 and 0.64 for the red meat and poultry slaughterhouse, respectively). A previous study comparing ATP-bioluminescence with TAB after C&D in a health care setting (Luick et al., 2013) observed higher positive predictive value (PPV = 0.90) and lower negative predictive value (NPV = 0.20) than in the present study (PPV 0.54 and NPV 0.89 for the red meat slaughterhouse, and PPV 0.71 and NPV 0.93 for the poultry slaughterhouse). This was not surprising, since ATP-bioluminescence detects not only bacterial cells but also other cells from organic debris such as blood cells, fat cells, etc. This means that even if bacteria were killed during the C&D process, remaining organic residues could still be detected on the surfaces sampled. This was observed for the cutting blade for carcasses, which was partly covered with burned residues after C&D, which had low TAB values (1.5 log CFU/100 cm^2) but high ATP values (3.9 log RLU/100 cm^2). Another issue to be aware of when using ATP-bioluminescence is that, depending on the type of organic debris present on a surface, the results vary greatly (Lane et al., 2020; Møretrø et al., 2019). Therefore, this method should not be used to assess microbial cleanliness, but can determine the efficacy of C&D, indicating whether or not a surface is clean (Griffith, 2016). Additionally, ATP-bioluminescence is a fast method for monitoring cleanliness, since it provides a result within seconds and thereby enables immediate corrective action, so it is very useful for monitoring C&D before slaughter starts in the morning.

The scald water in the poultry slaughterhouse had higher TAB and RLU values than that in the red meat slaughterhouse, both before and after C&D. One explanation could be the lower temperature of scald water in the poultry slaughterhouse. High bacterial loads pose a risk of cross-contamination of carcasses submerged in the scald water. Studies on the level of hygiene indicator bacteria in scald water during the past 15 years are lacking and few samples have been analyzed in previous studies. In two small-scale poultry slaughterhouses in South Africa (Geornaras et al., 1995, 1997), scald water with similar temperature as in the present study (52–54°C) sampled during production (approximately 2 h after start-up) showed approximately 1.0 log CFU/mL higher EB values, but 1.0 log CFU/mL lower TAB values than scald water sampled in the poultry slaughterhouse in the present study. In another previous study of a small-scale poultry slaughterhouse (Whyte et al., 2004), the levels of TAB and EB in scald water before and after slaughter were approximately 1.0 log CFU/mL higher than observed in the present study. Thresholds for an acceptable level of cleanliness of scald water are lacking, so the scald water was excluded from the comparative assessment of the different sampling methods.

In conclusion, the results obtained in this study highlight the main difficulties for FBOs using common hygiene monitoring methods for assessing surface cleanliness after C&D. These include the risk of missing bacteria when using only dipslides and the difficulty of interpreting the ATP-bioluminescence results, as this method does not only measure the microbial load. Since our results indicate that neither ATP-bioluminescence nor dipslides provide accurate estimates of C&D efficacy when used separately, a possibility would be to combine them. Swabbing and plating (TAB) could be used to verify the reliability of the other methods. Additionally, there is difficulty in interpreting the results of monitoring operations, due to the absence of commonly agreed guidelines on when a surface is sufficiently cleaned. It is also concerning that the slaughterhouses included in this study put less effort into monitoring the cleanliness of FCS in slaughter areas, even though these surfaces may constitute the greatest risk to meat cross-contamination, considering direct contact with the product. This may increase the risk of epidemiological spread of bacterial foodborne pathogens to consumers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Swedish Farmers' Foundation for Agricultural Research (grant numbers O-18-20-158 and O-20-20-447) and the Royal Swedish Agricultural Academy (grant number CF2020-0011). The authors gratefully acknowledge the slaughterhouses that participated in the study, Lise-Lotte Fernström for excellent laboratory assistance and Claudia von Brömssen for statistical advice.

References

3M Science Applied to Life. (2019). Technical Bulletin: Setting Pass/Fail Limits for the M Clean-Trace Hygiene Monitoring and Management system. https://multimedia.m.com/mws/media/1767520O/clean-trace-setting-pass-fail-limits-pdf-lm1-implementation.pdf

Adams, M. R., & Moss, M. O. (1995). In *Food microbiology* (p. 35). The Royal Society of Chemistry.

Althaus, D., Zweifel, C., & Stephan, R. (2017). Analysis of a poultry slaughter process: Influence of process stages on the microbiological contamination of broiler

carcasses. *Italian Journal of Food Safety, 4*, 7097. https://doi.org/10.4081/ijfs.2017.7097.

Arguello, H., Carvajal, A., Collazos, J. A., García-Feliz, C., & Rubio, P. (2012). Prevalence and serovars of *Salmonella enterica* on pig carcasses, slaughtered pigs and the environment of four Spanish slaughterhouses. *Food Research International, 45*, 905–912. https://doi.org/10.1016/j.foodres.2011.04.017.

Bonita, R., Beaglehole, R., & Kjellström, T. (2006). *Basic epidemiology* (2nd ed. p. 112). Geneva, Switzerland: WHO Library Cataloguing-in-Publication Data.

Brusa, V., Restovich, V., Galli, L., Arias, R., Linares, L., Costa, M., Ruíz, Díaz V., Pugin, D., & Leotta, G. (2021). Reduction of Shiga toxin-producing *Escherichia coli* in a beef abattoir. *Food Science and Technology International., 28*, 50–59. https://doi.org/10.1177/1082013221991258.

Carrascosa, C., Saavedra, P., Millán, R., Jaber, J., Pérez, E., Grau, R., Raposo, A., Mauricio, C., & Sanjuán, E. (2012). Monitoring of cleanliness and disinfection in dairies: Comparison of traditional microbiological and ATP bioluminescence methods. *Food Control, 28*, 368–373. https://doi.org/10.1016/j.foodcont.2012.05.001.

Ching, C. L., Kamaruddin, A., & Rajangan, C. S. (2021). Assessing the performance of a real-time total adenylate (ATP + ADP + AMP) detection assay for surface hygiene monitoring in food manufacturing plants and commercial kitchens. *Journal of Food Protection, 84*, 973–983. https://doi.org/10.4315/JFP-20-294.

Codex Alimentarius. (2020). General principles of food hygiene. CXC 1-1969. Adopted in 1969. Amended in 1999. Revised in 1997, 2003, 2020. Editorial corrections in 2011. https://www.fao.org/fao-who-codexalimentarius/codex-texts/codes-of-practice/pt/

Eurofins Food & Feed Testing Sweden AB. (2021). Instructions for hygiene monitoring with dipslides. https://cdnmedia.eurofins.com/european-east/media/681593/provtagningsanvisning-tryckplattor.pdf

Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union, L 139 of 30th of April 2004 (pp. 1). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri = CELEX:32004R0852

Fagerlund, A., Langsrud, S., Schirmer, B. C. T., Møretrø, T., & Heir, E. (2016). Genome analysis of *Listeria monocytogenes* sequence type 8 strains persisting in salmon and poultry processing environments and comparison with related strains. *PLoS One, 11*. https://doi.org/10.1371/journal.pone.0151117 e0151117.

Fagerlund, A., Møretrø, T., Heir, E., Briandet, R., & Langsrud, S. (2017). Cleaning and disinfection of biofilms composed of *Listeria monocytogenes* and background microbiota from meat processing surfaces. *Applied and Environmental Microbiology, 83*. https://doi.org/10.1128/AEM.01046-17 e01046–17.

García-Sánchez, L., Melero, B., Jaime, I., Hänninen, M. L., Rossi, M., & Rovira, J. (2017). *Campylobacter jejuni* survival in a poultry processing plant environment. *Food Microbiology, 65*, 185–192. https://doi.org/10.1016/j.fm.2017.02.009.

Geornaras, I., De Jesus, A., Van Zyl, E., & Von Holy, A. (1995). Microbiological survey of a South African poultry processing plant. *Journal of Basic Microbiology, 35*, 73–82. https://doi.org/10.1002/jobm.3620350204.

Geornaras, I., De Jesus, A., Van Zyl, E., & Von Holy, A. (1997). Bacterial populations of different sample types from carcasses in the dirty area of a South African poultry abattoir. *Journal of Food Protection, 60*, 551–554. https://doi.org/10.4315/0362-028X-60.5.551.

Ghafir, Y., China, B., Dierick, K., De Zutter, L., & Daube, G. (2008). Hygiene indicator microorganisms for selected pathogens on beef, pork, and poultry meats in Belgium. *Journal of Food Protection, 71*, 35–45. https://doi.org/10.4315/0362-028X-71.1.35.

Gómez, D., Ariño, A., Carramiñana, J. J., Rota, C., & Yangüela, J. (2012). Sponge versus mini-roller for the surface microbiological control of *Listeria monocytogenes*, total aerobic mesophiles and *Enterobacteriaceae* in the meat industry. *Food Control, 27*, 242–247. https://doi.org/10.1016/j.foodcont.2012.03.031.

Griffith, C. (2016). Surface sampling and the detection of contamination (Chapter 44). In *Handbook of hygiene control in the food industry*, pp. 673–696). Cambridge, United Kingdom: Woodhead Publishing Limited https://doi.org/10.1016/B978-0-08-100155-4.00044-3.

Griffith, C. (2005). Improving surface sampling and detection of contamination (Chapter 36). In *Handbook of hygiene control in the food industry*, pp. 588–618). Cambridge, United Kingdom: Woodhead Publishing Limited https://doi.org/10.1533/9781845690533.3.588.

Hansson, I., Pudas, N., Harbom, B., & Engvall, E. O. (2010). Within-flock variations of *Campylobacter* loads in caeca and on carcasses from broilers. *International Journal of Food Microbiology, 141*, 51–55. https://doi.org/10.1016/j.ijfoodmicro.2010.04.019.

Hauge, S. J., Johannessen, G. S., Haverkamp, T. H. A., Bjørkøy, S., Llarena, A. K., Spilsberg, B., Leithaug, M., Økland, M., Holthe, J., Røtterud, O.-J., Alvseike, O., & Nagel-Alne, G. E. (2023). Assessment of poultry process hygiene and bacterial dynamics along two broiler slaughter lines in Norway. *Food Control, 146*. https://doi.org/10.1016/j.foodcont.2022.109526 109526.

Hutchison, M. L., Thomas, D. J. I., Small, A. H., Buncic, S., & Howell, M. (2007). Implementation of compulsory hazard analysis critical control point system and its effect on concentrations of carcass and environmental surface bacterial indicators in United Kingdom red meat slaughterhouses. *Journal of Food Protection, 70*, 1633–1639. https://doi.org/10.4315/0362-028X-70.7.1633.

Khamisse, E., Firmesse, O., Christieans, S., Chassaing, D., & Carpentier, B. (2012). Impact of cleaning and disinfection on the non-culturable and culturable bacterial loads of food-contact surfaces at a beef processing plant. *International Journal of Food Microbiology, 158*, 163–168. https://doi.org/10.1016/j.ijfoodmicro.2012.07.014.

Lane, K., McLandsborough, L. A., Autio, W. R., & Kinchla, A. J. (2020). Efficacy of ATP monitoring for measuring organic matter on postharvest food contact surfaces. *Journal of Food Protection, 83*, 1829–1837. https://doi.org/10.4315/0362-028X.JFP-19-443.

Lindblad, M., Hansson, I., Vågsholm, I., & Lindqvist, R. (2006). Postchill *Campylobacter* prevalence on broiler carcasses in relation to slaughter group colonization level and chilling system. *Journal of Food Protection, 69*, 495–499. https://doi.org/10.4315/0362-028X-69.3.495.

