UNIVERSITY DE SAO PAULO

FACULTY OF PHARMACEUTICAL SCIENCES

Graduate Program in Food Science

Cross contamination of *Listeria monocytogenes* in ready-to-eat meat product during slicing: a predictive approach

Janaina Thais Lopes

Thesis for obtaining the title of

DOCTOR

Supervisor:

Profa. Dra. Bernadette Dora Gombossy de Melo Franco

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Janaina Thais Lopes

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Profa. Dra.

Sao Paulo, March 2017

Let your ear be open to suggestion and take teaching, so that at the end you may be wise.

A man's heart may be full of designs, but the purpose of the Lord is unchanging.

Proverbs 19:20-21

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ABSTRACT

LOPES, J.T. **Cross contamination of *Listeria monocytogenes* in ready-to-eat meat product during slicing: a predictive approach.** 2017 70p. Thesis (Doctoral) - Faculty of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, 2017.

Ready to eat (RTE) meat products are subject to recontamination after industrial processing, mainly by *Listeria monocytogenes*, a pathogenic microorganism that can persist for a long time in the environment. A RTE meat product that is contaminated with *L. monocytogenes* due to cross contamination during some stage after industrial processing, such as weighing, slicing or wrapping, can be an important causer of disease, due to absence of a kill step before consumption. The objective of this project was to measure the transfer of *L. monocytogenes* during slicing of cooked ham (cross contamination) at retail, simulating in the laboratory the practices in commercial slicing, and to develop a predictive model capable of describing this transfer. It was observed that in the first slices obtained after the experimental contamination of the slicer, the counts and the transfer rates of *L. monocytogenes* were higher than in the subsequent slices, and the counting curves presented a long tail as the slices were obtained. The data demonstrate that the slicer may be a relevant source of cross contamination of *L. monocytogenes* for RTE meat products, regardless of the level of contamination of the slicer. With the data obtained, a new transfer model was proposed called 4p-2se, as it contained four parameters (4p) and two environments (2se), and was independent of the quantification of the pathogen transferred to the slicer. The proposed model was compared to two pathogen transfer models previously described, and the predicted data presented lower RMSE (Root Mean Sum of squared errors) values than the other models. The 4p-2se model was able to satisfactorily predict the pathogen transfer data during slicing of cooked ham, which could assist the food retail establishments and regulatory agencies in the evaluation and control of cross contamination of RTE foods and in the design of proper risk management strategies.

Key words: Cooked ham, cross contamination, *Listeria monocytogenes*, predictive modeling

RESUMO

LOPES, J.T. **Contaminação cruzada de *Listeria monocytogenes* em produto cárneo pronto para o consumo durante o fatiamento: uma abordagem preditiva.** 2017 70p. Tese (Doutorado) - Faculdade de Ciências Farmacêuitcas, Universidade of São Paulo, São Paulo, 2017.

Os produtos derivados da carne que são prontos para consumo estão sujeitos à recontaminação após o processamento industrial, principalmente por *Listeria monocytogenes*, um microrganismo patogênico capaz de persistir por longo tempo no ambiente. Um produto cárneo pronto para consumo que se contamina com *L. monocytogenes* devido à contaminação cruzada durante alguma etapa após o processamento industrial, tal como pesagem, fatiamento ou acondicionamento, pode ser um importante causador de enfermidade, pois não há uma etapa de eliminação do patógeno antes do consumo. Este projeto teve por objetivo mensurar a transferência de *L. monocytogenes* durante o fatiamento de presunto cozido (contaminação cruzada), simulando em laboratório práticas adotadas nos estabelecimentos comerciais de fatiamento de produtos prontos para o consumo, e desenvolver um modelo preditivo capaz de descrever esta transferência. Foi observado que nas primeiras fatias obtidas após a contaminação experimental do fatiador, as contagens e as taxas de transferência de *L. monocytogenes* eram mais altas que nas subsequentes, observando-se que as curvas de contagem apresentavam uma longa cauda ao longo do fatiamento. Os dados demonstram que o fatiador pode ser uma fonte importante de contaminação cruzada de *L. monocytogenes* para produtos cárneos prontos para o consumo fatiados, independentemente do nível de contaminação do fatiador. Com os dados obtidos, foi possível sugerir um novo modelo de transferência, denominado 4p-2se, formado por uma equação com apenas quatro parâmetros (4p) e dois ambientes (2se,) sendo esse modelo independente da quantificação do patógeno transferido para o fatiador. O modelo sugerido foi comparado a outros dois modelos de transferência previamente descritos, observando os dados preditos no modelo 4p-2se apresentavam valores de RMSE (*Root Mean Sum of squared erros*) mais baixos que os demais modelos. O modelo proposto mostrou-se capaz de predizer satisfatoriamente os dados de transferência de patógeno durante o fatiamento de presunto cozido, podendo auxiliar os estabelecimentos comerciais de alimentos e as agências reguladoras na avaliação e controle da contaminação cruzada de alimento prontos para consumo e na concepção de estratégias adequadas de gestão de risco.

Palavras-chave: Presunto cozido, contaminação cruzada, *Listeria monocytogenes*, modelagem preditiva

SUMMARY

I. ORGANIZATION OF THE THESIS

This thesis was divided into chapters, as follows:

Chapter 1- Introduction and objectives

1.1 Ready-to-eat meat products

1.2 *Listeria monocytogenes:* importance in ready-to-eat foods

1.3 Predictive microbiology and microbial transfer (cross-contamination)

1.4 Objectives

Chapter 2. Quantitative assessment of *Listeria monocytogenes* transfer during slicing of ready-to-eat cooked ham (Manuscript in preparation for publication)

Chapter 3. A semi-mechanistic modeling approach to describe the transfer of *Listeria monocytogenes* during slicing of ready-to-eat cooked ham (Manuscript in preparation for publication).

References

II. CHAPTER 1

1. INTRODUCTION AND OBJECTIVES

1.1 Ready-to-eat meat products

Ready-to-eat (RTE) meat products are defined as products consisting of or containing meat such as meats in sauce, smoked meat, fried meat, sausage and cooked dried meat. Microbiological surveillance of RTE meat products provides empirical data to inform scientific guidance for improving food safety. Surveillance data may be used to inform the development of food safety standards and define research priorities based on risk assessments. Also it may provide an indication of direct consumer exposure. The growing preference for convenience is one of the major trends in food consumption, and RTE products have increasingly gained share (BRASIL, 2000; ACNIELSEN, 2004; YANG et al., 2016).

Due to its intrinsic characteristics, meat is an excellent substrate for survival and growth of numerous microorganisms and many factors may favor this microbial development. Meat is exposed to microbial contamination at all stages of processing, particularly in the operations where it is most handled and where special care is not taken in relation to Good Manufacturing Practices (PARDI et al., 2001; SOFOS, 2008).

Processing can greatly reduce or even eliminate the microbial load in meat, but many ready-to-eat meat products are susceptible to post-processing contamination that occur during operations such as weighing, grinding, mixing and storage, affecting their safety and shelf life (ANONYMOUS, 2006; SISKOS et al., 2007). As these products do not need to be cooked before consumed it is extremely important to keep them at proper storage temperature to avoid microbial multiplication (DONDERO et al., 2004; FRANCO et al., 2010).

The transfer of pathogenic microorganisms from a contaminated food to an uncontaminated one, directly or indirectly, through contact with contaminated equipment, air, manipulators and / or food, is called cross contamination (PÉREZ-RODRIGUÉZ et al., 2008). Cross-contamination is more important when it occurs with ready-to-eat foods, which are not subjected to a microbial inactivation process prior to consumption, putting the health of the consumer at risk (SHEEN, HWANG, 2010; CHEN, ZHAO & DOYLE, 2014). There are numerous potential sources of cross-contamination during the production and/or preparation of ready-to-eat foods, such as work surfaces, equipment, handlers, utensils, etc. (MUÑOZ et al., 2013).

In the case of ready-to-eat meat products, the slicing area in food marketing establishments corresponds to the most critical area in relation to the occurrence of cross contamination due to deficiency in good hygiene practices. The degree of cross-contamination of these foods is related to the temperature of the slicing environment and the process of cleaning the surfaces in contact with the products (ORGANIZAÇÃO PAN-AMERICANA DA SAÚDE, 2006; ROUCOURT: BUCHIESER, 2007; NASCIMENTO et al., 2010).

Surface cross-contamination of foodborne pathogens in food products during processing or preparation is a major concern to consumers and food manufacturers. Although risk assessment and analyses have been applied to monitor and to reduce the hazards, some knowledge gaps, including surface cross-contamination after the industrial processing stage, need to be addressed for proper risk assessments (GALLAGER et al., 2003). Surface foodborne pathogen transfer during slicing of RTE meat products, such as ham, salami, bologna and other restructured meat, is one of the most important factors to impact their safety. These products are sold in supermarkets in the refrigerated section, either produced by brand-named manufacturers or prepared in-store, made-to-order. Sliced RTE products are also commonly sold in delicatessen and fast food restaurants, where a retail-scale slicer is often used on site for meal or sandwich preparations. The slicing equipment, if not properly cleaned and sanitized, can cause microbial cross-contamination (SHEEN & HWANG, 2010; HENRIQUES et al., 2017).

1.2 *Listeria monocytogenes:* importance in ready-to-eat foods

Listeria monocytogenes is easily found in foods. Its presence has been reported in raw and processed foods such as bovine meat, poultry meat, seafood, vegetables, milk, dairy products, refrigerated and ready-to-eat products, soft cheeses, processed and smoked meats (MARTINS, 2009; GONZÁLES et al., 2013; SYNE et al., 2013). Several outbreaks of foodborne diseases have been associated to heat treated ready-to-eat (RTE) meat products (GOTTLIEB et al., 2006; CARTWRIGHT et al., 2013; THOMAS et al., 2015), and occurrence of pathogens such as *Listeria monocytogenes* and *Salmonella* spp is common (GARRIDO et al., 2009; MEYER et al., 2012; MODZELEWSKA-KAPITULA & MAJ-SOBOTKA, 2014; OSAILI et al., 2014; GOMEZ et al., 2015).

According to the CDC (Centers for Disease Control and Prevention, USA), *L. monocytogenes* was responsible for 28% of food-related deaths in 2000 (MURPHY et al., 2005). Fresh high moisture cheeses and meat products, especially those ready for consumption as sausages and pates, are the foods most commonly associated with this

disease (SWAMINATHAN & GERNER-SMIDT, 2007). Warriner & Namvar (2009) reported an outbreak of listeriosis caused by consumption of sliced meat products in 2008 in Canada, with 53 confirmed cases and 20 deaths. A risk assessment of 23 selected categories of RTE foods, published in 2003, identified deli meats as the food category with the greatest associated public health risk (FDA/FSIS, 2003). Risk assessments estimate that more than 70% of those listeriosis cases attributable to deli meats are associated with deli meats sliced at retail and formulated without growth inhibitors (ENDRIKATet al., 2010; PRADHAN et al., 2010).

Very little is known on the characteristics of *L. monocytogenes* in humans in Brazil, certainly due to under-notification of foodborne diseases and difficulties in diagnosis of listeriosis (GERMANO AND GERMANO, 2008, MARTINS & GERMANO, 2011). The current Brazilian food regulations are vague in relation to *L. monocytogenes,* and testing of this pathogen is required only in high humidity cheeses (absence in 25g) (BRASIL, 2001). Hofer, Reis, and Hofer (2006) studied 255 strains isolated from the spinal fluid and blood of patients showing possible invasive disease and healthy individuals from several regions of Brazil (1969 – 2000) and observed predominance of serovars 4b (60.3%) and 1/2a (29%). Schwab & Edelweiss, 2003, tested 148 placentas obtained from abortions or premature deliveries and *L. monocytogenes* was detected in 33.8% of them.

Different foods have been associated to isolated cases and outbreaks of listeriosis worldwide, but some types of food products, such us those that are ready-to-eat and have long shelf-life under refrigeration are considered more important as they pose a higher risk (MARTINS & GERMANO, 2011). Several studies have investigated the prevalence of *L. monocytogenes* in Brazilian meat products. Borges et al. (1999) tested four types of salami from different commercial brands, and *L. monocytogenes* was found in 13.3% of the samples. Araújo et al. (2002) tested two types of products formulated with turkey meat (ham and blanquet), comparing positivity for *L. monocytogenes* in whole products acquired in their original package and in samples of the same type of products sliced in the store. The pathogen was not detected in whole products, but 60.0% and 50.0% of sliced ham and blanquet, respectively, were positive. Sakate et al. (2003) investigated *L. monocytogenes* in different types and brands of sliced salami sold in the city of Sao Paulo (Brazil), and detected the pathogen in 6.7% (3/45) of the samples. These findings are in agreement with observations of EFSA (European Food Safety Authority 2013), indicating that the prevalence of *L. monocytogenes* in retail sliced meat products is approximately seven times higher than that observed in products that are sliced and packed in processing facilities under federal inspection. Some authors reported that inadequate food handling in the slicing process was

likely responsible for the positivity of *L. monocytogenes* in sliced products (ARAUJO et al., 2002; GARRIDO et al., 2009; PÉREZ-RODRÍGUEZ et al., 2010; LAKICEVIC et al., 2015).

Some studies have shown that *L. monocytogenes* and other *Listeria* species are able to remain adhered to equipment and surfaces throughout the processing of a food, as they are excellent biofilm-forming agents. When forming biofilms, they are protected from adversities such as dehydration and bacteriophage colonization, and present higher antimicrobial resistance. Biofilms may be responsible for the spread of microorganisms through food during the processing line, and a number of studies reported the importance of pathogen transfer (cross-contamination) during slicing of meat products, especially *Listeria monocytogenes*, *Salmonella* spp and *Escherichia coli* O157:H7 (PÉREZ-RODRIGUÉZ et al., 2006; SHEEN, HWANG, 2010; CHEN, ZHAO E DOYLE, 2014; ERICKSON et al., 2015, PALMER et al., 2007; RIEU et al., 2007; ADRIAO et al., 2008; OLIVEIRA et al., 2010; TAKAHASHI et al., 2010; LAKICEVIC et al., 2015).

Proper cleaning and disinfection of the contact surfaces of equipment and utensils in food processing play an important role in the control of biofilm formation and the spread of cross-contamination. Jemmi and Stephan (2006) reported that the *L. monocytogenes* is able to survive on different types of surfaces, creating the risk for cross-contamination between the environment and processing equipment. Cleaning involves the removal of organic and mineral residues by some mechanical process or application of detergent and then disinfection with appropriate chemical sanitizers which inactivate the microorganisms, which may be based on chlorine, iodine, quaternary ammonium compounds, and peracetic or other acids. These sanitizers generally cause a reduction of 2 to 4 Log CFU/cm^2 in the count of *L. monocytogenes* (BRASIL, 2002; KESKINEN et al., 2008; SHEEN & HWANG, 2010; BISBIROULAS *et al.*, 2011; YUN et al., 2012).

L. monocytogenes is a pathogenic microorganism widely distributed in nature, and can be found in soil, silage, food processing plants, and animal and human feces. Food is the main vehicle of this pathogen for humans. An important feature that contributes to this fact is its ability to survive and multiply even at refrigeration temperatures and in modified atmospheres (JAY, 2005; GRAY et al., 2006; MARTINS, 2009; STRAWN et al., 2013). This pathogen is a non-spore forming Gram-positive bacillus, facultative anaerobic, mobile due to peritrichous flagella and presents a positive reaction for catalase and negative for oxidase. It multiplies in temperatures from 0 to 45 °C and presents optimum multiplication in pH between 6 and 8. It is capable to multiply at Aw as low as 0.93 and is resistant to freezing and treatment with sodium nitrate (120mg/kg) and sodium chloride (3%) (FORSYTHE, 2002; JAY, 2005; GERMANO & GERMANO, 2008; BUCHANAN et al., 2017).

Once ingested through a contaminated food, the pathogen can invade the gastrointestinal epithelium and pass into the bloodstream, and reach the liver, spleen and lymphatic system. In more severe cases *L. monocytogenes* can cause septicemia, encephalitis and meningitis and be fatal (JEMMI & STEPHAN, 2006; GERMANO & GERMANO, 2008; MARTINS & GERMANO, 2011). This pathogen can cross the placenta barrier and infect the fetus, leading to premature delivery or even abortion. The incubation period of listeriosis is from 1 to 7 days, hampering the identification of the food vehicle. The main symptoms are similar to those of the flu, with abdominal pain and diarrhea. The disease is more serious in immunocompromised individuals, the elderly, pregnant women and newborns (CDC, 2013). It is believed that the infective dose capable of causing the disease in these individuals is 2 to 8 Log UFC/g (WILLIAMS et al., 2007; SMITH et al., 2008; WARRINER & NAMVAR, 2009).

1.3 Predictive Microbiology and microbial transfer (cross contamination)

Predictive microbiology (quantitative microbial food ecology) is an essential component of modern food microbiology. Predictive microbiology unifies microbiology, engineering and statistics to obtain predictions about the behavior of microorganisms in food systems using mathematical tools, that can be used to create models that estimate variations in microbial population as a function of time, effects of environmental conditions on the speed of multiplication or inactivation, and also the interference of the food matrix in microbiological growth. Modeling provides important information for decision making, risk analysis, shelf life assessment, as well as for the development of new products or processes (NAUTA et al., 2005; SARMENTO, 2006; AARNISALO et al., 2007; BILLOIR et al., 2011; FAKRUDDING et al., 2011).

Predictive microbiological models can be divided into two categories: mechanistic (or deterministic) and empirical. Mechanistic models describe the microbial response theoretically, and are rarely used because of the complexity of microbial physiology in food. Empirical models are more commonly used because they describe a set of data by means of a mathematical relationship that best fits microbial physiology (KRIST et al., 1998; McKEEKIN et al., 2002; MOLLER et al., 2016a; MOLLER et al., 2016b).

Knowledge of the environmental parameters that most influence microbial multiplication is essential for the development and practical use of the models. Among these parameters, the most important are temperature, pH and Aw (SCHAFFNER & LABUZA, 1997). As an example, Gill et al. (1991) used predictive modeling to verify the hygiene of meat processing operations by modeling the behavior of *E. coli* in meat carcasses, based

only on temperature response, ignoring other important factors, leading to imprecise prediction and possibly not in accordance with what actually occurs. Newer models were based on broader data sets (water activity, pH and lactate concentration), improving their prediction power (PRESSER et al., 1997). This example emphasizes that knowledge is cumulative and leads to the development of better and more complete models, increasing the scope of application (McMEEKIN et al., 2002; MOLLER et al., 2016).

Some studies have modeled the transfer of pathogens from stainless steel surfaces, such as kitchen utensils, slicers and meat grinders, to food (KESKINEN et al., 2008; KUSUMANINGRUM et al., 2003; SHEEN & HWANG, 2010; MOLLER et al., 2012, MOLLER et al., 2016). Recent bacterial transfer work with mechanical delicatessen slicers has shown that *L. monocytogenes* readily transfers both to and from slicer blades and meats (MIDELET et al., 2006; VORST et al., 2006; AARNISALO et al., 2007; CHAITIEMWONG et al., 2014).

The factors contributing to *L. monocytogenes* cross-contamination at retail, however, remain not well understood. Mathematical models of environmental cross-contamination offer a valuable alternative to studies in retail facilities as they allow for the expeditious and cost-effective evaluation of cross-contamination risks and enable exploration of the effects of different management practices. This work aimed at contributing with data for enlargement of the knowledge on mathematical models capable to describe the cross-contamination that occurs when ready-to-eat cooked ham is sliced, simulating in laboratory practices that are adopted in most retail stores.

This work was developed in the frame of the MEAT-CROSS-CON project (Meat safety: an innovative modelling approach to evaluate microbial pathogen transfer and cross contamination from farm to fork), supported by Sao Paulo Research Foundation (FAPESP) and Danish Research Science Foundation (DRSF ex-Innovation Fund Denmark - IFD), in partnership with researchers from the National Food Institute, Technical University of Denmark, Denmark (FAPESP 2012/50535-5).

1.4 OBJECTIVES

1.4.1 General objective

To study the transfer (cross-contamination) of *Listeria monocytogenes* in cooked ham during slicing in a manual slicer, and develop a predictive model capable of describing the observed cross contamination.

1.4.2 Specific objectives

a. To evaluate the transfer profile of *L. monocytogenes* during slicing in a manual slicer experimentally contaminated with the pathogen by slicing a piece of experimentally contaminated cooked ham, simulating in laboratory the procedures adopted at retail establishments in the city of São Paulo, SP, Brazil.

b. To develop a predictive model capable of describing the transfer of *L. monocytogenes* from the experimentally contaminated slicer to non-inoculated cooked ham during slicing and to estimate the transfer of the pathogen to the resulting slices.

III. Chapter 2

Quantitative evaluation of the transfer of *Listeria monocytogenes* during slicing of ready-to-eat cooked ham

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Abstract

Cross contamination of RTE foods with *Listeria monocytogenes* in operations at retail such as slicing can result in foodborne listeriosis. The aim of this study was to evaluate the transfer of *L. monocytogenes* to slices of ready-to-eat cooked ham during slicing, simulating in laboratory the practices observed in retail stores and assuming that the slicer was contaminated with the pathogen at two different levels of contamination (high and moderate). The effect of the quality of the slicing blade (new or damaged) was also investigated. Four challenge tests were performed: A) slicing of cooked ham in the slicing machine experimentally contaminated with *L. monocytogenes* at high inoculum level (8 log CFU/slice) using an old blind blade; B) slicing of cooked ham in the slicing machine experimentally contaminated with *L. monocytogenes* at high inoculum level (8 log CFU/slice) using a new blade; C) slicing of cooked ham in the slicing machine experimentally contaminated with *L. monocytogenes* at moderate inoculum level (6 log CFU/slice) using a new blade; D) slicing of cooked ham in the slicing machine experimentally contaminated with *L. monocytogenes* at high inoculum level (8 log CFU/slice) using an old blind blade, with a recontamination step during the slicing procedure. Results indicated that, once contaminated, the slicing machine can be source of *L. monocytogenes* up to the 190th slice obtained after the contamination. The level of contamination of the slicing machine, but not the type of blade, influenced significantly the Transfer Rates. Long tails on the cross-contamination graphs indicate that *L. monocytogenes* can be transferred to a large number of slices of RTE meat products via slicing machines, evidencing the need of good hygiene practices in the slicing environment.

Keywords: ready-to-eat meat, cooked ham, transfer pathogen, *Listeria monocytogenes.*

1. Introduction

Listeria monocytogenes is a foodborne pathogen of great concern to food industry. The disease, listeriosis, has a high case-fatality rate (~20 to 30% of cases) and affects primarily the elderly, pregnant women, neonates, debilitated and immunocompromised people. Clinical manifestations range from febrile gastroenteritis to more severe invasive forms, including septicemia, meningitis, perinatal infections and abortions. This bacterium is ubiquitous in the environment and can survive a wide range of stress conditions, such as high salt concentration, low temperature, low water activity and low pH, which makes it problematic for food producers. Presence of *L. monocytogenes* in the food processing environment is thought to be the primary source of post-processing contamination during food manufacturing and in retail or food service settings (Allerberger and Wagner, 2010; Buchanan et al, 2017).

Deli-meats and other types of ready-to-eat (RTE) meat products have been implicated in outbreaks of listeriosis in many parts of the world (Cartwright et al., 2013, Gormley et al, 2010). Based on data from a quantitative risk assessment on *L. monocytogenes* carried out in the United States for 23 categories of RTE (RTE) foods, deli meats were classified in the “very high risk” category (>1,500 cases domestically per annum) (FDA/FSIS, 2003).

RTE meat products became increasingly popular in the recent years due to the convenience they offer, in addition to the perceived healthiness (lean) and good taste (Jiang & Xiong, 2015). These meat products can be retailed in large pieces or pre-sliced in the processing industry, but it is very common that large pieces are sliced at the moment of purchase, in a consumer-demand fashion. This step is considered one of the most important routes of cross-contamination of RTE meats with *L. monocytogenes*, and may pose a risk for the consumers as there is no further kill treatment before consumption (Gallager, Ebel and Kause, 2003; Sheen, 2008).