Lofstedt, R. (2019). The management and communication of a food risk controversy: The Swedish campylobacter case. *Journal of Risk Research, 22*, 803–816. https://doi.org/10.1080/13669877.2019.1608287.

Luick, L., Thompson, P. A., Loock, M. H., Vetter, S. L., Cook, J., & Guerrero, D. M. (2013). Diagnostic assessment of different environmental cleaning monitoring methods. *American Journal of Infection Control, 41*, 751–752. https://doi.org/10.1016/j.ajic.2012.09.019.

Maes, S., Huu, S. N., Heyndrickx, M., van Weyenberg, S., Steenackers, H., Verplaetse, A., Vackier, T., Sampers, I., Raes, K., & Reu, K. D. (2017). Evaluation of two surface sampling methods for microbiological and chemical analyses to assess the presence of biofilms in food companies. *Journal of Food Protection, 80*, 2022–2028. https://doi.org/10.4315/0362-028X.JFP-17-210.

Maes, S., Heyndrickx, M., Vackier, T., Steenackers, H., Verplaetse, A., & Reu, K. D. (2019). Identification and spoilage potential of the remaining dominant microbiota on food contact surfaces after cleaning and disinfection in different food industries. *Journal of Food Protection, 82*, 262–275. https://doi.org/10.4315/0362-028X.JFP-18-226.

Marin, C., Cerdà-Cuéllar, M., González-Bodi, S., Lorenzo-Rebenaque, L., & Vega, S. (2022). Persistent *Salmonella* problems in slaughterhouses related to clones linked to poultry companies. *Poultry Science, 101*. https://doi.org/10.1016/j.psj.2022.101968 101968.

McHugh, M. L. (2012). Interrater reliability: The kappa statistic. *Biochemia Medica, 22*, 276–282. https://doi.org/10.11613/BM.2012.031.

Moazzami, M., Bergenkvist, E., Fernström, L.-L., Rydén, J., & Hansson, I. (2021). Reducing *Campylobacter jejuni, Enterobacteriaceae*, *Escherichia coli*, and total aerobic bacteria on broiler carcasses using combined ultrasound and steam. *Journal of Food Protection, 84*, 572–578. https://doi.org/10.4315/JFP-20-395.

Moore, G., & Griffith, C. (2002). A comparison of surface sampling methods for detecting coliforms on food contact surfaces. *Food Microbiology, 19*, 65–73. https://doi.org/10.1006/fmic.2001.0464.

Møretrø, T., Langsrud, S., & Heir, E. (2013). Bacteria on meat abattoir process surfaces after sanitation: Characterisation of survival properties of *Listeria monocytogenes* and the commensal bacterial flora. *Advances in Microbiology, 03*, 255–264. https://doi.org/10.4236/aim.2013.33037.

Møretrø, T., & Langsrud, S. (2017). Residential bacteria on surfaces in the food industry and their implications for food safety and quality. *Comprehensive Reviews in Food Science and Food Safety, 16*(5), 1022–1041. https://doi.org/10.1111/1541-4337.12283.

Møretrø, T., Normann, M. A., Sæbø, H. R., & Langsrud, S. (2019). Evaluation of ATP bioluminescence-based methods for hygienic assessment in fish industry. *Journal of Applied Microbiology, 127*, 186–195. https://doi.org/10.1111/jam.14292.

Ninios, T., Lundén, J., Korkeala, H., & Fredriksson-Ahomaa, M. (2014). *Meat inspection and control in the slaughterhouse* (1st ed.). Wiley Blackwell (Chapter 19).

Okpo, E., Leith, J., Smith-Palmer, A., Bell, J., Parks, D., Browning, F., Byers, L., Corrigan, H., Webster, D., Karcher, A. M., Murray, A., & Storey, T. (2015). An outbreak of an unusual strain of *Listeria monocytogenes* infection in North-East Scotland. *Journal of Infection and Public Health, 8*, 612–618. https://doi.org/10.1016/j.jiph.2015.05.009.

Peruzy, M. F., Houf, K., Joossens, M., Yu, Z., Proroga, Y. T., & Murru, N. (2021). Evaluation of microbial contamination of different pork carcass areas through culture-dependent and independent methods in small-scale slaughterhouses. *International Journal of Food Microbiology, 336*. https://doi.org/10.1016/j.ijfoodmicro.2020.108902 108902.

Rivas, T., Vizcaíno, J. A., & Herrera, F. J. (2000). Microbial contamination of carcasses and equipment from an Iberian pig slaughterhouse. *Journal of Food Protection, 63*, 1670–1675. https://doi.org/10.4315/0362-028X-63.12.1670.

Rodrigues, L. B., Dos Santos, L. R., Rizzo, N. N., Ferreira, D., De Oliveira, A. P., Levandowski, R., Webber, B., & Do Nascimento, V. P. (2018). ATP-Bioluminescence and conventional microbiology for hygiene evaluation of cutting room surfaces in poultry slaughterhouse. *Acta Scientiae Veterinariae, 46*, 1534. https://doi.org/10.22456/1679-9216.81882.

Saini, J. K., Marsden, J. L., Fung, D. Y. C., & Crozier-Dodson, B. A. (2012). Evaluation of potential for translocation of Listeria monocytogenes from floor drains to food contact surfaces in the surrounding environment using Listeria innocua as a surrogate. *Advances in Microbiology, 2*, 565–570. https://doi.org/10.4236/aim.2012.24073.

Samapundo, S., de Baenst, I., Aerts, M., Cnockaert, M., Devlieghere, F., & Van Damme, P. (2019). Tracking the sources of psychrotrophic bacteria contaminating chicken cuts during processing. *Food Microbiology, 81*, 40–50. https://doi.org/10.1016/j.fm.2018.06.003.

Stanga, M. (2010). *Sanitation: Cleaning and disinfection in the food industry* (pp. 514–540). Germany: Wiley-VCH. Weinheim.

Statutory Instrument (2002). The Meat (Hazard Analysis and Critical Control Point) Regulations 2002. UK Statutory Instrument 2002. No 889.

Stephan, R., Althaus, D., Kiefer, S., Lehner, A., Hatz, C., Schmutz, C., Jost, M., Gerber, N., Baumgartner, A., Hächler, H., & Mäusezahl-Feuz, M. (2015). Foodborne transmission of *Listeria monocytogenes* via ready-to-eat salad: A nationwide outbreak in Switzerland, 2013–2014. *Food Control, 57*, 14–17. https://doi.org/10.1016/j.foodcont.2015.03.034.

Swedish Food Agency (1998). The Swedish Statute Book. SLV FS, 1998:10. https://www.livsmedelsverket.se/globalassets/om-oss/lagstiftning/nummerordning-upphord-lagstiftning/1998/slvfs-1998-10_skannad.pdf

Swedish Standards Institute (SIS) (2019). SS-EN 1276:2019. Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2, step 1). https://www.sis.se/en/produkter/chemical-technology/products-of-the-chemical-industry/chemicals-for-industrial-and-domestic-disinfection-purposes/ss-en-12762019/

Swedish Standards Institute (SIS) (2018). SS-EN ISO 18593:2018. Microbiology of the food chain- Horizontal methods for surface sampling (ISO 18593:2018). https://www.sis.se/en/produkter/mathematics-natural-sciences/microbiology/food-microbiology/ss-en-iso-185932018/

Swedish Standards Institute (SIS) (2012). SS-EN 14349:2012. Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on non-porous surfaces without mechanical action - Test method and requirements (phase2, step 2). https://www.sis.se/en/produkter/health-care-technology/sterilization-and-disinfection/disinfectants-and-antiseptics/ssen143492012/

Tutenel, A. V., Pierard, D., Van Hoof, J., & De Zutter, L. (2003). Molecular characterization of *Escherichia coli* O157 contamination routes in a cattle slaughterhouse. *Journal of Food Protection, 66*, 1564–1569. https://doi.org/10.4315/0362-028x-66.9.1564.

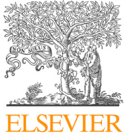
Wang, H., He, A., & Yang, X. (2018). Dynamics of microflora on conveyor belts in a beef fabrication facility during sanitation. *Food Control, 85*, 42–47. https://doi.org/10.1016/j.foodcont.2017.09.017.

Whyte, P., McGill, K., Monahan, C., & Collins, J. D. (2004). The effect of sampling time on the levels of micro-organisms recovered from broiler carcasses in a commercial slaughter plant. *Food Microbiology, 21*, 59–65. https://doi.org/10.1016/S0740-0020(03)00040-6.

Zeng, H., De Reu, K., Gabriël, S., Mattheus, W., De Zutter, L., & Rasschaert, G. (2021). *Salmonella* prevalence and persistence in industrialized poultry slaughterhouses. *Poultry Science, 100*. https://doi.org/10.1016/j.psj.2021.01.014 100991.

III

Food Control 119 (2021) 107424



Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Reducing *Campylobacter jejuni*, *Enterobacteriaceae* and total aerobic bacteria on transport crates for chickens by irradiation with 265-nm ultraviolet light (UV–C LED)

Madeleine Moazzami*, Lise-Lotte Fernström, Ingrid Hansson

Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health (BVF), Division of Food Safety, Campus Ultuna, Ulls Väg 26, Box 7036, 750 07, Uppsala, Sweden

ARTICLE INFO

Keywords:
Ultraviolet light
Campylobacter
Enterobacteriaceae
Total aerobic bacteria
Transport crate

ABSTRACT

It is critical to maintain low levels of microbes in the whole food production chain. Due to high speed of slaughter, lack of time, and structural characteristics of crates, sufficient cleaning and disinfection of crates used for transporting chickens to abattoirs is a challenge. Inadequately cleaned transport crates for broiler chickens caused a major outbreak of campylobacteriosis in Sweden in 2016–2017, when the contaminated crates introduced *Campylobacter* to the chickens during thinning. This study evaluated the antibacterial efficacy of 265-nm ultraviolet (UV–C) LED light on artificially contaminated chicken transport crates. In a laboratory study, a transport crate artificially contaminated with *Campylobacter* and cecum contents was irradiated with 265-nm UV-C light by a continuous LED array in a treatment cabinet. The transport crate was sampled 52 times by cotton swabs before and after UV-C treatment for 1 min (20.4 mJ/cm^2) and 3 min (61.2 mJ/cm^2). The swab samples were analysed for *Campylobacter jejuni (C. jejuni)*, bacteria belonging to the family *Enterobacteriaceae*, and total aerobic bacteria. After irradiation with UV-C LED light for 1 min, a mean reduction in *C. jejuni* of log 2.0 ± 0.5 CFU/mL was observed, while after irradiation for 3 min the reduction was log 3.1 ± 1.0 CFU/mL. The mean reduction in *Enterobacteriaceae* was log 1.5 ± 0.3 CFU/mL after 1 min of irradiation and log 1.8 ± 0.8 CFU/mL after 3 min. The mean reduction in total aerobic bacteria was log 1.4 ± 0.4 CFU/mL after 1 min of irradiation and log 1.6 ± 0.5 CFU/mL after 3 min. Significant reductions in bacterial load were observed in all samples after UV-C treatment and extending the treatment time from 1 to 3 min significantly increased the reduction in *C. jejuni*. However, before implementation of UV-C LED treatment in commercial chicken abattoirs, the irradiation unit would need to be extended and/or the washing procedure before UV-C treatment, to reduce the amount of organic matter on transport crates, would need to be improved.