This study aimed at performing a quantitative evaluation of the transfer of *Listeria monocytogenes* to slices of RTE cooked ham during slicing, simulating in laboratory the practices observed in retail stores and assuming that the slicer was contaminated with the pathogen at two different levels of contamination (high and moderate). The effect of the quality of the slicing blade (new or damaged) was also investigated.

2. Materials and methods

2.1 *Listeria monocytogenes* strain and inoculum preparation

The challenge strain used in this study was the meat isolate *L. monocytogenes* 046/77 (serotype 1/2c), from the culture collection of the Food Microbiology Laboratory of the Faculty of Pharmaceutical Sciences of University of Sao Paulo, Sao Paulo, Brazil. The frozen culture (-80 °C) was transferred to 2 mL of Brain Heart Infusion broth (BHI; Difco, Becton Dickinson, Maryland, USA), incubated overnight at 37 °C, transferred again to BHI broth and incubated overnight at 37 °C. Cells were washed twice (2810 × g for 5 min at 4 ºC) and the pellet suspended in 0.1% peptone water (Oxoid Ltd, Basingstoke, UK) to achieve ca. 8 log CFU/mL. Exact counts were determined by serial dilution and plating on Listeria Selective Agar Base supplemented with Listeria Selective Supplement SR 0140F (Oxoid Ltd, Basingstoke, UK).

2.2 Slicing machine and cleaning procedure

The study was conducted with a delicatessen type manual slicing machine (Urano SMS-048,Sao Paulo, Brazil)) containing a 210 mm diameter stainless steel (#-304) cutting blade. Before use, the slicing machine was cleaned with Extran neutral detergent (Merck, São Paulo, Brazil), rinsed with water, dried with clean paper towels and sprayed with 70% ethanol. The cutting blade was autoclaved at 121 ^{o}C for 15 min. Before use, the machine was assembled and exposed to UV light (260 nm) for 1 h, and then the blade, cutter and base were swabbed (Absorve, São Paulo, Brazil) and tested for *L. monocytogenes*. The study was conducted with two types of blades: an old blind blade and a new blade.

2.3 Experimental contamination of the slicing machine

Pieces of cooked ham (~3500 g) were purchased in local supermarkets in the city of Sao Paulo, Brazil, and tested for absence of *L. monocytogenes* using ISO 11290-2:1998/Amd.1:2004 (International Organization for Standardization, 2004). The Listeria negative cooked ham pieces were immersed for 30 min in a suspension containing the challenge strain at 8 log CFU/mL (high level of contamination) or 6 log CFU/mL (moderate level of contamination), and dried at room temperature in a safety hood. These experimentally contaminated pieces were sliced in the slicer until five slices (app 10g each) were obtained. Prior to this slicing procedure, a meat matrix was created in the slicing machine by means of slicing a Listeria-free sample of cooked ham. The five slices that

resulted in the experimental contamination of the slicing machine were submitted to enumeration of *L. monocytogenes* (see 2.5).

2.4 Transfer of *L. monocytogenes* from the contaminated slicer to the cooked ham during slicing

Pieces of Listeria-free cooked ham were sliced in the experimentally contaminated slicing machine until 190 slices were obtained. Each slice was introduced in separate sterile bags (Nasco, Fort Atkinson, USA), put on ice until tested, and submitted to enumeration of *L. monocytogenes* (see 2.5). Counts of *L. monocytogenes* (see 2.6) were performed in the first ten slices of cooked ham, every five slices between the 10^{th} up to the 60^{th} slice and every ten slices from the 60^{th} slice up to the 190^{th} slice. The experiments were repeated three times for each level of contamination (high and moderate) and for each type of blade (old blind blade and new blade). An additional experiment was performed simulating a recontamination during the slicing procedure (high level of contamination and old blind blade).

In summary, four challenge tests were performed:

A) slicing of cooked ham in the slicing machine experimentally contaminated with *L. monocytogenes* at high inoculum level (8 log CFU/slice) using an old blind blade;

B) slicing of cooked ham in the slicing machine experimentally contaminated with *L. monocytogenes* at high inoculum level (8 log CFU/slice) using a new blade;

C) slicing of cooked ham in the slicing machine experimentally contaminated with *L. monocytogenes* at moderate inoculum level (6 log CFU/slice) using a new blade;

D) slicing of cooked ham in the slicing machine experimentally contaminated with *L. monocytogenes* at high inoculum level (8 log CFU/slice) using an old blind blade, with a recontamination step during the slicing procedure.

2.5 Enumeration of *Listeria monocytogenes* in the cooked ham slices

The slices in the bags were weighed and added of 0.1% PW (Oxoid, England) to obtain a 1:10 proportion and mixed in a Stomacher 400 Lab-blender (Seward Medical, London, England) for 1 min. Serial decimal dilutions were prepared in 0.1% PW, plated (1 x 100 µl or 3 x 333 µl) onto supplemented Listeria Selective Agar Base and incubated at 37 °C for 24-48 h. Results were expressed as log CFU/slice.

2.6 Data analysis

The experiments were done in triplicate (trials 1, 2 and 3). Enumerations of *L. monocytogenes* were expressed as log mean ± standard deviation. Transfer rates (TR) were calculated according to Montville and Schaffner (2003):

TR (%) = (CFU destination/CFU source) x 100

where “CFU destination” corresponds to counts of *L. monocytogenes* in a given contaminated slice, while “CFU source” corresponds to counts of *L. monocytogenes* in the first slice after the experimental contamination of the slicing machine. Student's t-test was used to determine significant differences ($p \leq 0.05$) in the TR calculated for high and moderate inoculum levels and also for old blind and new blades. The software Sigma Stat version 3.11 (Systat Software Inc., USA) was used for the statistical treatments.

3. Results and Discussion

Listeria monocytogenes was not detected in the purchased cooked ham pieces nor in the slicer surfaces after cleaning.

Figure 1 shows the counts *of L. monocytogenes* (log CFU/slice) in the challenge tests with cooked ham slices obtained after experimental contamination of the slicer at high inoculum level using the old blind and the new blades (Fig 1A and 1B, respectively), at moderate inoculum level using the new blade (Fig 1C) and the high inoculum level and new blade, with a recontamination step during the slicing operation (Fig 1D).

Results in Fig 1A and 1B indicate that the counts of *L. monocytogenes* in slices obtained after experimental contamination of the slicing machine with high level of contamination decreased gradually, from an average of 6.7±0.2 CFU/slice in the first slice to 1.9±0.1 log CFU/slice in the last slice. Student’s test indicated that for this level of contamination, no significant difference was observed for the two types of blades ($p \geq 0.05$).

Fig 1C indicates that the counts of *L. monocytogenes* in slices obtained after experimental contamination of the slicing machine with moderate level of contamination also decreased gradually, from an average of 5.3±0.1 CFU/slice in the first slice to 1.2±0.2 log CFU/slice in the last slice. In this case, Student’s test indicated that for this level of contamination, the difference observed for the two types of blades was significant ($p < 0.05$).

The *L. monocytogenes* transfer rates (RT), expressed as log transfer (%), are shown in Table 1 and Figure 2. The values ranged from 2.27 to -1.17% for challenge test A, from 3.43 to -1.39 for challenge test B and from 2.09 to -2.06% for challenge test C. For challenge

test D, the values ranged from 1.57 to -1.51% after the initial contamination and from 1.30 to -1.19% after the recontamination. Using Student's t-test to compare the RTs, the level of contamination of the slicing machine influenced significantly the RTs ($p<0.05$), but the type of blade did not influence the RTs ($p\geq0.05$). These findings deserve attention as they agree with those reported by Montville and Schaffner (2003) who observed that the inoculum size had a highly significant influence on cross contamination of surfaces with *Enterobacter aerogenes,* but disagree with those reported by Kusumaningrum et al (2002) who observed that the inoculum size did not influence the surface transfer of *Staphylococcus aureus*, *Campylobacter jejuni* and *Salmonella* Enteritidis.

Data presented in this study demonstrate that slicing machines can be the source of cross contamination of RTE meat products, such as cooked ham, with *L. monocytogenes*, once they become contaminated when used to slice other products containing the pathogen, regardless the level of contamination of these products. The cross contamination seems to be influenced by the quality of the blade, as higher Transfer Rates were observed for the slices obtained when a new and sharp blade was used. Long tails on the cross-contamination graphs indicate that *L. monocytogenes* can be transferred to a large number of slices of RTE meat products via slicing machines, evidencing the need of good hygiene practices in the slicing environment.

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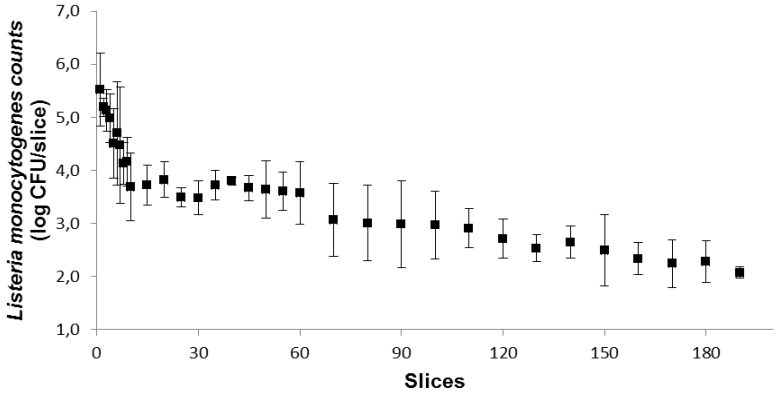
Table 1. *Listeria monocytogenes* transfer rates according to the challenge test (see text for experimental details).

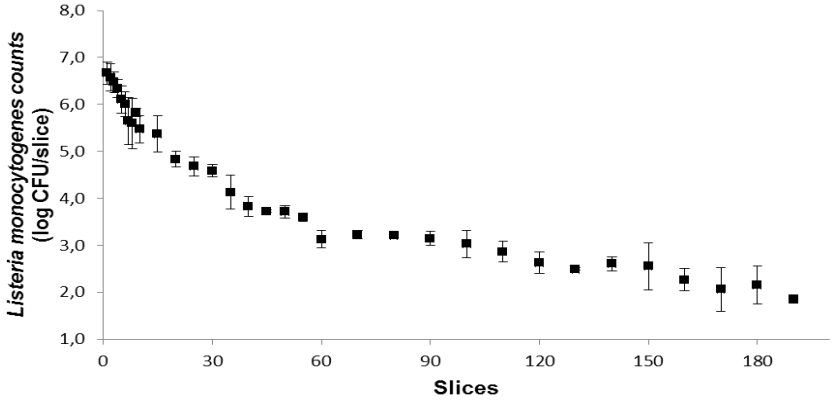
Challenge test	Log transfer (%)		
	Min	Max	Mean
A	-1.17	2.27	0.31
B	-1.39	3.43	0.89
C	-2.06	2.09	0.06
D*	-1.51	1.57	0.28
D**	-1.19	1.30	0.11

D* - values obtained after the initial contamination
D** - values obtained after the recontamination

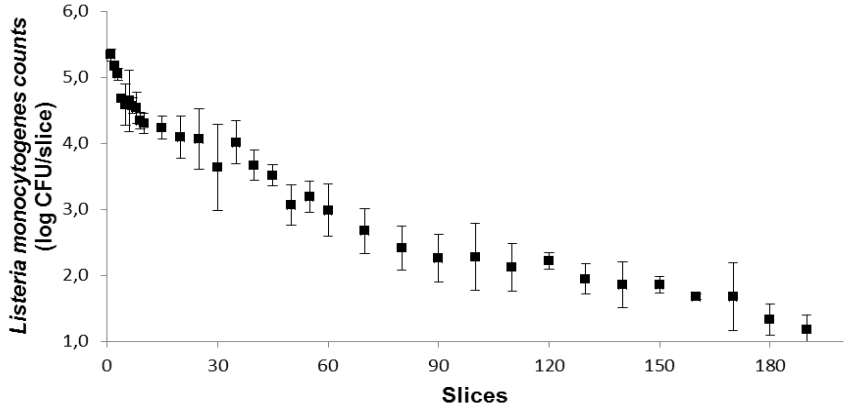
Challenge test A: *Listeria monocytogenes* transfer at high inoculum level (8 log CFU/mL) using an old blind blade during slicing of cooked ham.



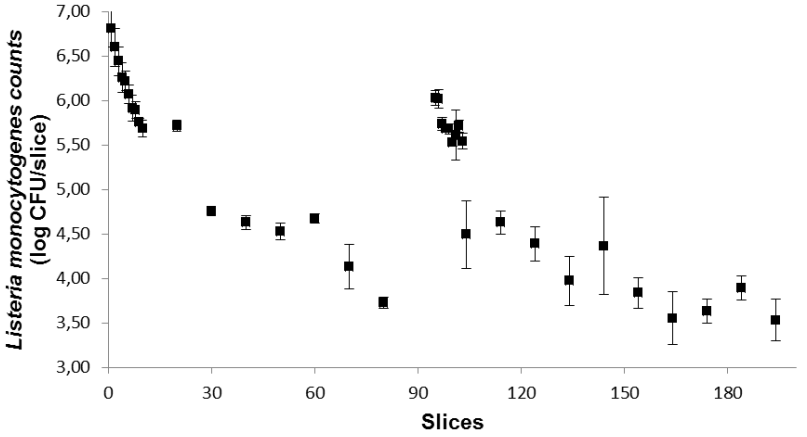
Challenge test B: *Listeria monocytogenes* transfer at high inoculum level (8 log CFU/mL) using a new blade during slicing of cooked ham.



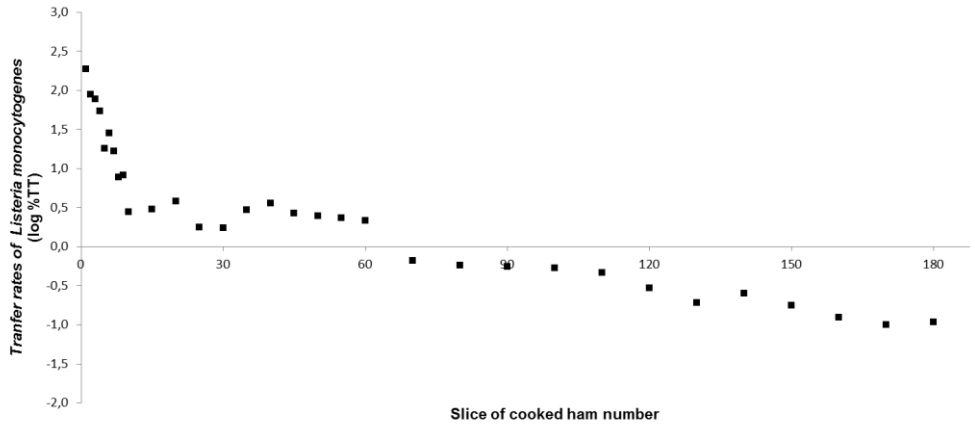
Challenge test C: *Listeria monocytogenes* transfer at moderate inoculum level (6 log CFU/mL) using a new blade during slicing of cooked ham.



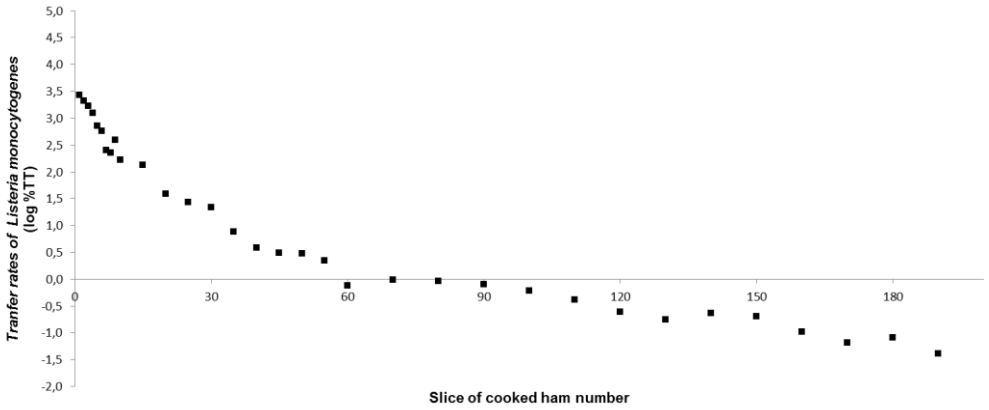
Challenge test D: *Listeria monocytogenes* transfer at high inoculum level (8 log CFU/mL) using an old blind blade, with recontamination at the 90th slice of cooked ham.



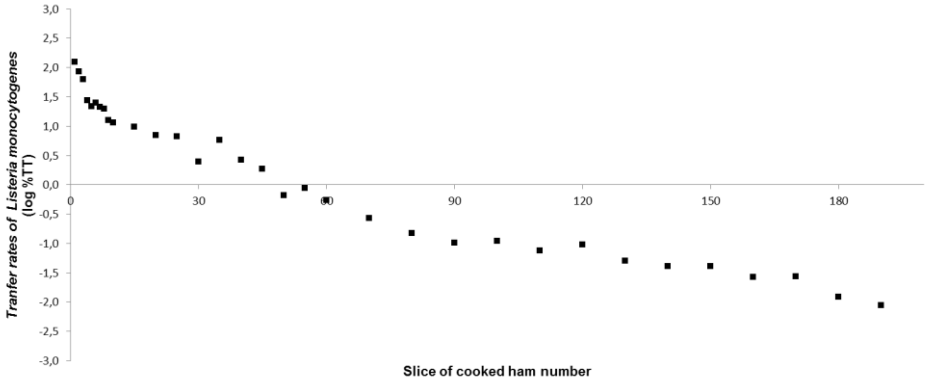
Challenge test A: *Listeria monocytogenes* transfer rates at high inoculum level (8 log CFU/mL) using an old blind blade during slicing of cooked ham.



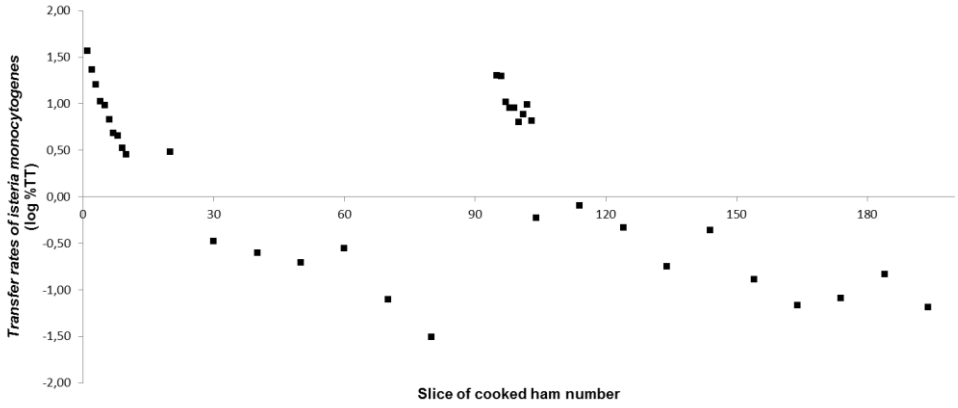
Challenge test B: *Listeria monocytogenes* transfer rates at high inoculum level (8 log CFU/mL) using a new blade during slicing of cooked ham.



Challenge test C: *Listeria monocytogenes* transfer at moderate inoculum level (6 log CFU/mL) using a new blade during slicing of cooked ham.



Challenge test D: *Listeria monocytogenes* transfer at high inoculum level (8 log CFU/mL) using an old blade, with recontamination at the 90th slice of cooked ham.



Attachments

Table 1- Counts of *Listeria monocytogenes* (log CFU/slice) in challenge test A (Trials 1, 2 and 3)

	Slice number	Trials			Mean±SD
		1	2	3	
Slices of cooked ham used for experimental contamination of the slicer	C1	7.8	7.6	7.9	7.8±0.1
	C2	7.1	7.4	7.3	7.2±0.2
	C3	71	6.8	7.2	7.0±0.2
	C4	6.7	5.5	7.4	6.5±0.9
	C5	6.6	5.0	5.9	5.8±0.8
Slices of cooked ham obtained after experimental contamination of the slicer	1	6.3	5.0	5.3	5.5±0.7
	2	5.4	5.1	5.1	5.2±0.2
	3	5.4	4.7	5.3	5.1±0.4
	4	5.2	4.5	5.3	5.0±0.5
	5	5.3	4.2	4.1	4.5±0.7
	6	5.8	4.2	4.0	4.7±1.0
	7	5.7	3.5	4.3	4.5±1.1
	8	4.5	3.7	4.2	4.1±0.4
	9	4.6	3.7	4.2	4.2±0.5
	10	4.4	3.3	3.3	3.7±0.6
	15	4.1	3.4	3.7	3.7±0.4
	20	4.2	3.5	3.8	3.8±0.3
	25	3.6	3.3	3.6	3.5±0.2
	30	3.7	3.1	3.6	3.5±0.3
	35	3.5	3.7	4.0	3.7±0.3
	40	3.7	3.9	3.8	3.8±0.1
	45	3.4	3.8	3.8	3.7±0.2
	50	3.9	3.0	3.9	3.6±0.5
	55	3.9	3.7	3.2	3.6±0.4
	60	4.2	3.1	3.4	3.6±0.6
	70	3.8	2.5	2.9	3.1±0.7
	80	3.6	2.2	3.1	3.0±0.7
	90	3.7	2.1	3.1	3.0±0.8
	100	3.7	2.4	2.9	3.0±0.6
	110	3.3	2.6	2.8	2.9±0.4
	120	3.1	2.4	2.6	2.7±0.4
	130	2.7	2.2	2.7	2.5±0.3
	140	3.0	2.4	2.5	2.6±0.3
	150	3.3	2.1	2.2	2.5±0.7
	160	2.7	2.1	2.2	2.3±0.3
	170	2.7	2.1	1.9	2.2±0.4
	180	2.7	2.0	2.1	2.3±0.4
	190	2.0	2.1	2.2	2.1±0.1

Table 2- Counts of *Listeria monocytogenes* (log CFU/slice) in challenge test B (Trials 1, 2 and 3)

	Slice number	Trials			Mean±SD
		1	2	3	
Slices of cooked ham used for experimental contamination of the slicer	C1	7.2	7.2	7.3	7.2±0.1
	C2	6.9	7.0	7.1	7.0±0.1
	C3	6.7	6.6	6.9	6.7±0.2
	C4	6.1	6.3	6.4	6.2±0.2
	C5	5.6	6.1	6.0	5.9±0.3
Slices of cooked ham obtained after experimental contamination of the slicer	1	6.8	6.8	6.4	6.7±0.2
	2	6.8	6.7	6.2	6.6±0.3
	3	6.7	6.5	6.2	6.5±0.2
	4	6.5	6.3	6.1	6.3±0.2
	5	6.3	6.2	5.8	6.1±0.3
	6	6.2	6.1	5.7	6.0±0.3
	7	6.0	5.9	5.1	5.6±0.5
	8	5.9	5.9	5.0	5.6±0.5
	9	5.9	5.9	5.7	5.8±0.1
	10	5.7	5.6	5.1	5.5±0.3
	15	5.0	5.7	5.5	5.4±0.4
	20	4.9	5.0	4.6	4.8±0.2
	25	4.7	4.9	4.5	4.7±0.2
	30	4.5	4.7	4.5	4.6±0.1
	35	4.0	4.5	3.8	4.1±0.4
	40	3.9	4.0	3.6	3.8±0.2
	45	3.8	3.7	3.7	3.7±0.0
	50	3.8	3.8	3.6	3.7±0.1
	55	3.6	3.6	3.5	3.6±0.1
	60	3.2	3.3	2.9	3.1±0.2
	70	3.3	3.2	3.1	3.2±0.1
	80	3.2	3.2	3.2	3.2±0.0
	90	3.1	3.1	3.3	3.1±0.1
	100	2.8	2.9	3.4	3.0±0.3
	110	2.7	2.7	3.1	2.9±0.2
	120	2.5	2.5	2.9	2.6±0.2
	130	2.5	2.5	2.5	2.5±0.0
	140	2.6	2.5	2.8	2.6±0.1
	150	2.4	2.1	3.1	2.6±0.5
	160	2.2	2.1	2.5	2.3±0.2
	170	2.0	1.6	2.6	2.1±0.5
	180	2.0	1.8	2.6	2.2±0.4
	190	1.8	1.9	1.9	1.9±0.1