1. Introduction

Campylobacteriosis is the most commonly reported bacterial gastrointestinal disease in humans in the United States, Australia, Sweden, and other European countries. The most important risk factor for humans contracting campylobacteriosis is consumption of contaminated chicken products (Australian Government Department of Health, 2019; Center for Disease Control and Prevention, 2019; EFSA, 2017). It is critical to maintain low levels of microbes in the whole food production chain. Proper cleaning and disinfection is essential during the process, to reduce the occurrence of pathogenic bacteria on production animals, on food products, and on surfaces. This is of great importance, since cleaning and disinfection shortcomings have been shown to cause food poisoning. In one example, contaminated chicken transport crates caused a large outbreak of campylobacteriosis in Sweden in 2016–2017. The contaminated crates introduced *Campylobacter* to the chickens during thinning, when part of the flock was delivered to slaughter (Lofstedt, 2019). Studies show that pathogenic bacteria such as *Campylobacter* spp. and *Salmonella* spp. can remain on chicken transport crates even after cleaning and disinfection (Atterbury, Gigante, Tinker, Howell, & Allen, 2020; Hansson, Ederoth, Andersson, Vågsholm, & Engvall, 2005; Northcutt & Berrang, 2006; Peyrat, Soumet, Maris, & Sanders, 2008; Slader et al., 2002). Bacteria belonging to the family *Enterobacteriaceae* and total aerobic bacteria can be used to assess general cleanliness and to detect pathogenic bacteria at the abattoir (Haughton et al., 2011; Roccato et al., 2018). Various

* Corresponding author..
E-mail address: madeleine.moazzami@slu.se (M. Moazzami).
URL: http://www.slu.se (M. Moazzami).

https://doi.org/10.1016/j.foodcont.2020.107424
Received 24 April 2020; Received in revised form 9 June 2020; Accepted 11 June 2020
Available online 03 July 2020
0956-7135/ © 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

pathogens, total aerobic bacteria, coliforms, and *E. coli* have been analysed to evaluate the efficacy of different disinfection methods on transport crates for chickens (Atterbury et al., 2020; Berrang & Northcutt, 2005; Hinojosa et al., 2018; Northcutt & Berrang, 2006).

The demand for innovative disinfection approaches in the food industry is increasing (Morales-de la Peña, Welti-Chanes, & Martín-Belloso, 2019). There are alternative methods based on physical disinfection, such as ultraviolet light (UV). The forms UV-A (400–320 nm) and UV-B (320–290 nm) appear naturally as solar radiation, while UV-C radiation (290–100 nm) occurs mainly in artificial light (Giordano & Romano, 2015). It is known that UV-C light induces DNA damage in bacterial cells (Cheigh, Park, Chung, Shin, & Park, 2012). Although disinfection by conventional 254-nm UV-C light can be used to reduce the amount of *Campylobacter* (Haughton et al., 2011; Haughton, Lyng, Cronin, Fanning, & Whyte, 2012; Isohanni & Lyhs, 2009), the germicidal effect should increase at a wavelength of 265 nm (Kowalski, 2009). Comparison of the germicidal effect of 265 and 280-nm LEDs against *E. coli* in petri dishes showed that 265 nm was more efficient (Li, Wang, Huo, Lu, & Hu, 2017). However, to the best of our knowledge, there has been no research on the inactivating effect of 265-nm UV-C light on *Campylobacter*.

The poultry industry is calling for alternative approaches for disinfecting transport crates for chickens, since the cleaning and disinfection methods currently used are insufficient to consistently reduce *Campylobacter* and other bacteria. The aim of this study was to evaluate whether 265-nm UV-C LED light can be used to reduce *Campylobacter jejuni (C. jejuni)*, *Enterobacteriaceae*, and total aerobic bacteria on transport crates for chickens.

2. Materials and methods

2.1. LED light source

The UV unit used in the study was a WiSDOM DS (LED TAILOR INNOVA7ION, Salo, Finland) consisting of light-emitting diodes (LEDs), housed in an enclosed steel cabinet with external dimensions 615 mm × 445 mm × 330 mm and internal dimensions 500 mm × 325 mm × 140 mm (length × width × height) (Fig. 1). The internal dimensions represented the maximum size of the object to be irradiated. The object was placed in the middle of the cabinet, on a glass shelf at a distance of approximately 140 mm from the LEDs, which were installed in the roof and floor of the cabinet. Full 360° irradiation was ensured by the placement of the LEDs and reflective surfaces inside the cabinet. Samples were irradiated with a continuous LED array (Crystal IS, NY, USA) with wavelength 260–270 nm, an emission peak of 265 nm, and 12-nm bandwidth at full-width half maximum. The heat produced by the complete light system was low and had no effect on the test samples exposed to the UV-C light inside the cabinet. UV-C intensity was measured with a Gigaherz-Optik UV 3719–4 optometer (Gigahertz, Germany). The UV-C intensity was on average 0.34 mW/cm^2 (± 0.06 mW/cm^2) inside the cabinet, with good uniformity across the whole irradiated area. The energy received by a sample was 20.4 mJ/cm^2 (± 3.6 mJ/cm^2) when irradiated for 1 min and 61.2 mJ/cm^2 (± 10.8 mJ/cm^2) when irradiated for 3 min. The irradiation cabinet was equipped with a time control and safety features necessary when working with high-intensity UV-C light.

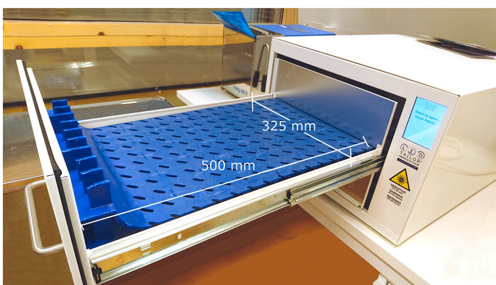


Fig. 1. Part of a transport crate in the UV-C irradiation cabinet (WiSDOM DS, LED TAILOR INNOVA7ION, Salo, Finland). Internal dimensions of the cabinet are indicated.

2.2. Transport crates

A cleaned used chicken transport crate (Linco food systems, Trige, Denmark) of the material high density polyethylene was donated by an abattoir in Sweden. The upper surface of the crate was relatively smooth but contained small scratches. It was also composed of multiple holes (18 mm length × 7 mm width) to facilitate ventilation and removal of feces during transport, and feces and water during cleaning (Fig. 1). The crate was cut into four pieces with dimensions 480–500 mm × 240–300 mm (length × width) to fit into the LED cabinet (Fig. 1). The upper surface of each of the pieces was measured and divided by a line into two equal parts. One piece of the crate at a time was submerged in a plastic box containing a mixture of chicken cecum contents and *C. jejuni* (see section 2.3). A lid was placed on the plastic box and the contents were mixed by tilting the box from side to side 10 times, after which it was left to stand for 10 min. The crate was then removed from the box and any visible caecal material on the surface of the crate was removed. The caecal mixture on the surface of the crate was evenly distributed with a sterile cotton swab pre-moistened in the caecal mixture. Samples were then taken by gloved hand, using a sterile cotton swab measuring 10 cm × 10 cm (Wellkang Ltd. t/a Wellkang Tech Consulting Suite B, London, UK) moistened with 30 mL buffered peptone water (BPW) (Oxoid CM0509; Basingstoke, UK). When swabbing the first part of the upper surface, the entire area was swabbed with overlapping horizontal and vertical strokes. The cotton swab was then placed in a sterile plastic bag, 90 mL BPW was added, and the suspension was homogenized in a stomacher for 1 min at 240 rpm (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, New Jersey, USA). This sample served as an untreated control of the number of bacteria on the crates (A-samples). The crate was then placed in the LED cabinet and treated by UV-C light for 1 or 3 min. After UV-C treatment, the other part of the crate was sampled (B-sample) in exactly the same way as the control. A total of 52 untreated (A) + 52 treated (B) samples were collected from the crate surfaces, 25 (A) + 25 (B) after treatment with UV-C light for 1 min and 27 (A) + 27 (B) after treatment with UV-C light for 3 min.

2.3. Bacteriological analyses

2.3.1. Quantification of C. jejuni

Analysis for *C. jejuni* was performed according to ISO 10272–2 (2017). Briefly, a 10-fold serial dilution in 0.1% (v/v) peptone water (Dilucups, LabRobot Products AB, Stenungsund, Sweden) was prepared. To ensure the possibility to estimate low numbers of *Campylobacter,* 1 mL from the initial suspension was distributed on the surface of four regular (90 mm) plates of modified charcoal cephoperazone desoxycholate agar (mCCDA) (Oxoid, Basingstoke, UK). For the other dilutions, 0.1 mL was surface plated onto each mCCDA plate. The plates were incubated at 41.5 ± 0.5 °C for 44 ± 4 h in a jar with microaerobic atmosphere generated by use of CampyGen™ (Oxoid, Basingstoke, UK). A blood agar plate (National Veterinary Institute (SVA), Uppsala, Sweden) with a *C. jejuni* strain (CCUG 43594) was also placed in each jar, as a positive control of the microaerobic atmosphere. After incubation, colonies characteristic of *C. jejuni* were quantified and the number of *Campylobacter* was expressed as log CFU per mL. The detection limit was log 1.0 CFU/mL.

2.3.2. Quantification of bacteria belonging to the family Enterobacteriaceae

Analysis for bacteria belonging to the family *Enterobacteriaceae* was performed according to NMKL 144 (3rd Ed. 2005). The previously prepared 10-fold dilutions were also used to estimate counts of *Enterobacteriaceae* in samples. From each dilution, 1.0 mL was mixed carefully with 10–15 mL violet red bile glucose agar (VRBG) (Becton, Dickinson and Company, Sparks USA) in a Petri dish and left to solidify, and then an overlay of 5 mL VRBG was added. Plates were then incubated at 37 ± 1 °C for 24 ± 2 h. Bacterial counts were performed on plates with 15–150 colonies. Five colonies preliminarily identified as *Enterobacteriaceae* were cultured on blood agar and incubated at 37 ± 1 °C for 24 ± 2 h. Presence of bacteria belonging to the family *Enterobacteriaceae* was confirmed by oxidase test and the number of *Enterobacteriaceae* was expressed as log CFU per mL. The detection limit was log 1.0 CFU/mL.

2.3.3. Quantification of total aerobic bacteria

Total aerobic bacteria was quantified according to NMKL 86 (5th Ed. 2013). From the initial dilution series prepared for each sample, 1.0 mL aliquots of each dilution were mixed with 15–20 mL of plate count agar (PCA) (Oxoid, Basingstoke, UK) and left to solidify, and then an overlay of 5–10 mL PCA was added. Plates were then incubated at 30 ± 1 °C for 72 ± 6 h. Bacterial counts were performed on plates with 25–250 colonies and total aerobic bacteria was expressed as log CFU per mL. The detection limit was log 1.0 CFU/mL.

2.4. Simulation of inadequately cleaned transport crates

Intestinal contents from 10 to 15 broiler caeca from *Campylobacter*-negative flocks according to the Swedish Campylobacter program (Hansson et al., 2007) were used to simulate caecal contamination of transport crates. The caeca were stored at −20 °C and thawed by storage in the refrigerator the day before analyses. The caeca were cut into 1–2 cm pieces using sterile scissors and tweezers, placed in a bottle with 0.5 L BPW, a lid was added, and the bottle was shaken. The contents were transferred to sterile plastic bags and homogenized for 1 min at 240 rpm in a stomacher. The contents of the bags were then poured into a clean and disinfected 40 L plastic box. An additional 5 L BPW and 40 mL of an overnight culture of *C. jejuni* (CCUG 43594) in brain heart infusion broth (BHI) (CM1135; Oxoid. Basingstoke, United Kingdom) were poured into the plastic box.