Table 3- Counts of *Listeria monocytogenes* (log CFU/slice) in challenge test C (replicates 1, 2 and 3)

	Slice number	Trials			Mean ± SD
		1	2	3	
Slices of cooked ham used for experimental contamination of the slicer	C1	.	5.9	5,8	5.7±0.3
	C2	5.5	5.6	5.9	5.6±0.2
	C3	5.3	5.5	5.7	5.5±0.2
	C4	5.1	5.5	5.6	5.4±0.3
	C5	5.1	5.3	5.4	5.2±0.1
Slices of cooked ham obtained after experimental contamination of the slicer	1	5.4	5.4	5.2	5.3±0.1
	2	5.1	5.3	5.1	5.2±0.1
	3	4.9	5.1	5.1	5.0±0.1
	4	4.7	4.6	4.7	4.7±0.0
	5	4.8	4.2	4.7	4.6±0.3
	6	5.1	4.2	4.6	4.6±0.5
	7	4.7	4.4	4.6	4.6±0.1
	8	4.7	4.3	4.6	4.5±0.2
	9	4.4	4.2	4.5	4.3±0.1
	10	4.4	4.1	4.4	4.3±0.2
	15	4.3	4.0	4.4	4.2±0.2
	20	4.0	3.8	4.4	4.1±0.3
	25	3.6	4.1	4.5	4.1±0.5
	30	3.3	3.2	4.4	3.6±0.6
	35	3.8	3.9	4.4	4.0±0.3
	40	3.5	3.5	3.9	3.7±0.2
	45	3.6	3.6	3.3	3.5±0.2
	50	2.8	3.4	3.0	3.1±0.3
	55	3.1	3.5	3.0	3.2±0.2
	60	3.0	3.3	2.6	3.0±0.4
	70	2.4	3.0	2.6	2.7±0.3
	80	2.1	2.8	2.4	2.4±0.3
	90	1.9	2.6	2.2	2.3±0.4
	100	2.5	2.6	1.7	2.3±0.5
	110	1.9	2.5	1.9	2.1±0.4
	120	2.1	2.3	2.3	2.2±0.1
	130	1.8	2.2	1.8	1.9±0.2
	140	1.9	2.2	1.5	1.9±0.3
	150	2.0	1.8	1.8	1.9±0.1
	160	1.6	1.7	1.7	1.7±0.0
	170	2.3	1.5	1.3	1.7±0.5
	180	1.6	1.1	1.3	1.3±0.2
	190	1.4	0.9	1.2	1.2±0.2

Table 10a - Counts of *Listeria monocytogenes* (log CFU/slice) in challenge test D (Trials 1, 2 and 3)

	Slice number	Trials			Mean±SD
		1	2	3	
Slices of cooked ham used for experimental contamination of the slicer	C1	7.6	7.3	7.5	7.5±0.2
	C2	7.7	7.4	7.5	7.6±0.1
	C3	7.4	7.5	7.2	7.4±0.1
	C4	7.5	7.4	7.6	7.5±0.1
	C5	7.6	6.6	7.5	7.2±0.5
Slices of cooked ham obtained after experimental contamination of the slicer	1	7.0	6.9	6.6	6.8±0.2
	2	6.7	6.7	6.4	6.6±0.2
	3	6.6	6.4	6.3	6.4±0.2
	4	6.4	6.1	6.3	6.3±0.2
	5	6.3	6.1	6.2	6.2±0.1
	6	6.2	6.1	6.0	6.1±0.1
	7	6.1	5.8	5.9	5.9±0.1
	8	6.0	5.9	5.8	5.9±0.1
	9	5.8	5.7	5.7	5.8±0.0
	10	5.8	5.6	5.7	5.7±0.1
	20	5.8	5.7	5.6	5.7±0.1
	30	4.8	4.8	4.7	4.8±0.0
	40	4.6	4.7	4.5	4.6±0.1
	50	4.6	4.6	4.4	4.5±0.1
	60	4.7	4.6	4.7	4.7±0.1
	70	4.0	4.0	4.4	4.1±0.3
	80	3.7	3.8	3.7	3.7±0.1

Table 10b- Counts of *Listeria monocytogenes* (log CFU/slice) in challenge test D after recontamination of the slicer (Trials 1, 2 and 3)

	Slice number	Trials			Mean±SD
		1	2	3	
Slices of cooked ham used for experimental recontamination of the slicer	C1	7.9	7.1	7.1	7.3±0.4
	C2	7.9	7.0	7.2	7.4±0.4
	C3	7.1	6.9	6.1	6.7±0.5
	C4	6.6	6.9	6.7	6.8±0.1
	C5	6.7	6.7	6.7	6.7±0.0
Slices of cooked ham obtained after experimental recontamination of the slicer	95	6.1	6.1	5.9	6.0±0.1
	96	6.1	6.1	5.9	6.0±0.1
	97	5.7	5.8	5.7	5.7±0.1
	98	5.7	5.7	5.7	5.7±0.0
	99	5.7	5.7	5.6	5.7±0.1
	100	5.5	5.6	5.5	5.5±0.0
	101	5.5	5.9	5.4	5.6±0.3
	102	5.7	5.8	5.7	5.7±0.1
	103	5.6	5.5	5.5	5.5±0.1
	104	4.4	4.9	4.1	4.5±0.4
	114	4.5	4.8	4.6	4.6±0.1
	124	4.2	4.6	4.4	4.4±0.2
	134	4.1	4.2	3.7	4.0±0.3
	144	5.0	4.2	3.9	4.4±0.5
	154	3.8	4.0	3.7	3.8±0.2
	164	3.6	3.8	3.2	3.6±0.3
	174	3.6	3.8	3.5	3.6±0.1
	184	4.0	3.7	4.0	3.9±0.1
	194	3.6	3.3	3.7	3.5±0.2

IV. Chapter 3

A semi-mechanistic modeling approach to describe the transfer of *Listeria monocytogenes* during slicing of ready-to-eat cooked ham

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Abstract

Cross contamination of RTE meat products with *Listeria monocytogenes* in operations at retail such as slicing can result in foodborne listeriosis. Slicing of cooked ham containing *Listeria monocytogenes* may contaminate the slicing machine and cross contaminate a large number of slices obtained afterwards. A mathematical model capable to satisfactorily predict the pathogen transfer data during slicing of cooked ham is very useful to assist the food retail establishments and regulatory agencies in the evaluation and control of cross contamination of RTE foods. In this way, this study developed a semi-mechanistic model to describe the transfer of *L. monocytogenes* (cross contamination) that occurs during slicing of cooked ham when practices at retail were simulated in the laboratory. The new model was called 4p-2se, as it contained four parameters (4p) and two environments (2se), and was independent of the quantification of the pathogen transferred to the slicer. The proposed model was compared to two pathogen transfer models previously described, and the predicted data presented lower RMSE (Root Mean Sum of squared errors) values than the other models. The proposed model was able to satisfactorily predict the pathogen transfer data during slicing of cooked ham, helping retailers in the design of proper risk management strategies.

Keywords: predictive modeling, cooked ham, cross-contamination, *Listeria monocytogenes.*

1. Introduction

Listeria monocytogenes is resistant to high salt concentration and has the capability to survive and grow in refrigerated foods, aerobically and anaerobically. This pathogen is the causative agent of listeriosis, a severe disease that affects immunocompromised individuals, pregnant women, the elderly and young children (Buchanan et al, 2017). Different from most pathogens, *L. monocytogenes* is ubiquitous and a common contaminant in the food processing environment, mainly due to formation of resistant biofilms on surfaces (Manios & Skandamis, 2014). Absence of competing microbiota in heat processed foods favors its growth (Gombas et al., 2003). Many studies have shown that *L. monocytogenes* are widely distributed in food processing environments (Ferreira et al., 2014; Carpentier & Cerf, 2011). Listeria may enter food processing environment from raw materials or the movement of people or equipment and can persist due to ineffective cleaning and sanitation, poor design or condition of food equipment or environment or insufficient controls of movement of people or equipment. Its presence has also been reported in farm (Fox et al., 2011), retail (Hoelzer et al., 2015; Wang et al., 2015) and home environments (Evans & Redmond, 2015).

Foods are considered the major vehicle for listeriosis. In the food chain from the farm to the consumer, the processing, transportation, and storage of meat products stages potentially provide growth conditions and nutrient content to support unwanted growth of *L. monocytogenes*. Because ready-to-eat (RTE) foods are normally eaten raw or handled, processed, mixed, cooked, or otherwise prepared without further bactericidal steps before consumption, the microbial contamination of these products continues to draw attention (Yang et al., 2016).

The food-processing environment is an important source of *L. monocytogenes* but still poorly recognized and understood. The number of publications on investigations of processing environments is increasing, but data on the rate of transfer of pathogens from food to contact surfaces during processing remain limited (Moller et al., 2012; Chaitiemwong et a., 2014). Challenge tests, frequently used in these studies, have been criticized as an expensive, labour intensive, time consuming and non-cumulative research tool, besides giving only modest assurance on product safety. Instead, cross-contamination mathematical models are becoming more and more popular, and some of them describe bacterial transfer between surfaces (Kusumaningrum et al., 2004; Schaffner, 2004; Pérez-Rodríguez et al., 2006; Nauta, 2008; Hoelzer et al., 2012) while others predict the transfer of *L. monocytogenes* during slicing of RTE products (Vorst et al., 2006; Aarnisalo et al., 2007; Keskinen et al., 2008; Sheen, 2008; Chaitiemwong et a., 2014). Models able to describe and explain the transfer are essential to assist food processors and authorities to access risks and to improve food safety. Studies that simulate and model the distribution of pathogens

during processing operations are of major relevance to risk analysts to ascertain the importance of equipment sanitation, sources of potential product contamination and improved equipment design (Flores et al., 2006).

In this study, a semi-mechanistic model is proposed to describe the transfer of *L. monocytogenes* (cross contamination) that occurs during slicing of a ready-to-eat meat product (cooked ham), based on data generated (Table 1) in a previous laboratory study (Lopes et al., submitted) where practices adopted in most retail stores that slice RTE meat products for the consumer were simulated.

2. Materials and Methods

2.1 Model development

The results of the challenge tests described the manuscript Lopes et al. 2017 (submitted, see chapter 2), were analyzed and fitted to a model previously developed by Nauta et al. (2005) for predicting cross-contamination of *Campylobacter* in poultry processing. As results were unsatisfactory, the Moller et al. (2012) model to predict cross-contamination by *Salmonella* Typhimurium DT104 (log CFU/g) during grinding of pork was also tested for fitting as this model assumes that *S. Typhimurium* in the grinder is organized in two different matrices (the grinder and the meat), and theoretically can also be applied to describe the transfer of *Listeria monocytogenes* from a contaminated slicer to non-inoculated cooked ham slices after consecutive slicing. For the development of the new model and estimation of its parameters, the residual sum of squares (RSS) was minimized using the solver function in MS Excel (Microsoft® Office Excel® 2007). The suggested model was compared to the models of Nauta et al. (2005) e Moller et al. (2012) by *F*-tests (Zwietering et al., 1990). The root mean sum of squared errors (RMSE) (Ratkowsky, 2004) was used to calculate the goodness of fit of the proposed model (Hurvich and Tsai, 1989), considering the RSS, the number of observations and the number of parameters. The predictive capacity of the model and robustness of the model were evaluated by the acceptable zone simulation (AZS) method described by Oscar (2005) also used by Moller et al., 2013.

2.2. Validation of the model

To validate the best-fit model, two challenge tests were performed. Validation challenge tests 1 and 2 corresponded, respectively, to the high and moderate level of experimental contamination (10^8 CFU/ml) of the cooked ham used for contamination of the slicing machine.