2.5. Initial concentrations of C. jejuni, bacteria belonging to the family Enterobacteriaceae, and total aerobic bacteria

The initial concentrations of bacteria in the caecal mixture containing chicken caeca, the overnight culture of *C. jejuni*, and BPW were quantified as described in section 2.3. Mean concentration of *C. jejuni*, *Enterobacteriaceae* and total aerobic bacteria in the caecal mixture were log 5.3 ± 0.5 CFU/mL, log 5.2 ± 0.3 CFU/mL, and log 6.1 ± 0.5 CFU/mL respectively (Fig. 2).

2.6. Statistical analyses

The data obtained in the study were compiled and analysed using Microsoft Office Excel and R studio (RStudio® version 1.2.1335 - Windows 7+). Bacterial counts (CFU/mL) were $\log_{10}$ transformed. Standard deviations of bacterial reductions following 1 and 3 min treatments were calculated. Statistical significance was determined by the paired *t*-test, which was performed for both treatment durations for each of the three bacterial groups under investigation. The Welch two-sample *t*-test was conducted to determine significant differences between treatment times. Differences before and after treatment, and between treatment times, were deemed significant at $p < 0.05$.

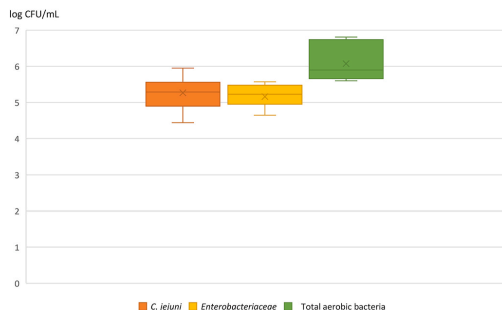


Fig. 2. Initial concentrations of *C. jejuni*, members of the family *Enterobacteriaceae*, and total aerobic bacteria in the mixture used to simulate caecal contamination of inadequately cleaned transport crates in the 52 analyses.

3. Results

3.1. Quantification of C. jejuni

The transport crate was irradiated 52 times and reductions in the number of *C. jejuni* were observed in all swab samples. Following irradiation of transport crate sections with UV-C light for 1 min, the mean reduction in *C. jejuni* was log 2.0 ± 0.5 CFU/mL. The concentration varied between log 4.4 and 5.8 CFU/mL before treatment, and between log 2.2 and 3.8 CFU/mL after treatment. A significant increase in the reduction of *C. jejuni* was observed on extending the treatment time from 1 to 3 min. In the treatment where the transport crates were irradiated with UV-C light for 3 min, the mean reduction in *C. jejuni* was log 3.1 ± 1.0 CFU/mL. The concentration varied between log 4.5 and 7.1 CFU/mL before treatment, and between log 2.0 and 3.8 CFU/mL after treatment, in that case (Fig. 3). The difference in the numbers of *C. jejuni* on the crate before and after treatment with UV-C light was highly significant ($P < 0.0001$) for both treatment times (1 min and 3 min).

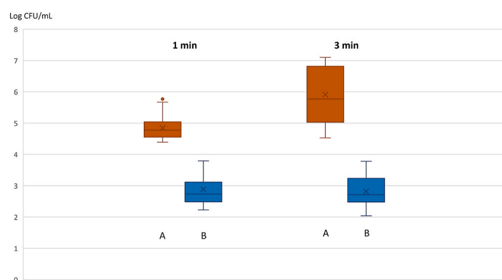


Fig. 3. Concentration of *C. jejuni* in swab samples from an artificially caeca-contaminated chicken transport crate taken: (A) before treatment and (B) after irradiation with UV-C light for 1 or 3 min.

3.2. Quantification of bacteria belonging to the family Enterobacteriaceae

The transport crate was irradiated 52 times and reductions in bacteria belonging to the family *Enterobacteriaceae* were observed in all swab samples. Countable numbers were present after treatment in all samples except one, in which the amount was reduced below the limit of detection after 3 min of irradiation. This value is excluded from Fig. 4. Following treatment of transport crate sections with UV-C light for 1 min, the mean reduction in *Enterobacteriaceae* was log

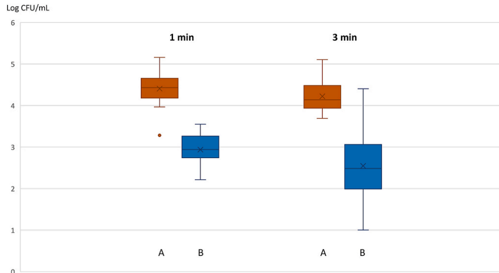


Fig. 4. Concentration of bacteria belonging to the family *Enterobacteriaceae* in swab samples from an artificially caeca-contaminated chicken transport crate taken: (A) before treatment and (B) after irradiation with UV-C light for 1 or 3 min.

1.5 ± 0.3 CFU/mL. The concentration varied between log 3.3 and 5.2 CFU/mL before treatment, and between log 2.2 and 3.6 CFU/mL after treatment.

No significant increase in the reduction in *Enterobacteriaceae* was observed on extending the treatment time from 1 to 3 min. Following irradiation with UV-C light for 3 min, the mean reduction in *Enterobacteriaceae* was log 1.8 ± 0.8 CFU/mL. The concentration varied between log 3.7 and 5.1 CFU/mL before treatment, and between log 1 and 4.4 CFU/mL after treatment (Fig. 4). The difference in the number of bacteria belonging to the family *Enterobacteriaceae* on the crate before and after treatment with UV-C light was highly significant ($P < 0.0001$) for both treatment times (1 min and 3 min).

3.3. Quantification of the total aerobic bacteria

The transport crate was irradiated 51 times and reductions in the total aerobic bacteria were observed in all swab samples, while one sample was excluded due to contamination. The number of bacteria present was countable in all samples subjected to the treatment at both treatment times. After treatment of the crate with UV-C light for 1 min, the mean reduction in total aerobic bacteria was log 1.4 ± 0.4 CFU/mL. The concentration varied between log 5.2 and 6.2 CFU/mL before treatment, and between log 3.8 and 4.6 CFU/mL after treatment. No significant increase in the reductions in total aerobic bacteria was observed on extending the treatment time from 1 to 3 min. In the treatment where the crate was irradiated with UV-C light for 3 min, the mean reduction in total aerobic bacteria was log 1.6 ± 0.8 CFU/mL. The concentration varied between log 4.9 and 5.5 CFU/mL before

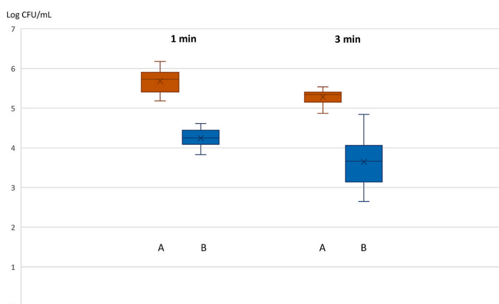


Fig. 5. Concentration of total aerobic bacteria in swab samples from an artificially caeca-contaminated chicken transport crate taken: (A) before treatment and (B) after irradiation with UV-C light for 1 or 3 min.

treatment, and between log 2.7 and 4.8 CFU/mL after treatment (Fig. 5). The difference in total aerobic bacteria before and after treatment with UV-C light was highly significant ($P < 0.0001$) for both treatment times (1 min and 3 min).

4. Discussion

Significant reductions in bacterial numbers were observed in all samples after irradiation of the artificially caeca-contaminated transport crate sections with 265-nm UV-C light. However, the bacterial reductions observed were lower than those reported in other studies examining the effect of 265-nm UV-C light on other bacteria and materials, e.g., suspensions of *Salmonella* spp. and *Staphylococcus aureus* in petri dishes (Lee, Yoon, Park, & Ryu, 2018) and *E. coli* in petri dishes (Li et al., 2017). In the present study, total inactivation of *C. jejuni* was not achieved. One explanation could be a “shadow” effect caused by the holes and cracks in the crate, in which the bacteria could have been protected from irradiation (Hinojosa et al., 2018). The upper surface of the crate was not completely smooth because it was composed of multiple holes for ventilation and removal of feces. Additionally, the surface consisted of small scratches from the claws of the chickens due to its previous use. The “shadow” effect has been observed in experiments performed on chicken meat (Haughton et al., 2011; Haughton, Lyng, Cronin, Fanning, & Whyte, 2012). Another reason for the failure to achieve total inactivation of *C. jejuni* could have been that the caecal mixture in which the crate sections were submerged was opaque and UV-light has low penetrating capacity (Ninios, Lundén, Korkeala, & Fredriksson-Ahomaa, 2014).

It has been observed that the antibacterial efficacy of UV irradiation treatment may depend on the initial concentration of bacteria (Isohanni & Lyhs, 2009). In that case, the reduction in bacteria might have been different if a lower concentration of bacteria had been used to simulate inadequately cleaned transport crates in the present study. Interestingly, the initial number of bacteria on the transport crate was at the same level or lower than that found on naturally contaminated transport crates from an abattoir in the UK (Atterbury et al., 2020).

Extending the treatment time from 1 to 3 min resulted in greater reductions in *C. jejuni* of log 1.1 CFU/mL being observed, which was statistically significant ($p < 0.05$). However, considerable numbers of *C. jejuni* were still present on the transport crates after the 3 min treatment (log 2.0–3.8 CFU/mL). No significant differences were observed in *Enterobacteriaceae* or total aerobic bacteria when 1 and 3 min treatments were compared. This might be due to *Campylobacter* being more sensitive to UV-light than other Gram-negative bacteria associated with poultry, such as *E. coli* and *Salmonella* Enteritidis (Haughton et al., 2011; Murdoch, Maclean, MacGregor, & Anderson, 2010). In the 3 min treatment, the standard deviation in counts was higher for *C. jejuni* than for *Enterobacteriaceae* and total aerobic bacteria. Fluctuations in the amount of *Campylobacter* have been observed previously, leading to the suggestion that *Campylobacter* is less robust to environmental conditions than the other bacteria tested (Atterbury et al., 2020).

As treatments to reduce *Campylobacter*, conventional 254-nm UV-C light and 405-nm blue light have been studied. In studies where chicken meat was irradiated with 254-nm UV-C light for less than 1 min, the reduction in *Campylobacter* was only log 0.6–0.8 CFU/g (Haughton et al., 2011; Isohanni & Lyhs, 2009). In another study, micro-plates inoculated with *Campylobacter* and irradiated with 405-nm blue light did not show any significant reductions when treated for less than 5 min (Murdoch et al., 2010). This indicates that short treatment times using conventional 254-nm UV-C light and 405-nm blue light may not be sufficient to achieve acceptable reductions in *Campylobacter*.

Introducing UV-C light as a disinfection method at a commercial abattoir could be a challenge, due to the high speed of slaughter and the humidity of the crates. In one of the largest abattoirs in Sweden, around 50 million broilers are slaughtered per year, equivalent to five broilers

per second. This means that the rate of cleaning and disinfection of transport crates also has to be very high and that there is just a limited time for any disinfection procedure. Disinfection of the crates is currently performed after the cleaning process, and the treatment only takes a few seconds.

When using UV light for disinfection of objects, the energy received by a sample depends on treatment time and distance from the light source. In the present study, only 1 min was needed to reduce *C. jejuni* by 2 log CFU/mL (0.02 J/cm^2). To reduce *C. jejuni* by 2.3 log CFU/mL on micro-plates with 405-nm blue light, 25 min (15 J/cm^2) was needed in a previous study (Murdoch et al., 2010). Levels of *C. jejuni* can be reduced below the limit of detection when packaging materials and food contact surfaces are irradiated for less than 1 min with 254-nm UV-C light with similar energy dosage to that used in the present study (Haughton et al., 2011). However, when porous material (polyethylene-polypropylene) was tested in that study, the energy dose had to be increased to reduce *C. jejuni* below the limit of detection. Since chicken transport crates have a porous surface, this suggests that more LED diodes should be added to the UV unit to increase the energy exerted on the samples. This could be a way to achieve greater reductions in bacteria without having to increase the treatment time.

To enable longer treatment times under commercial conditions, a longer tunnel lined with UV-C LED lights, through which the crates would pass during 1 min, could be built. However, this might be a costly investment for the abattoir and not practically possible in a commercial setting. Another solution could be to reduce the amount of fecal matter, and thereby decrease the initial concentration of bacteria on the crates, by improving the washing procedure prior to UV-C treatment (Atterbury et al., 2020). Alternatively, the efficiency of the UV-C unit could be improved by decreasing the distance between the transport crate and the light source (Haughton, Grau et al., 2012; Lee et al., 2018) or by adding more LED diodes.

5. Conclusions

Disinfection by UV-C light is not a new technology, but to our knowledge it has not been used previously for cleaning and disinfection of transport crates for chickens. Evaluation of the antibacterial efficacy of 265-nm UV-C LED light on artificially contaminated chicken transport crates in this study revealed significant reductions in *C. jejuni*, *Enterobacteriaceae*, and total aerobic bacteria. Irradiation treatment for 1 or 3 min effectively reduced *C. jejuni* on the crates, although considerable numbers of bacteria were still present on the crates after the treatment. Thus UV-C LED light may have good potential for reducing microbial loads on transport crates for chickens, but if UV-C light treatment is used in abattoirs, the UV unit would need to be extended and/or the washing process prior to UV-C treatment would need to be improved.

CRediT authorship contribution statement

Madeleine Moazzami: Conceptualization, Writing - original draft. **Lise-Lotte Fernström:** Formal analysis. **Ingrid Hansson:** Conceptualization, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that there is no conflict of interest.

Acknowledgments

The authors gratefully acknowledge the abattoir that participated in the study and LED TAILOR INNOVA7ION for lending the LED light source. The authors also thank the Ivar and Elsa Sandbergs Research Foundation for project funding.

References

Atterbury, R. J., Gigante, A. M., Tinker, D., Howell, M., & Allen, V. M. (2020). An improved cleaning system to reduce microbial contamination of poultry transport crates in the United Kingdom. *Journal of Applied Microbiology,* 1–9. https://doi.org/10.1111/jam.14576.

Australian Government Department of Health (2019). Australia ' s notifiable disease status , 2015 : Annual report of the national notifiable diseases surveillance system. *Australia's notifiable disease status, 2015*: Annual report of the national notifiable diseases surveillance system. *NNDSS Annual Report Working Group, 43*, 10.

Berrang, M. E., & Northcutt, J. K. (2005). Use of water spray and extended drying time to lower bacterial numbers on soiled flooring from broiler transport coops. *Poultry Science, 84*(11), 1797–1801. https://doi.org/10.1093/ps/84.11.1797.

Center for Disease Control and Prevention (2019). Campylobacter (Campylobacteriosis). Retrieved November 11, 2019, from https://www.cdc.gov/campylobacter/faq.html.

Cheigh, C. I., Park, M. H., Chung, M. S., Shin, J. K., & Park, Y. S. (2012). Comparison of intense pulsed light- and ultraviolet (UVC)-induced cell damage in Listeria monocytogenes and Escherichia coli O157:H7. *Food Control, 25*(2), 654–659. https://doi.org/10.1016/j.foodcont.2011.11.032.

EFSA (2017). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal, 15*(12), https://doi.org/10.2903/j.efsa.2017.5077.

Giordano, A., & Romano, G. (2015). TM cell cycle control and dysregulation. *Cell Cycle, 285*, 133. https://doi.org/10.1385/1-59259-822-6.

Hansson, I., Ederoth, M., Andersson, L., Vågsholm, I., & Engvall, E. O. (2005). Transmission of Campylobacter spp. to chickens during transport to slaughter. *Journal of Applied Microbiology, 99*(5), 1149–1157. https://doi.org/10.1111/j.1365-2672.2005.02689.x.

Hansson, I., Plym Forshell, L., Gustafsson, P., Boqvist, S., Lindblad, J., Olsson Engvall, E., et al. (2007). Summary of the Swedish Campylobacter program in broilers, 2001 through 2005. *Journal of Food Protection, 70*(9), 2008–2014. https://doi.org/10.4315/0362-028X-70.9.2008.

Haughton, P. N., Grau, E. G., Lyng, J., Cronin, D., Fanning, S., & Whyte, P. (2012a). Susceptibility of Campylobacter to high intensity near ultraviolet/visible 395 ± 5nm light and its effectiveness for the decontamination of raw chicken and contact surfaces. *International Journal of Food Microbiology, 159*(3), 267–273. https://doi.org/10.1016/j.ijfoodmicro.2012.09.006.

Haughton, P. N., Lyng, J., Cronin, D., Fanning, S., & Whyte, P. (Lyng, Cronin, Fanning, & Whyte, 2012b). Effect of crust freezing applied alone and in combination with ultraviolet light on the survival of Campylobacter on raw chicken. *Food Microbiology, 32*(1), 147–151. https://doi.org/10.1016/j.fm.2012.05.004.

Haughton, P. N., Lyng, J. G., Cronin, D. A., Morgan, D. J., Fanning, S., & Whyte, P. (2011). Efficacy of UV light treatment for the microbiological decontamination of chicken, associated packaging, and contact surfaces. *Journal of Food Protection, 74*(4), 565–572. https://doi.org/10.4315/0362-028x.jfp-10-356.

Hinojosa, C., Caldwell, D., Byrd, J., Droleskey, R., Lee, J., Stayer, P., et al. (2018). Use of foaming disinfectants and cleaners to reduce aerobic bacteria and salmonella on poultry transport coops. *Animals, 8*(11), https://doi.org/10.3390/ani8110195.

Isohanni, P. M. I., & Lyhs, U. (2009). Use of ultraviolet irradiation to reduce Campylobacter jejuni on broiler meat. *Poultry Science, 88*(3), 661–668. https://doi.org/10.3382/ps.2008-00259.

Kowalski, W. (2009). Ultraviolet germicidal irradiation handbook: UVGI for air and surface disinfection. *Ultraviolet germicidal irradiation handbook: UVGI for air and surface disinfection* (pp. 17–18). . https://doi.org/10.1007/978-3-642-01999-9.

Lee, Y. W., Yoon, H. D., Park, J.-H., & Ryu, U.-C. (2018). Application of 265-nm UVC LED lighting to sterilization of typical Gram negative and positive bacteria. *Journal of the Korean Physical Society, 72*(10), 1174–1178. https://doi.org/10.3938/jkps.72.1174.

Li, G. Q., Wang, W. L., Huo, Z. Y., Lu, Y., & Hu, H. Y. (2017). Comparison of UV-LED and low pressure UV for water disinfection: Photoreactivation and dark repair of Escherichia coli. *Water Research, 126*, 134–143. https://doi.org/10.1016/j.watres.2017.09.030.

Lofstedt, R. (2019). The management and communication of a food risk controversy: The Swedish campylobacter case. *Journal of Risk Research, 22*(6), 803–816. https://doi.org/10.1080/13669877.2019.1608287.

Morales-de la Peña, M., Welti-Chanes, J., & Martín-Belloso, O. (2019). Novel technologies to improve food safety and quality. *Current Opinion in Food Science, 30*, 1–7. https://doi.org/10.1016/j.cofs.2018.10.009.

Murdoch, L. E., Maclean, M., MacGregor, S. J., & Anderson, J. G. (2010). Inactivation of *Campylobacter jejuni* by exposure to high-intensity 405-nm visible light. *Foodborne Pathogens and Disease, 7*(10), 1211–1216. https://doi.org/10.1089/fpd.2010.0561.

Ninios, T., Lundén, J., Korkeala, H., & Fredriksson-Ahomaa, M. (2014). *Meat inspection and control in the slaughterhouse* (First). Wiley Blackwell441.

Northcutt, J. K., & Berrang, M. E. (2006). Influence of a chicken transport cage-washing

system on wastewater characteristics and bacteria recovery from cage flooring. *The Journal of Applied Poultry Research, 15*(3), 457–463. https://doi.org/10.1093/japr/15.3.457.

Peyrat, M. B., Soumet, C., Maris, P., & Sanders, P. (2008). Recovery of Campylobacter jejuni from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination. *International Journal of Food Microbiology, 124*(2), 188–194. https://doi.org/10.1016/j.ijfoodmicro.2008.03.030.

Roccato, A., Mancin, M., Barco, L., Cibin, V., Antonello, K., Cocola, F., et al. (2018). Usefulness of indicator bacteria as potential marker of Campylobacter contamination in broiler carcasses. *International Journal of Food Microbiology, 276*(April), 63–70. https://doi.org/10.1016/j.ijfoodmicro.2018.04.003.

Slader, J., Domingue, G., Mcalpine, K., Owen, R. J., Bolton, F. J., & Humphrey, T. J. (2002). Impact of transport crate reuse and of catching and processing on. *Society, 68*(2), 713–719. https://doi.org/10.1128/AEM.68.2.713.

IV

Journal of Food Protection, Vol. 84, No. 4, 2021, Pages 572–578
https://doi.org/10.4315/JFP-20-395
Published 2021 by the International Association for Food Protection
This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/)

Research Note

Reducing *Campylobacter jejuni, Enterobacteriaceae, Escherichia coli,* and Total Aerobic Bacteria on Broiler Carcasses Using Combined Ultrasound and Steam

MADELEINE MOAZZAMI,[1]*† EMMA BERGENKVIST,[1]† LISE-LOTTE FERNSTRÖM,[1] JESPER RYDÉN,[2] AND INGRID HANSSON[1]

[1]*Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Ulls väg 26, 750 07 Uppsala, Sweden (ORCID: https://orcid.org/0000-0001-7038-911X [M.M.]; https://orcid.org/0000-0003-3764-2341 [I.H.]); and* [2]*Department of Energy and Technology, Swedish University of Agricultural Sciences, Lennart Hjelms väg 9, 750 07 Uppsala, Sweden (ORCID: https://orcid.org/0000-0002-5451-4563 [J.R.])*

MS 20-395: Received 30 September 2020/Accepted 9 November 2020/Published Online 12 November 2020

ABSTRACT

Campylobacteriosis is the most frequently reported foodborne illness in Europe and many other parts of the world. *Campylobacter* can colonize the intestines of broilers, mostly in large amounts. Broilers are usually slaughtered in a high-speed automated system that could cause rupture of the intestines during evisceration, resulting in contamination of carcasses with intestinal bacteria like *Campylobacter*. This study evaluated the combined effects of ultrasound and steam (SonoSteam) on naturally contaminated chicken carcasses at a large-scale abattoir in Sweden. Ultrasound at 30 to 40 kHz and steam at 84 to 85°C or 87 to 88°C were used at slaughter, with a line speed of 18,000 birds per hour. The amounts of *Campylobacter* spp., *Enterobacteriaceae, Escherichia coli,* and total aerobic bacteria on neck skins from 103 chicken carcasses, sampled before and after treatment by ultrasound-steam, were analyzed. *Campylobacter* spp. were quantified in 58 (56%) of the neck skins, from birds belonging to four of the seven flocks represented. All 58 isolates were identified as *Campylobacter jejuni*. After the ultrasound-steam treatment, the mean reductions in *C. jejuni, Enterobacteriaceae, E. coli* and total aerobic bacteria were 0.5 ± 0.8, 0.6 ± 0.6, 0.5 ± 0.6, and 0.4 ± 0.7 log CFU/g, respectively. No significant differences in reduction between the two different treatment temperatures were observed for any of the bacteria. Although the bacterial reductions were significant, large amounts of bacteria remained on the carcasses after treatment. Further studies are needed to identify optimal measures at slaughter to reduce food spoilage bacteria and pathogenic bacteria, which should be considered in a One Health perspective.