2.2.1. Preparation of *Listeria monocytogenes* culture

The meat isolate *L. monocytogenes* 046/77 (serotype 1/2c), from the culture collection of the Food Microbiology Laboratory of the Faculty of Pharmaceutical Sciences of University of Sao Paulo, Sao Paulo, Brazil, was used. The preparation of the culture for the tests was the same described in Lopes et al. 2017 (manuscript in preparation, see chapter 2).

2.2.2. Slicing machine

A delicatessen type manual slicing machine (Urano SMS-048, Sao Paulo, Brazil) containing a 210 mm diameter stainless steel (#-304) cutting blade was used. Two types of blades were used: old blind blade and new blade. Cleaning, assembling of the slicing machine and surface sampling for testing for *L. monocytogenes* were the same described in Lopes et al. 2017 (manuscript in preparation, see chapter 2).

2.2.3 Experimental contamination of the slicing machine

Pieces of cooked ham (~3500 g) were purchased in local supermarkets in the city of Sao Paulo, Brazil, tested for absence of *L. monocytogenes* using ISO 11290-2:1998/Amd.1:2004 (International Organization for Standardization, 2004), and used for contamination of the slicing machine as described in Lopes et al. 2017 (manuscript in preparation, see chapter 2).

2.2.4 Transfer of *L. monocytogenes* from the contaminated slicer to the cooked ham during slicing

Pieces of Listeria-free cooked ham were sliced in the experimentally contaminated slicing machine until 190 slices were obtained, following the same procedure described in Lopes et al. 2017 (manuscript in preparation, see chapter 2). The extent of transfer (cross-contamination) was determined counting *L. monocytogenes* in the first five slices and then in every 5th slice until de last slice. The experiments were done in triplicate.

2.2.5 Enumeration of *Listeria monocytogenes* in the cooked ham slices

Enumerations of *Listeria monocytogenes* in the slices were done as described in Lopes et al. 2017 (manuscript in preparation, see chapter 2). Results were expressed as log mean ± standard deviation.

Table 1- Summary of data on *Listeria monocytogenes* transfer during slicing of cooked ham (Lopes et al. 2017 - manuscript in preparation, see chapter 2).

Challenge Tests[a]	Cutting blade	Level of experimental inoculation (log CFU/piece)	Slices of cooked ham		
			Mean weight per slice (gr)	Total number of slices obtained	Number of slices submitted to counts of *L. monocytogenes*
A1. ■	Old[b]	8.3*	12.9	200	38
A2. ■	Old[b]	8.5*	10.9	200	38
A3. ■	Old[b]	8.4*	10.4	200	38
B1. ◊	New[c]	8.4*	7.4	200	38
B2. ◊	New[c]	8.4*	9.9	200	38
B3. ◊	New[c]	8.2*	6.7	200	38
C1. ●	New[c]	6.2**	9.3	200	38
C2. ●	New[c]	6.3**	11.9	200	38
C3. ●	New[c]	6.3**	8.4	200	38
D1. □	Old[b]	8.5*	12.6	209	46
D2. □	Old[b]	8.3*	12.5	209	46
D3. □	Old[b]	8.5*	10.3	209	46

[a] Tests with the same symbol are replicates
[b] Old blind blade
[c] New blade

3. Results and Discussion

3.1 *Listeria* transfer model and fitting of models and goodness of fit

It is important to know that a single slice of pathogen contaminated meat product may be responsible for cross-contamination throughout the slicing process. The transfer of pathogens during slicing of ready-to-eat products was investigated by (Vorst et al., 2006; Aarnisalo et al., 2007; Sheen, 2008; Sheen and Hwang, 2010; Moller et al., 2012; Chaitiemwong et al., 2014). However, a mathematically model able to describe the *Listeria monocytogenes* cross-contamination by slicing of RTE products without input value and considering two environment was development in this study. The structure of the model, and particularly its ability to predict the tailing phenomenon, seems relevant for different cross-contamination processes. Nevertheless, it has yet to be elucidated what the two suggested environmental matrices consist of and how the transfer takes place at the physical level. The tailing phenomenon could also be interpreted as two subpopulations behaving differently in one environment, that is having different susceptibility to the environmental stress experienced during slicing and, thereby, different transfer abilities, as also proposed by Moller et al., 2012. More investigations are needed to determine the exact cause of the observed two-phase transfer of *Listeria* during slicing.

The data of the challenges tests were adjusted to the *L. monocytogenes* transfer model built based on results described in Lopes et al. 2017 (manuscript in preparation, see chapter 2). The transfer profiles were similar in all tests, with higher transfer in the first slices, followed by a lower and persistent transfer, forming a tail. The tail formation phenomenon during cross-contamination is a common occurrence, also observed in the other studies on transfer of pathogens during the processing of ready-to-eat meat products and *in natura* meat (VORST et al., 2006; AARNISALO et al., 2007; SHEEN, 2008; SHEEN & HWANG, 2010; MOLLER et al., 2012; CHAITIEMWONG et al., 2014; MOLLER et al., 2016).

Tables 2, 3, 4 and 5 indicate the results of data fittings to three models: 4p-1se (4 parameters and one single environment) corresponds to the model of Nauta et al. (2005), 5p-2se (5 parameters and two environments) corresponds to the model of Moller et al. (2012) and 4p-2se (4 parameters and two environments) corresponds to the new model proposed in this study. The model 4p-1se (Nauta et al, 2005) was effective in describing the transfer of *Campylobacter* in poultry processing, but was not able to predict the transfer of *Listeria* during slicing of cooked ham. The second model (5p-2se), a cross-contamination model described by Moller et al. (2012) based on Nauta et al, 2005, was also inappropriate to describe the transfer observed in the cooked ham study because it contained a 5^{th} parameter correspondent to the number of microbial cells transferred to the experimentally

contaminated slicer (*input*). Moller et al. (2012) model considers two distinct environments (2se): the first environment is the one in which the bacterial cells are weakly adhered to the surface of the slicer and can be easily removed, characterizing a high transferability, and the second environment is one in which the pathogen is strongly adhered to the surface and the transfer rate from the slicer to the meat product occurs at a slower rate. However, in this study with slicing of cooked ham, it was not possible to determine the number of *L. monocytogenes* transferred to the slicer (*input*), so that a new transfer model was suggested and named 4p-2se, with four parameters, represented in the diagram in Figure 1 and described by the following equation:

$$\text{Log } (Li+k) = \log(b1(1-b1)k\text{-}1E1.i + b2(1-b2)k\text{-}1E2.i)$$

where E1, E2, b1 and b2 represent the probability of transfer of the pathogen during slicing, i.e., if k slices of cooked ham are obtained (i= 1. 2. ...k), the slice number k contains Li CFU of *L. monocytogenes.* The organization of *L. monocytogenes* in two environments is represented by E1 and E2. The probability of transfer of *L. monocytogenes* from the slicer (environments 1 and 2) to the non-inoculated cooked ham is represented by b1 and b2. The model did not consider possible inactivation of *L. monocytogenes* in the slicing process.

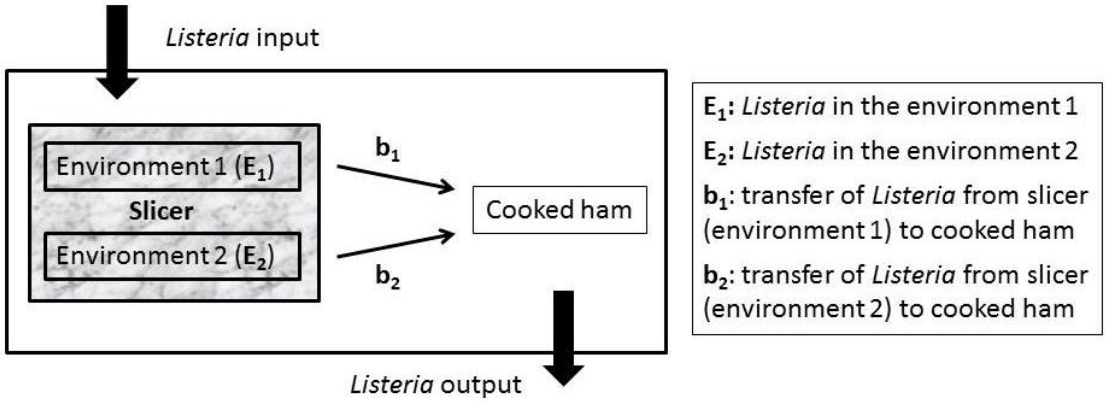


Figure 1- Diagram of the suggested *Listeria monocytogenes* transfer model (4p-2se model) during slicing of cooked ham.

Table 2- Comparison of the performance of models 4p-2se, 4p-1se and 5p-2se describing the transfer of *Listeria monocytogenes* during slicing of cooked ham, based on results of challenge tests A (Trials 1, 2 and 3). The lowest RMSE for each model are in **bold**.

Model	Trials	a1	b1	E1	gamma (1-c1)	a2	b2	E2	sigma (1-c3)	delta (1-c2)	log RSS	N	P	RMSE
4p-2se	A1	-	0.0196	5.8836	-	-	0.3154	6.4444	-	-	3.2951	33	4	0.06259
4p-1se	A1	0.0471	0.0223	-	0.9803	-	-	-	-	0.3298	21.3204	38	4	0.13581
5p-2se	A1	0.0038	0.0208	-	1.0000	0.0600	0.8518	-	4.1251	1.0000	3.7611	38	5	**0.05877**
4p-2se	A2	-	0.0216	5.2622	-	-	0.4694	5.4920	-	-	2.8858	33	4	**0.05858**
4p-1se	A2	0.0165	0.0063	-	0.9672	-	-	-	-	0.1414	25.0210	38	4	0.14712
5p-2se	A2	0.0007	0.0232	-	1.0000	0.8609	0.0228	-	0.4322	1.0000	4.3822	38	5	0.06344
4p-2se	A3	-	0.0252	5.6114	-	-	0.4862	5.8148	-	-	2.1253	33	4	**0.05027**
4p-1se	A3	0.0146	0.0236	-	0.9798	-	-	-	-	0.2598	27.1682	38	4	0.15330
5p-2se	A3	0.0017	0.0267	-	1.0000	0.1172	0.9721	-	13.6953	1.0000	3.3579	38	5	0.05553

a1, b1, Gamma (1-c1), a2, b2, Sigma (1-c3) e Delta (1-c2) Parameters of the Moller et al. (2012) model
a1, b1, Gamma (1-c1) e Delta (1-c2) Parameters of the Nauta et al. (2005) model
b1, E1, b2 e E2 Parameters of the suggested model
N Number of slices analyzed
P Number of parameters of the model
RMSE Root Mean Sum of Squared Errors

Table 3- Comparison of the performance of models 4p-2se, 4p-1se and 5p-2se describing the transfer of *Listeria monocytogenes* during slicing of cooked ham, based on results of challenge tests B (Trials 1, 2 and 3). The lowest RMSE for each model are in **bold**.

Model	Trials	a1	b1	E1	gamma (1-c1)	a2	b2	E2	sigma (1-c3)	delta (1-c2)	log RSS	N	P	RMSE
4p-2se	B1	-	0.0305	5.8095	-	-	0.1974	7.3885	-	-	0.7504	33	4	**0.02987**
4p-1se	B1	0.1596	0.0288	-	0.9660	-	-	-	-	0.0824	12.6777	38	4	0.10472
5p-2se	B1	0.0020	0.0297	-	1.0000	0.9306	0.0236	-	0.8804	1.0000	1.2283	38	5	0.03358
4p-2se	B2	-	0.0300	5.7057	-	-	0.1495	7.3566	-	-	0.7869	33	4	**0.03059**
4p-1se	B2	0.0997	0.0377	-	0.9749	-	-	-	-	0.0630	12.6777	38	4	0.10472
5p-2se	B2	0.0017	0.0297	-	1.0000	0.9432	0.0191	-	0.8763	1.0000	1.2283	38	5	0.03358
4p-2se	B3	-	0.0182	5.5919	-	-	0.1556	6.9214	-	-	2.4208	33	4	0.05365
4p-1se	B3	0.0857	0.0320	-	0.9813	-	-	-	-	0.1298	18.2301	38	4	0.12558
5p-2se	B3	0.0028	0.0201	-	1.0000	0.8809	0.0259	-	0.8462	1.0000	3.0903	38	5	**0.05327**

a1, b1, Gamma (1-c1), a2, b2, Sigma (1-c3) e Delta (1-c2) Parameters of the Moller et al. (2012) model
a1, b1, Gamma (1-c1) e Delta (1-c2) Parameters of the Nauta et al. (2005) model
b1, E1, b2 e E2 Parameters of the suggested model
N Number of slices analyzed
P Number of parameters of the model
RMSE Root Mean Sum of Squared Errors

Table 4- Comparison of the performance of models 4p-2se, 4p-1se and 5p-2se describing the transfer of *Listeria monocytogenes* during slicing of cooked ham, based on results of challenge tests C (Trials 1, 2 and 3). The lowest RMSE for each model are in **bold**.