HIGHLIGHTS

- Ultrasound-steam treatment reduced the numbers of bacteria on broiler neck skin.
- *Campylobacter* was present at >3 log CFU/g on some treated chicken neck skins.
- Reductions in bacterial levels were greatest on carcasses with high initial amounts.
- Higher steam temperature did not result in higher bacterial reductions.

Key words: Broiler carcass; *Campylobacter*; *Enterobacteriaceae*; *Escherichia coli*; Total aerobic bacteria; Ultrasound-steam

---

Campylobacteriosis has been the most frequently reported foodborne illness in the European Union since 2005 *(6)*. During 2016 to 2018, almost 250,000 confirmed cases were reported each year in Europe *(6),* although the actual number of cases is estimated to be around 9 million per year *(7)*. The total costs related to campylobacteriosis in the European Union are estimated to be around 2.4 billion euros per year *(7)*. *Campylobacter* is highly prevalent in broiler flocks worldwide, and handling and consumption of chicken and contaminated food present a high risk of campylobacteriosis in humans *(3, 13)*. In a baseline study performed in Europe in 2008, *Campylobacter* prevalence in cecum samples was detected in 71% of flocks on average, but it varied considerably (range, 2 to 100%) between different countries *(5)*.

Campylobacter can colonize the intestine of broilers, often in very high amounts (up to 8 log CFU/g), without the birds showing any symptoms of illness *(11, 26, 27)*. The prevalence of contaminated carcasses postchill has been shown to increase with higher degrees of intestinal colonization in the slaughter group *(16)*. High concentrations of *Campylobacter* on chicken meat are associated with an increased risk of disease in consumers *(17, 21)*. Risk assessment studies have shown that the risk of consumers developing campylobacteriosis would be reduced by 50% at the European Union level if all broiler flocks met the

* Author for correspondence. Tel: (0046) 18672317; E-mail: madeleine.moazzami@slu.se.
† Shared first authorship.

microbiological criterion of <3.0 log CFU *Campylobacter* per gram on neck skin *(28)*.

In large-scale broiler processing plants, the slaughter process is highly automated and occurs at very high speed. This can cause rupture of the intestines during evisceration, which can result in contamination of carcasses with intestinal bacteria like *Campylobacter*. The numbers of *Campylobacter* on carcasses from slaughter groups with high levels of intestinal colonization have been shown to increase 10-fold compared with those on *Campylobacter*-positive carcasses from slaughter groups with no positive cloacal samples *(16)*.

Different interventions can be performed to reduce the numbers of *Campylobacter* on broilers after slaughter. The efficiency of steam as a disinfection method has been evaluated in several studies *(1, 15, 18, 29)*. Steam pasteurization has been successfully used in a beef processing plant *(25)* and has also been tested on broiler carcasses *(15, 18, 29)*. In some of those tests, the treatment gave significant reductions (up to 3.3 log CFU/cm^2) in the numbers of *Campylobacter* on carcasses but also impaired carcass quality *(15, 29)*. Thus, there is still no solution to this quality problem, and further research is needed *(12)*. To reduce the treatment time, a technology that combines steam with ultrasound has been developed. The ultrasound destroys the protective sublayer of air that is present around all objects, facilitating rapid heat transfer. The steam can then immediately reach the carcass skin. This technology has been evaluated in a few studies and has been found to reduce the numbers of *Campylobacter* on broiler carcasses by 2.5 log CFU/mL *(9),* 1.0 log CFU/g *(20),* and 2.5 log CFU per carcass *(2)*.

The aim of the present study was to evaluate the effects of full-scale ultrasound-steam treatment on broiler carcasses naturally contaminated with bacteria in a slaughterhouse in Sweden.

MATERIALS AND METHODS

Broiler flocks. In order to select appropriate broiler flocks, flocks from producers with a previous history of often delivering *Campylobacter*-positive flocks at slaughter and enrolled in the Swedish Campylobacter program were selected *(10)*. Some of the flocks that were sent to slaughter had previously been tested (using sock samples in the broiler house) for the presence of *Campylobacter* 1 to 2 weeks before slaughter, as part of another study *(8)*. A total of seven flocks from six farms were included in the present study, and 10 to 25 carcasses from each flock were sampled.

Ultrasound-steam treatment. The SonoSteam equipment (European patent EPO; 116 02 722 020.12113, FORCE Technology/Sanovo Technology Group A/S, Brøndby, Denmark) uses a combination of ultrasound at 30 to 40 kHz (25 to 30 kHz) and steam. In this study, steam temperatures of 84 to 85°C and 87 to 88°C were used. The ultrasound-steam chamber was installed at the end of the slaughter line (Fig. 1) in a large-scale broiler chicken processing plant in Sweden. The chamber was positioned to allow whole carcasses on processing-line shackles to be treated before chilling. The ultrasound waves were produced simultaneously with the steam. The chamber contained two rows of nozzles, supplying steam for inside and outside treatment of the carcasses. The carcasses were treated for 1.2 to 1.5 s. After the treatment, the carcasses were sprayed with water and chilled with forced air for approximately 2.5 h.

Sample collection. Carcasses were randomly selected within the flocks selected for the study, but only carcasses without visible contamination and with a sufficient amount of neck skin were sampled. The line speed at the slaughterhouse was around 18,000 birds per hour. The carcasses were removed from the slaughter line and sampled before they entered the ultrasound-steam chamber. For this, approximately 10 g of neck skin was cut aseptically from the left or right side of alternate carcasses and weighed. The carcasses were then each labeled with a red band and placed back on the slaughter line. Each neck skin sample was placed in a separate stomacher bag (Blender bags Standard 400, Grade Products, Coalville, England) without transport medium and was labeled with an individual number and the letter A. The labeled carcasses were sampled in a similar way after treatment, and the bags were marked with the individual number of the carcass and the letter B. In this way, each carcass acted as its own control. Owing to the high line speed and short distance between the exit of the ultrasound-steam chamber and the entrance to the chilling room, the second sampling had to be performed after the chilling area and not immediately after ultrasound-steam treatment. In total, neck skins from 103 individual carcasses were sampled before and after treatment.

The samples were transported on the day of sampling, in an insulated box with refrigerant gel packs, to the laboratory at the Swedish University of Agricultural Sciences. The temperature was checked upon arrival. Only samples with a temperature of 2 to 8°C were accepted for analysis.

Bacteriological analysis. Samples were kept at a temperature of 2 to 8°C at the laboratory until analysis, which began within a maximum of 48 h after sampling at the processing plant. From each sample, 10 g of neck skin was aseptically weighed and placed in a stomacher bag, together with 90 g of buffered peptone water (CM0509, Oxoid, Basingstoke, UK), and homogenized for 1 min at 240 rpm (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, NJ). A 10-fold serial dilution of the fluid in peptone water (salt from VWR, peptone from Oxoid) was then prepared.

Enumeration and identification of thermotolerant *Campylobacter* spp. Quantification of thermotolerant *Campylobacter* spp. was determined according to ISO 10272-2 *(14)*. Modified charcoal cefoperazone desoxycholate agars (mCCDA; CM0739, Oxoid) were preincubated at 41.5 ± 0.5°C for 30 min before use. From the initial dilution, 1.0 mL was surface plated equally on four mCCDA plates (9 cm in diameter). For further dilutions, 0.1 mL was surface plated on a single mCCDA plate. All plates were incubated at 41.5 ± 0.5°C for 44 ± 4 h in a microaerobic atmosphere, using gas jars containing CampyGen sachets (Oxoid). A blood agar plate (SVA, Uppsala, Sweden) with *Campylobacter jejuni* (CCUG 43594) was placed in each jar, for qualitative control of the microaerobic atmosphere. After incubation for 44 ± 4 h, colonies characteristic of *Campylobacter* were counted. Bacterial counts were performed on plates with less than 150 colonies, and the number of *Campylobacter* bacteria was expressed as log CFU per gram, with a detection limit of 1.0 log CFU/g.

When *Campylobacter* was detected, at least three typical colonies from each agar plate were recultured on blood agar and incubated at 41.5 ± 1°C in a microaerobic atmosphere for 48 ± 4

FIGURE 1. *Ultrasound-steam chamber installed in the slaughter line of a large-scale broiler chicken processing plant.*

h. The colonies were then identified to species level using matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Daltonics, Billerica, MA).

Enumeration of bacteria belonging to the family *Enterobacteriaceae*. Enumeration of bacteria belonging to the family *Enterobacteriaceae* was performed according to NMKL 144, 3rd ed. *(22),* using the 10-fold serial dilution described above. A 1.0-mL sample from each dilution was mixed carefully with 10 to 15 mL of violet red bile glucose agar (BD, Sparks, MD) in a petri dish (9 cm in diameter) and left to solidify, and then an overlay of around 5 mL of violet red bile glucose agar was added. The plates were incubated at 37 ± 1°C for 24 ± 2 h. Bacterial counts were performed on plates with 15 to 150 colonies. Five colonies preliminarily identified as *Enterobacteriaceae* were cultured on blood agar and incubated at 37 ± 1°C for 24 ± 2 h. The identity of bacteria belonging to the family *Enterobacteriaceae* was confirmed by oxidase test, and the number of *Enterobacteriaceae* bacteria was expressed as log CFU per gram, with a detection limit of 1.0 log CFU/g.

Enumeration and identification of *E. coli*. Enumeration of *Escherichia coli* was performed according to NMKL 125, 4th ed. *(23)*. In brief, a 1.0-mL aliquot of each dilution in the initial dilution series prepared for each sample was mixed carefully with 5 mL of tryptic soy agar (TSA; Oxoid) in a petri dish (9 cm in diameter) and preincubated at room temperature (20 to 25°C) for 1 to 2 h. An overlay of 10 mL violet red bile agar (Oxoid) was then added. After solidification, the plates were incubated at 44°C for 24 ± 2 h. Bacterial counts were performed on plates with 10 to 100 colonies. Five colonies preliminarily identified as *E. coli* were cultured on TSA agar and incubated at 37°C for 24 ± 2 h. The colonies were identified to species level using matrix-assisted laser desorption ionization–time of flight mass spectrometry. The number of *E. coli* bacteria was expressed as log CFU per gram, with a detection limit of 1.0 log CFU/g.

Enumeration of total aerobic bacteria. Enumeration of total aerobic bacteria was performed according to NMKL 86, 5th ed. *(24),* on the same 10-fold serial dilution described above. A 1.0-mL aliquot from each dilution was mixed carefully with 15 to 20 mL of plate count agar (Oxoid) in a petri dish (9 cm in diameter) and left to solidify. After agar solidification, the plates were incubated at 30 ± 1°C for 72 ± 6 h. Bacterial counts were performed on plates with 25 to 250 colonies, and the total aerobic bacteria content was expressed as log CFU per gram, with a detection limit of 3.0 log CFU/g.

Statistical analysis. The results were compiled and analyzed using Microsoft Office Excel and R Studio (RStudio version 1.2.1335, Windows 7+). Bacterial counts (CFU per gram) were log transformed. Standard deviations of bacterial reductions following treatments were calculated. Statistical significance was determined by the paired *t* test, which was performed for each of the four bacterial groups studied. The Welch two-sample *t* test was conducted to determine significant differences between the two treatment temperatures (84 to 85°C and 87 to 88°C) for each bacterial group. Analysis with simple linear regression was performed to determine whether the initial amounts of *Campylobacter, Enterobacteriaceae, E. coli,* and total aerobic bacteria on the neck skin influenced the level of reduction achieved by ultrasound-steam treatment. Differ-

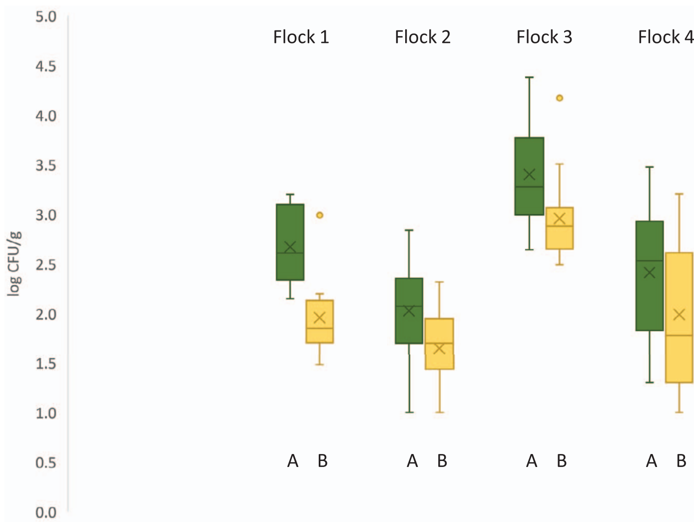


FIGURE 2. *Numbers of* Campylobacter jejuni *bacteria on neck skins from broilers in each of the four flocks that gave positive samples before (A) and after (B) ultrasound-steam treatment.*

ences before and after treatment and between treatment temperatures were deemed significant at a *P* value of <0.05.

RESULTS

Enumeration and identification of thermotolerant *Campylobacter* spp. *Campylobacter* was detected on 58 (56%) of the 103 neck skins tested, originating from four of the seven flocks sampled. All 58 isolates were identified as *C. jejuni*. One neck skin sample was found to be contaminated with *Campylobacter* at 1.7 log CFU/g before treatment, but no *Campylobacter* was quantified after the treatment. Therefore, this sample was removed from the statistical analysis.

The amounts of *Campylobacter* before treatment by ultrasound-steam varied between 1.0 and 4.4 log CFU/g and after treatment between 1.0 and 4.2 log CFU/g (Fig. 2). The mean reduction in *C. jejuni* was 0.5 ± 0.8 log CFU/g. A reduction in *C. jejuni* after the ultrasound-steam treatment was observed in 46 of the 58 samples, an increase was observed in 7, and 4 of the samples did not show either any increase or reduction. Samples with *Campylobacter* levels above 3 log CFU/g consisted of 14 (24%) of the positive samples before the ultrasound-steam treatment and 7 (12%) after the treatment (Fig. 2). The difference in numbers of *C. jejuni* before and after the treatment was highly significant ($P < 0.0001$).

Enumeration of bacteria belonging to the family *Enterobacteriaceae*. The amounts of bacteria belonging to the family *Enterobacteriaceae* before treatment by ultrasound-steam varied between 1.6 and 5.2 log CFU/g, while after treatment, the amounts varied between 1.1 and 3.7 log CFU/g (Fig. 3). In one pretreatment sample, the amount of *Enterobacteriaceae* was not countable, and therefore, that sample was removed from the analysis. The mean reduction in *Enterobacteriaceae* was 0.6 ± 0.6 log CFU/g. A reduction in the number of *Enterobacteriaceae* after ultrasound-steam treatment was observed for 83 of the 100 samples, while an increase was observed for 16 of the samples. In 17% of the samples, the number of *Enterobacteriaceae* was above 3 log CFU/g after treatment. The difference in numbers of bacteria belonging to the *Enterobacteriaceae* before and after treatment was highly significant ($P < 0.0001$).

Enumeration of *E. coli*. The amounts of *E. coli* before treatment by ultrasound-steam varied between 1.7 and 5.0 log CFU/g, and after treatment, the amounts varied between 1.7 and 4.2 log CFU/g (Fig. 3). The mean reduction in *E. coli* was 0.5 ± 0.6 log CFU/g. A reduction in *E. coli* after the ultrasound-steam treatment was observed in 80% of the samples, while an increase was observed in 20% of the samples. After the treatment, the number of *E. coli* was above 3 log CFU/g in 36% of the samples and above 4 log CFU/g in 3% of the samples. *E. coli* was isolated from all 100 samples, both before and after treatment by ultrasound-steam. The difference in numbers of *E. coli* before and after treatment was highly significant ($P < 0.0001$).

Enumeration of total aerobic bacteria. The amounts of total aerobic bacteria before treatment by ultrasound-steam varied between 3.7 and 7.0 log CFU/g, and after treatment, the amounts varied between 3.4 and 6.2 log CFU/g (Fig. 3). The mean reduction in total aerobic bacteria was 0.4 ± 0.7 log CFU/g. A reduction in total aerobic bacteria after the ultrasound-steam treatment was observed in 77% of the samples, while an increase was observed in 23% of the samples. In 15% of the treated samples, the total aerobic bacterial count was above 5 log CFU/g, and in two treated samples, the count was above 6.0 log CFU/g. The highest value before treatment was 7 log CFU/g, and the lowest value after treatment was 3.5 log CFU/g. Total aerobic

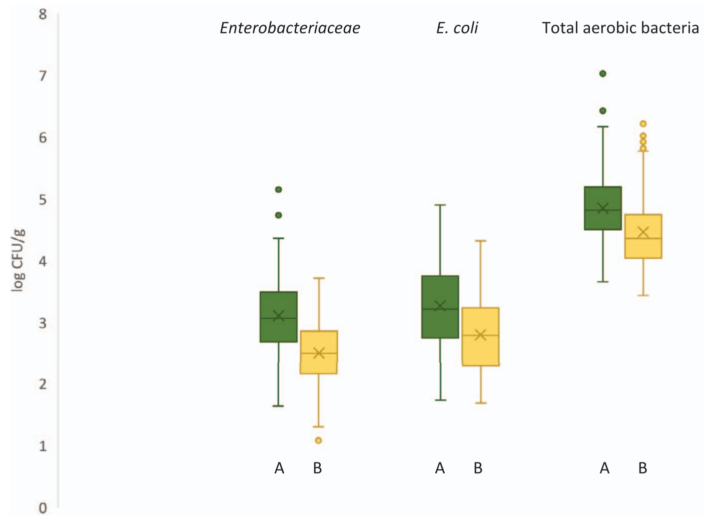


FIGURE 3. *Distribution of bacteria belonging to the* Enterobacteriaceae, E. coli, *and total aerobic bacteria on broiler neck skins before (A) and after (B) ultrasound-steam treatment.*

bacteria were found in all 103 samples both before and after treatment. The difference in numbers of total aerobic bacteria before and after treatment was highly significant ($P < 0.0001$).

Different treatment temperatures. Ten of the 58 samples where *C. jejuni* could be enumerated were treated at 84 to 85°C, and the remaining 48 samples were treated at 87 to 88°C. Of the 100 samples where *E. coli* and *Enterobacteriaceae* could be enumerated, 34 were treated at 84 to 85°C and 66 at 87 to 88°C. For total aerobic bacteria, 34 samples were treated at 84 to 85°C and 69 samples at 87 to 88°C. No significant difference in the reduction in any of the bacteria was observed between the different temperatures ($P = 0.1$ to 1.0).

Reduction of bacteria. Analysis of *C. jejuni* with simple linear regression showed that in general, there was a weak correlation ($R^2 = 0.27$) between the levels of *C. jejuni* bacteria on the neck skin samples before the treatment and the reduction achieved in this bacterial species (Fig. 4). The regression model resulted in a weak positive slope (0.2) that was statistically significant ($P = 0.04$, $R^2 = 0.08$). For the other parameters, the correlation was stronger ($R^2 = 0.7$, 0.6, and 0.6 for *Enterobacteriaceae, E. coli,* and total aerobic bacteria, respectively). The regression models for these groups resulted in a positive slope (0.6, 0.6, and 0.7, respectively) and were all highly significant ($P < 0.0001$; $R^2 = 0.4$, 0.3, and 0.7, respectively).

DISCUSSION

The reduction in *Campylobacter* achieved by the ultrasound-steam treatment in this study was not as high as reported in previous studies. In a Danish study, the mean reduction in *Campylobacter* on carcasses following ultrasound-steam treatment was around 1.0 log CFU/g *(20)*. The line speed in that slaughterhouse was, on average, 8,500 chickens per hour, while in our study, it was 18,000 chickens per hour. Higher line speed meant that the carcasses were exposed to the ultrasound-steam treatment for a shorter period. Other studies have indicated that longer treatment times result in higher reductions in bacteria *(2, 9, 19)*. Ultrasound-steam treatment for 5 s on the inside of the carcasses and 10 s on the outside has been found to result in a reduction in *Campylobacter* of around 2.5 log CFU/mL *(9)* or 2.5 log CFU per carcass *(2)*. The reductions in total viable bacteria also seem to be dependent on the duration of treatment, with, e.g., increased treatment time from 0.5 to 4 s on pork skin resulting in a final reduction in total viable bacteria of up to 3.3 log CFU/cm^2 *(19)*. Other studies examining steam pasteurization without the ultrasound component have also found a relationship between longer treatment times and higher reductions in bacteria *(15, 29)*.

The ultrasound-steam equipment can produce steam at up to 90 to 94°C. In this study, the temperature applied was either 84 to 85°C or 87 to 88°C, because the spices used later for some chicken products do not adhere properly to carcass skin treated at temperatures above 90°C. We did not find any significant difference in bacterial reductions between the two different treatment temperatures. In some cases, ultrasound-steam treatment at higher temperature has damaged the skin of the carcass, altering the product quality *(2, 20)*, whereas in other cases, no visual changes have been detected *(9)*.