Model	Trials	a1	b1	E1	gamma (1-c1)	a2	b2	E2	sigma (1-c3)	delta (1-c2)	log RSS	N	P	RMSE
4p-2se	C1	-	0.0128	4.5644	0.0128	-	0.0928	6.0087	-	-	2.0638	33	4	0.04954
4p-1se	C1	0.7378	0.0473	-	1.0000	-	-	-	-	0.6303	9.8210	38	4	0.09217
5p-2se	C1	0.0242	0.0132	-	1.0000	0.8307	0.1254	-	1.0321	1.0000	2.2441	38	5	**0.04539**
4p-2se	C2	-	0.0199	4.7446	0.0199	-	0.1820	5.7919	-	-	0.9337	33	4	0.03332
4p-1se	C2	0.6388	0.0471	-	1.0000	-	-	-	-	1.0000	5.6222	38	4	0.06974
5p-2se	C2	0.2549	0.0381	-	1.0000	0.4861	0.5936	-	1.6488	1.0000	1.1795	38	5	**0.03291**
4p-2se	C3	-	0.0158	4.4212	0.0158	-	0.0807	6.0980	-	-	1.9335	33	4	**0.04795**
4p-1se	C3	0.7533	0.0533	-	1.0000	-	-	-	-	1.0000	8.8049	38	4	0.08727
5p-2se	C3	0.0131	0.0167	-	1.0000	0.6813	0.1499	-	1.0685	1.0000	2.8826	38	5	0.05145

a1, b1, Gamma (1-c1), a2, b2, Sigma (1-c3) e Delta (1-c2) Parameters of the Moller et al. (2012) model
a1, b1, Gamma (1-c1) e Delta (1-c2) Parameters of the Nauta et al. (2005) model
b1, E1, b2 e E2 Parameters of the suggested model
N Number of slices analyzed
P Number of parameters of the model
RMSE Root Mean Sum of Squared Errors

Table 5- Comparison of the performance of models 4p-2se, 4p-1se and 5p-2se describing the transfer of *Listeria monocytogenes* during slicing of cooked ham, based on results of challenge tests D (Trials 1, 2 and 3). The lowest RMSE for each model are in **bold**.

Model	Trials	a1	b1	E1	gamma (1-c1)	a2	b2	E2	sigma (1-c3)	delta (1-c2)	log RSS	N	P	RMSE
4p-2se	D1	-	0.0402	6.6269	0.0402	-	0.2698	7.0374	-	-	5.6037	46	4	**0.05636**
4p-1se	D1	0.2103	0.0040	-	0.9216	-	-	-	-	0.0300	22.4945	46	4	0.11292
5p-2se	D1	0.0012	0.0332	-	1.0000	0.9091	0.0328	-	0.5322	1.0000	9.8705	46	5	0.07663
4p-2se	D2	-	0.0447	6.7178	0.0447	-	0.2632	6.9577	-	-	2.8186	46	4	**0.03997**
4p-1se	D2	0.1507	0.0071	-	0.9263	-	-	-	-	0.0120	13.9927	46	4	0.08906
5p-2se	D2	0.0012	0.0332	-	1.0000	0.9091	0.0328	-	0.6322	1.0000	9.9214	46	5	0.07683
4p-2se	D3	-	0.0313	6.3678	0.0313	-	0.1928	6.8859	-	-	5.8672	46	4	**0.05767**
4p-1se	D3	0.2314	0.0021	-	0.9257	-	-	-	-	0.0141	22.2775	46	4	0.11238
5p-2se	D3	0.0012	0.0332	-	1.0000	0.9091	0.0128	-	0.5400	1.0000	13.7237	46	5	0.09035

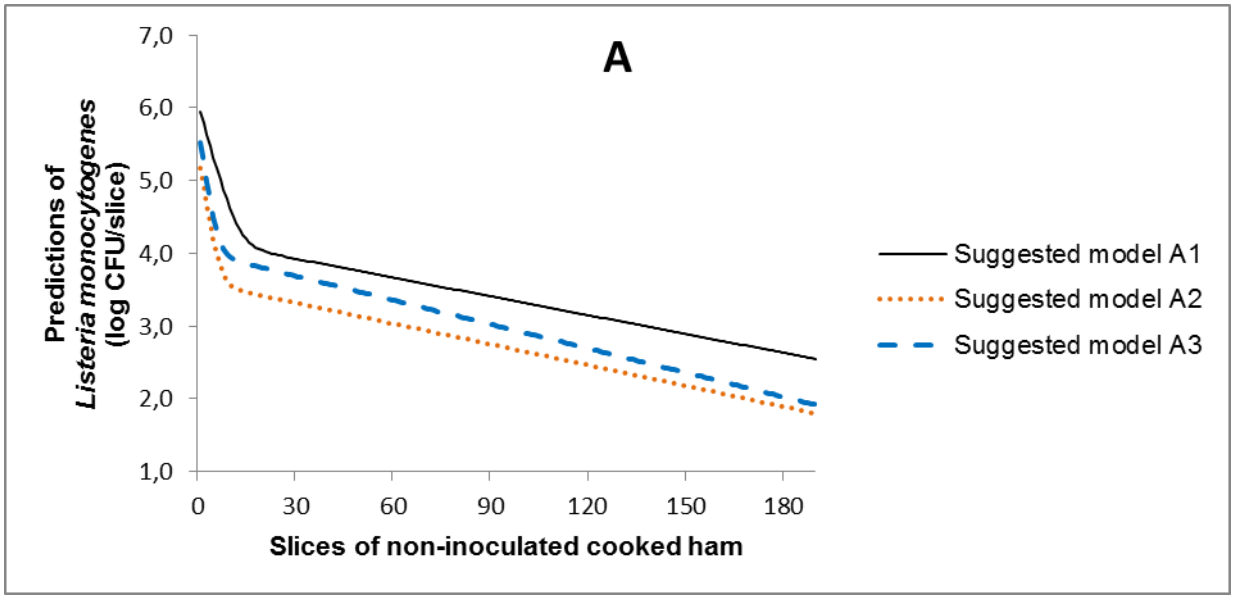
a1, b1, Gamma (1-c1), a2, b2, Sigma (1-c3) e Delta (1-c2) Parameters of the Moller et al. (2012) model
a1, b1, Gamma (1-c1) e Delta (1-c2) Parameters of the Nauta et al. (2005) model
b1, E1, b2 e E2 Parameters of the suggested model
N Number of slices analyzed
P Number of parameters of the model
RMSE Root Mean Sum of Squared Errors

3.2 Predictions of the *Listeria monocytogenes* transfers during slicing of cooked ham based on challenge tests adjustments

The estimates of the parameters E1, E2, b1 and b2 in the model 4p-2se were obtained based on the twelve independent challenges tests (4 tests with three trials each) described in Lopes et al. 2017 (manuscript in preparation, see chapter 2). The proposed model equation was adjusted for each challenge test and the parameters were estimated (predicted) using the Excel solver function (item 2.1) and the RMSE values calculated (Table 6). For each challenge test, the best fit of the proposed transfer equation model was investigated and the predicted curves of transfer are shown in Figure 2.

As shown in Table 6, the validation challenge test 1 presented the lowest RMSE values: 0.01295 (t2), 0.01573 (t3) and 0.01947 (t1), while the highest RMSE values were from challenge test A of the study in Lopes et al, 2017 (manuscript in preparation, see chapter 2): 0.06259 (A1). 0.05858 (A2) and 0.05027 (A3).

a)



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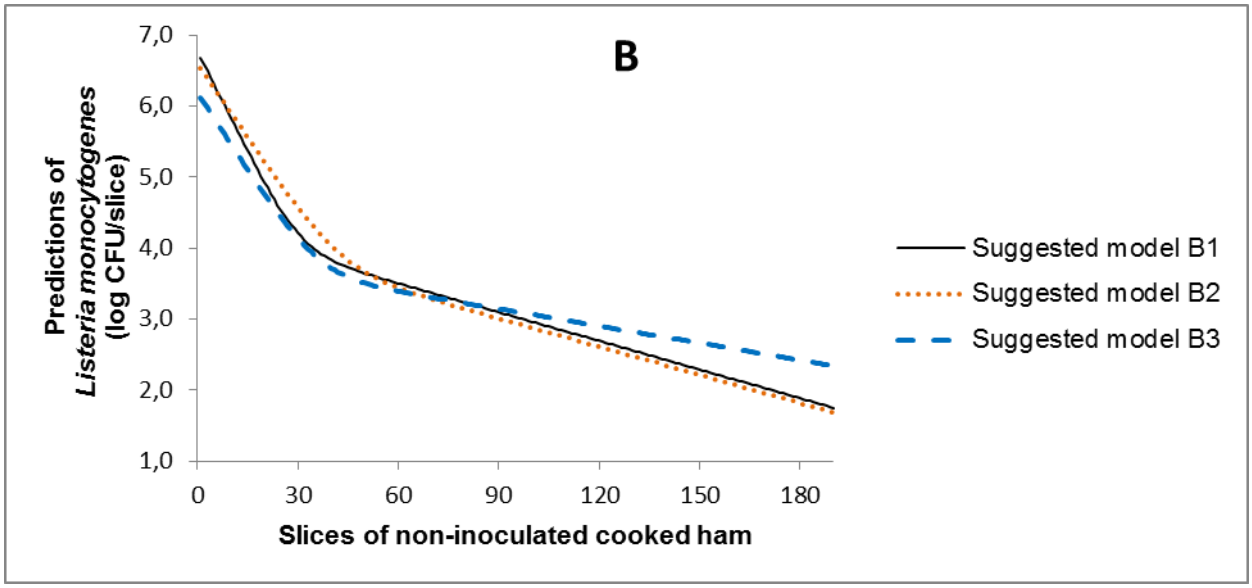
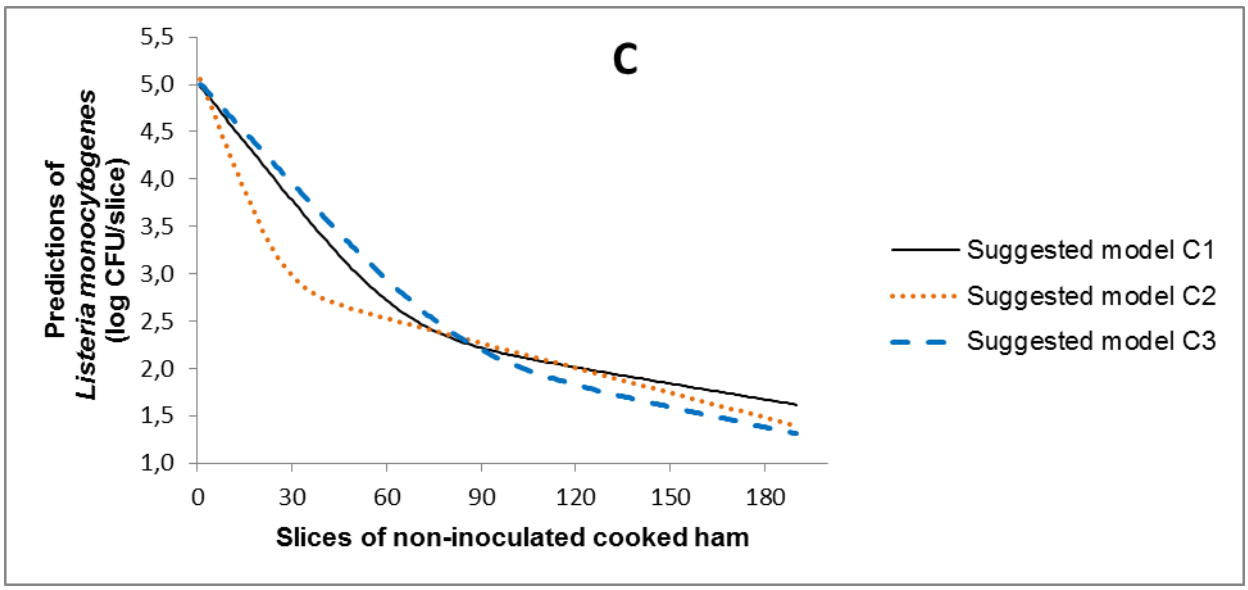