We found a weak correlation ($P = 0.04$) between the initial concentration of *Campylobacter* and the reduction achieved by treatment. However, this correlation was stronger ($P < 0.0001$) for the other bacteria studied (*Enterobacteriaceae, E. coli,* and total aerobic bacteria), probably because it is easier to reduce the amount of bacteria if the level is high from the beginning. Since the samples in our study were obtained after treatment by ultrasound-steam and air chilling, we cannot exclude the possibility that air chilling contributed to the reduction in bacteria. Other studies have shown varying results for the

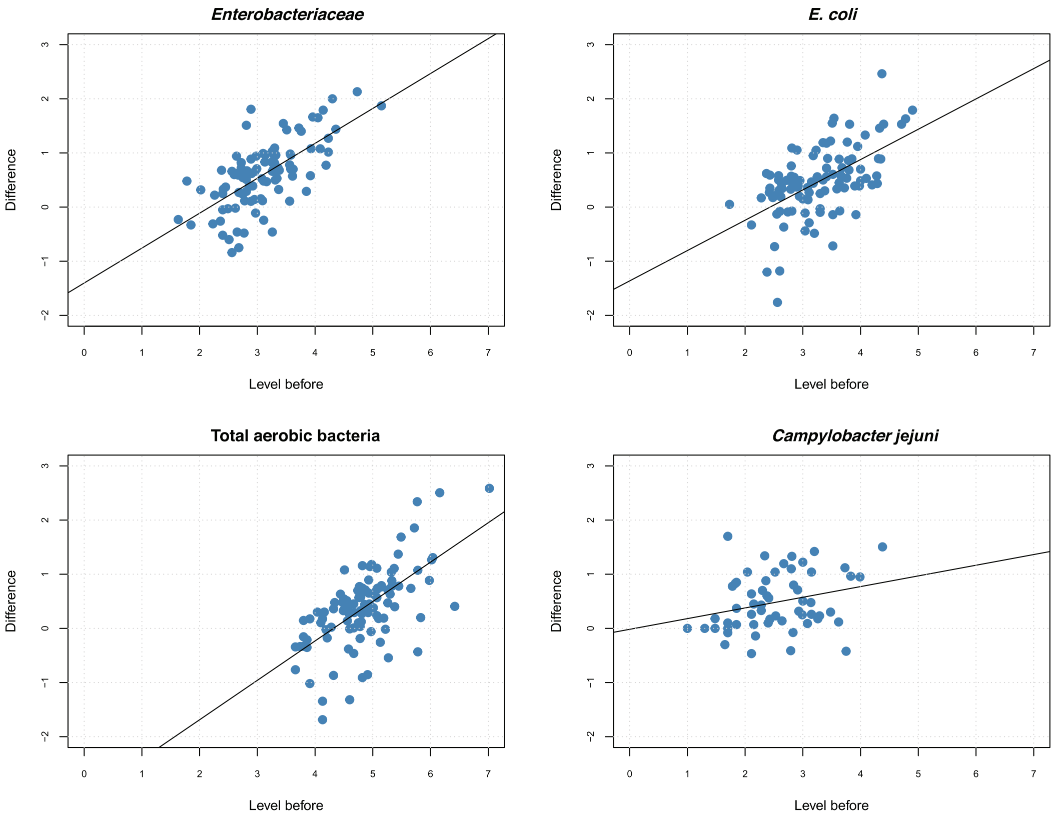


FIGURE 4. *Results of simple linear regression. The horizontal axis indicates the levels of* C. jejuni, Enterobacteriaceae, E. coli, *and total aerobic bacteria before the treatment, while the vertical axis shows the reductions (differences) in* C. jejuni, Enterobacteriaceae, E. coli, *and total aerobic bacteria numbers after the ultrasound-steam treatment.*

effect of air chilling on concentrations of bacteria. A significant reduction of 0.4 log CFU/g due to air chilling was found in one study *(2),* while another study found no significant effect of air chilling by itself without ultrasound-steam *(20)*.

The increase in *Campylobacter* observed in seven samples could be due to difficulties in enumeration of *Campylobacter* because the amounts of the other bacteria analyzed were reduced in these samples. Colonies of thermotolerant *Campylobacter,* especially *C. jejuni,* tend to spread, which results in difficulties in counting on some mCCDA plates because of swarming of *Campylobacter* colonies (ISO 10272-2 *(14)*). Another reason for the increase in *Campylobacter* observed in some samples could be variation in oxygen levels during transport, as *Campylobacter* spp. are microaerophilic and the atmosphere during transportation affects their survival. Although the reduction in *C. jejuni* was significant in this study, there were still large amounts of *Campylobacter* on the carcasses after treatment, with 12% of the samples having levels that exceeded 3 log CFU/g. It should be noted that the flocks chosen for this study were delivered from broiler producers that had previously often delivered chickens colonized with *Campylobacter* to slaughter. According to the process hygiene criterion for slaughter of broilers in the European Union *(4),* slaughterhouses have to ensure that the amount of *Campylobacter* bacteria on the neck skin does not exceed 3 log CFU/g, due to the risk of humans getting campylobacteriosis. However, in this study, we quantified the neck skins individually, whereas in the process hygiene criterion, a pool of at least 15 neck skins is analyzed. If the criterion is not fulfilled, the slaughterhouse must take action to improve slaughter hygiene, review process controls, identify the farm of origin, and review biosecurity measures on the farm of origin *(4)*.

This study examined full-scale ultrasound-steam treatment of broiler carcasses naturally contaminated with bacteria and found that the treatment achieved a significant reduction ($P < 0.0001$) in bacteria, but the levels of bacteria left on the carcasses after treatment were still high. Further studies are needed to determine whether the effectiveness of ultrasound-steam treatment is dependent on the duration of treatment and/or the temperature of the steam. In a One Health perspective, optimal measures must be implemented

at slaughter to reduce the numbers of food spoilage bacteria and pathogenic bacteria.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the slaughterhouse that participated in the study. The authors also thank the Ivar and Elsa Sandbergs Research Foundation for funding the project.

REFERENCES

1. Avens, J. S., S. N. Albright, A. S. Morton, B. E. Prewitt, P. A. Kendall, and J. N. Sofos. 2002. Destruction of microorganisms on chicken carcasses by steam and boiling water immersion. *Food Control* 13:445–450.
2. Boysen, L., and H. Rosenquist. 2009. Reduction of thermotolerant *Campylobacter* species on broiler carcasses following physical decontamination at slaughter. *J. Food Prot*. 72:497–502.
3. Centers for Disease Control and Prevention. 2019. Surveillance for foodborne disease outbreaks United States, 2017: annual report. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta.
4. European Commission. 2005. Commission Regulation (EC) No 2073/2005 of the European Parliament and of the Council of 15 November 2005 laying down rules on microbiological criteria for foodstuff. *Off. J. Eur. Union* L 338:1–26.
5. European Food Safety Authority. 2010. Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008—part A: *Campylobacter* and *Salmonella* prevalence estimates. *EFSA J*. 8:1503.
6. European Food Safety Authority, European Centre for Disease Prevention and Control. 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA J*. 16:5500.
7. European Food Safety Authority, Panel on Biological Hazards. 2011. Scientific opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA J*. 9:2105.
8. Frosth, S., O. Karlsson-Lindsjö, A. Niazi, L.-L. Fernström, and I. Hansson. 2020. Identification of transmission routes of campylobacter and on-farm measures to reduce campylobacter in chicken. *Pathogens* 9:363.
9. Hansen D., and B. S. Larsen. 2007. Reduction of *Campylobacter* on chicken carcasses by SonoSteam treatment. Proceedings of European Congress of Chemical Engineering (ECCE-6), Copenhagen, 16 to 20 September 2007.
10. Hansson, I., L. Plym Forshell, P. Gustafsson, S. Boqvist, J. Lindblad, E. Olsson Engvall, Y. Andersson, and I. Vågsholm. 2007. Summary of the Swedish *Campylobacter* program in broilers, 2001 through 2005. *J. Food Prot*. 70:2008–2014.
11. Hansson, I., N. Pudas, B. Harbom, and E. Olsson Engvall. 2010. Within-flock variations of *Campylobacter* loads in caeca and on carcasses from broilers. *Int. J. Food Microbiol*. 141:51–55.
12. Hansson, I., M. Sandberg, I. Habib, R. Lowman, and E. Olsson Engvall. 2018. Knowledge gaps in control of *Campylobacter* for prevention of campylobacteriosis. *Transbound. Emerg. Dis*. 65:30–48.
13. Health Protection NSW, Communicable Diseases Branch. 2019. NSW OzFoodNet annual surveillance report: 2018. Health Protection NSW, Sydney.
14. International Organization for Standardization. 2017. Microbiology of the food chain—horizontal method for detection and enumeration of *Campylobacter* spp.—part 2: colony-count technique. ISO 10272-2. International Organization for Standardization, Geneva.
15. James, C., S. J. James, N. Hannay, G. Purnell, C. Barbedo-Pinto, H. Yaman, M. Araujo, M. L. Gonzalez, J. Calvo, M. Howell, and J. E. L. Corry. 2007. Decontamination of poultry carcasses using steam or hot water in combination with rapid cooling, chilling or freezing of carcass surfaces. *Int. J. Food Microbiol*. 114:195–203.
16. Lindblad, M., I. Hansson, I. Vågsholm, and R. Lindqvist. 2006. Postchill *Campylobacter* prevalence on broiler carcasses in relation to slaughter group colonization level and chilling system. *J. Food Prot*. 69:495–499.
17. Luber, P., S. Brynestad, D. Topsch, K. Scherer, and E. Bartelt. 2006. Quantification of *Campylobacter* species cross-contamination during handling of contaminated fresh chicken parts in kitchens. *Appl. Environ. Microbiol*. 72:66–70.
18. McCann, M. S., J. J. Sheridan, D. A. McDowell, and I. S. Blair. 2006. Effects of steam pasteurisation on *Salmonella* Typhimurium DT104 and *Escherichia coli* O157:H7 surface inoculated onto beef, pork and chicken. *J. Food. Eng*. 76:32–40.
19. Morild, R. K., P. Christiansen, A. H. Sørensen, U. Nonboe, and S. Aabo. 2011. Inactivation of pathogens on pork by steam-ultrasound treatment. *J. Food Prot*. 74:769–775.
20. Musavian, H. S., N. H. Krebs, U. Nonboe, J. E. L. Corry, and G. Purnell. 2014. Combined steam and ultrasound treatment of broilers at slaughter: a promising intervention to significantly reduce numbers of naturally occurring campylobacters on carcasses. *Int. J. Food Microbiol*. 176:23–28.
21. Nauta, M. J., M. Sanaa, and A. H. Havelaar. 2012. Risk based microbiological criteria for *Campylobacter* in broiler meat in the European Union. *Int. J. Food Microbiol*. 158:209–217.
22. NordVal International. 2005. Enterobacteriaceae. Determination in foods and feeds. NMKL 144, 3rd ed. NordVal International, Bergen, Norway.
23. NordVal International. 2005. Thermotolerant coliform bacteria and *Escherichia coli*. Enumeration in food and feed. NMKL 125, 4th ed. NordVal International, Bergen, Norway.
24. NordVal International. 2013. Aerobic microorganisms. Determination in foods at 37 °C, 30 °C, 25 °C, 20 °C, 17/7 °C or 6.5 °C by the colony count method. NMKL 86, 5th ed. NordVal International, Bergen, Norway.
25. Nutsch, A. L., R. K. Phebus, M. J. Riemann, D. E. Schafer, J. E. Boyer, R. C. Wilson, J. D. Leising, and C. L. Kastner. 1997. Evaluation of a steam pasteurization process in a commercial beef processing facility. *J. Food Prot*. 60:485–492.
26. Rosenquist, H., H. M. Sommer, N. L. Nielsen, and B. B. Christensen. 2006. The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. *Int. J. Food Microbiol*. 108:226–232.
27. Stern, N. J., and M. C. Robach. 2003. Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. *J. Food Prot*. 66:1557–1563.
28. Swart, A., M.-J. Mangen, and A. H. Havelaar. 2013. Microbiological criteria as a decision tool for controlling *Campylobacter* in the broiler meat chain. RIVM letter report 330331008/2013. National Institute for Public Health and the Environment, Bilthoven, The Netherlands.
29. Whyte, P., K. McGill, and J. D. Collins. 2003. An assessment of steam pasteurization and hot water immersion treatments for the microbiological decontamination of broiler carcasses. *Food Microbiol*. 20:111–117.

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2023:88

Proper cleaning and disinfection in slaughterhouses and meat processing premises is a pre-requisite for production of safe, high-quality meat. This thesis evaluated the efficacy of cleaning and disinfection procedures in slaughterhouses and adjacent meat processing premises by testing for pathogenic and hygiene indicator bacteria on equipment, facilities and carcasses. The results showed that current cleaning and disinfection procedures in slaughterhouses and meat processing premises do not always reduce bacteria to an acceptable level.

Madeleine Moazzami received her graduate education at the Faculty of Veterinary Medicine. Her veterinary degree was obtained from the University of Las Palmas de Gran Canaria (ULPGC).

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

ISSN 1652-6880
ISBN (print version) 978-91-8046-230-3
ISBN (electronic version) 978-91-8046-231-0