B
Predictions of Listeria monocytogenes (log CFU/slice)
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0
30
60
90
120
150
180
Slices of non-inoculated cooked ham
Suggested model B1
Suggested model B2
Suggested model B3

c)


C
Predictions of Listeria monocytogenes (log CFU/slice)
5,5
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0
30
60
90
120
150
180
Slices of non-inoculated cooked ham
Suggested model C1
Suggested model C2
Suggested model C3

d)

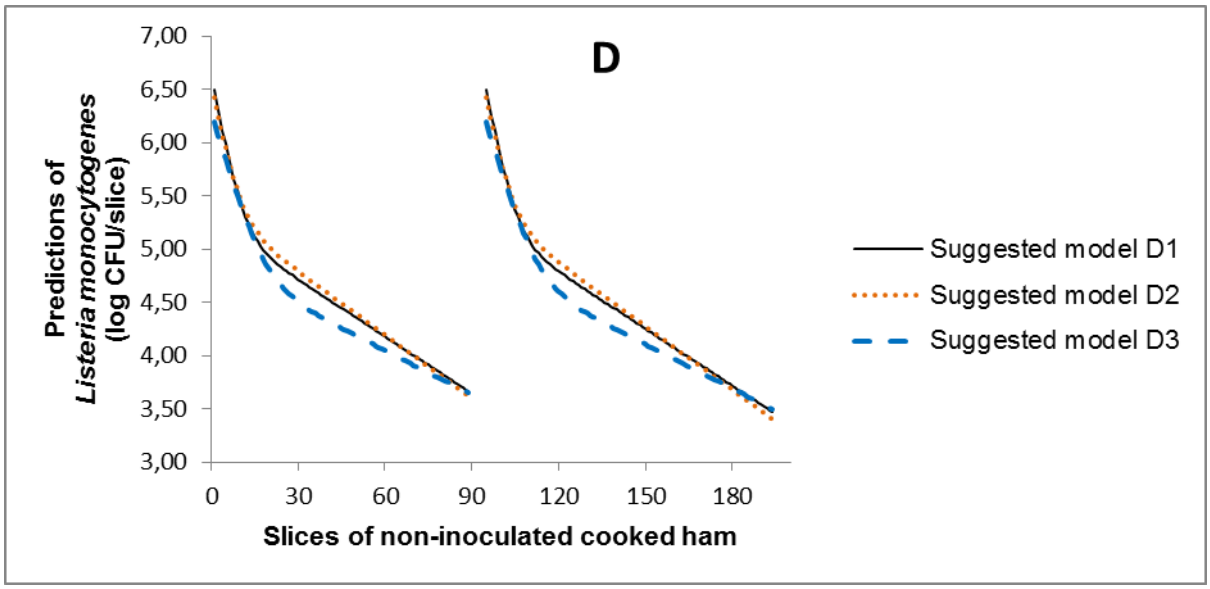


Figure 2- Predicted curves of *Listeria monocytogenes* transfer during slicing of cooked ham, based on data described in Lopes et al. 2017 (manuscript in preparation, see chapter 2).

a) Challenge test A: high level of inoculation and old blind blade, b) Challenge test B: high level of inoculation and a new blade, c) Challenge test C: intermediate level of inoculation and new blade, and d) Challenge test D: high level of inoculation, old blade and recontamination of the slicer during slicing.

Table 6- Estimates of the parameters obtained from the proposed transfer model 4p-2se

Trials*	Estimated Parameters				RMSE**
	E_1	E_2	b_1	b_2	
A1	5.88	6.44	0.02	0.32	0.06259
A2	5.26	5.49	0.02	0.47	0.05858
A3	5.61	5.81	0.03	0.49	0.05027
B1	5.81	7.39	0.03	0.20	0.02987
B2	5.71	7.36	0.03	0.15	0.03059
B3	5.59	6.92	0.02	0.16	0.05365
C1	4.56	6.01	0.01	0.09	0.04954
C2	5.68	5.73	0.04	0.53	0.03332
C3	4.42	6.10	0.02	0.08	0.04795
D1	6.63	7.04	0.04	0.27	0.05636
D2	6.72	6.96	0.04	0.26	0.03997
D3	6.37	6.89	0.03	0.19	0.05767
E1	0.00	5.92	0.02	0.06	0.01947
E2	0.00	6.01	0.02	0.05	0.01295
E3	0.00	6.06	0.02	0.05	0.01573

*Trials are described in Lopes et al. 2017 (manuscript in preparation, see chapter 2).

**Root Mean Sum of Squared Errors

3.3 Evaluation of the performance of the *Listeria monocytogenes* transfer model 4p-2se by the Acceptable Simulation Zone (ASZ)

The robustness of the transfer model proposed in this study was investigated in order to determine how well the model could predict the transfer of *L. monocytogenes* during the slicing of ready-to-eat meat product in conditions different of those used to create the model. For this, the real counts of *L. monocytogenes* in each trial were compared to the counts predicted by the suggested model, using the Acceptable Simulation Zone (ASZ) concept, also used by Velugoti et al., (2011) and Moller et al., (2013). According to this concept, a model can be considered applicable to a new data set when at least 70% of the observed data are located inside a zone of ± 0.5 log CFU per g in the predicted transfer curve. Table 7 shows the performance of the simulations derived from the proposed model, considering three ASZ: ±0.5 log CFU/slice, ±1.1 log CFU/slice and ±1.7 log CFU/slice.

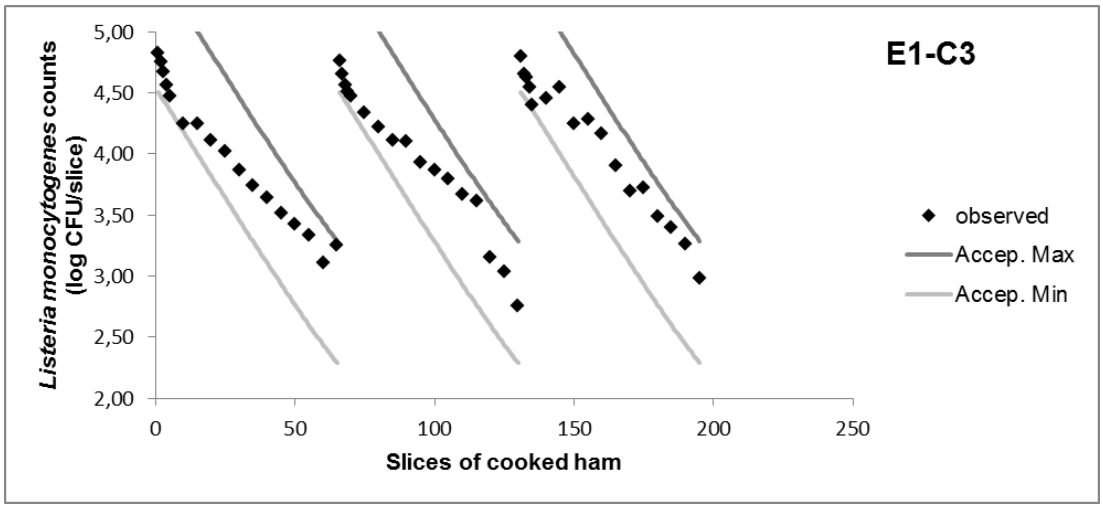
The application of the ASZ of ± 0.5 log CFU per slice of cooked ham to the results in Table 7 that presented the highest percentages in the three validation tests (challenge test C) indicated that 3-4 challenge tests (25-33%) presented predicted data that could be considered satisfactory by the model 4p-2se (Figure 3). When a larger range of ASZ (± 1.1 log CFU/slice) was considered, six-ten challenges tests (50-83%) presented predicted data inside this ASZ. The inclusion of all predicted data inside an ASZ was possible only when a larger deviation (± 1.7 log CFU/slice) was considered.

Table 7- Performance of the predicted data generated by the transfer model 4p-2se for slicing of cooked ham, considering three Acceptable Simulation Zones (ASZ). Challenges tests with at least 70% of observed data inside the ASZ are in **bold**.

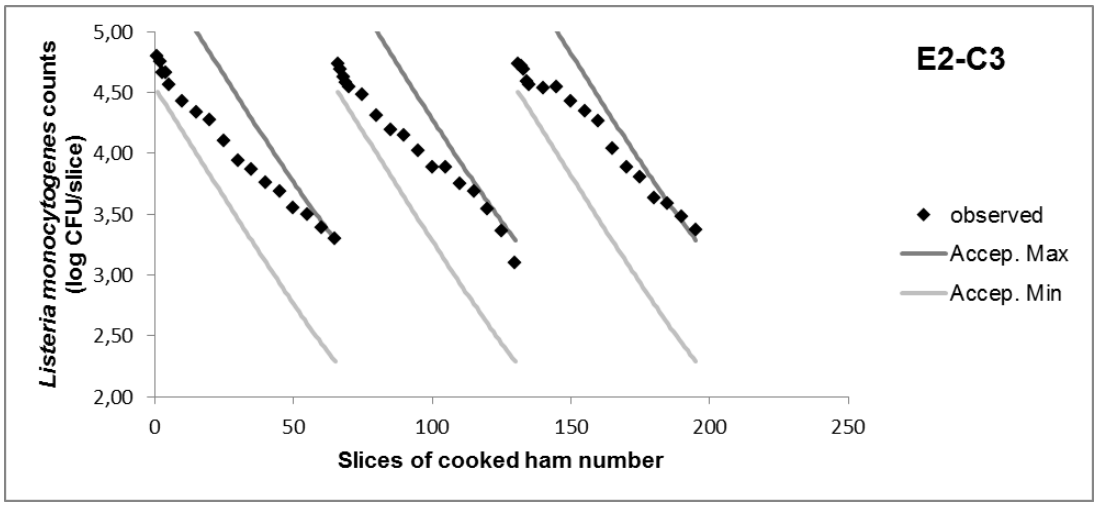
Trials	Level of contamination	Acceptable Simulation Zone (ASZ) %[a]								
		±0.5 log CFU/slice			±1.1 log CFU/slice[b]			±1.7 log CFU/slice[c]		
		E1	E2	E3	E1	E2	E3	E1	E2	E3
A1	HL	61	69	**71**	**90**	**94**	**96**	**100**	**100**	**100**
A2	HL	63	47	45	**100**	**100**	**98**	**100**	**100**	**100**
A3	HL	**82**	**78**	**75**	**100**	**100**	**100**	**100**	**100**	**73**
B1	HL	49	55	55	61	65	65	**71**	**76**	**84**
B2	HL	39	43	47	57	61	61	**71**	**100**	**100**
B3	HL	51	57	59	67	**71**	**71**	**100**	**100**	**100**
C1	ML	**90**	69	69	**100**	**100**	**100**	**100**	**100**	**100**
C2	ML	**88**	**92**	**88**	**100**	**100**	**100**	**100**	**100**	**100**
C3	ML	**100**	**94**	**88**	**100**	**100**	**100**	**100**	**100**	**100**
D1	HL	0	4	6	59	**71**	**71**	**94**	**96**	**98**
D2	HL	0	0	6	59	**71**	**71**	**100**	**100**	**100**
D3	HL	4	18	35	67	**71**	**71**	**100**	**100**	**100**
Total %[d]		25	25	33	50	83	83	100	100	100

*Trials are described in Lopes et al. 2017 (manuscript in preparation, see chapter 2).
HL – High level of contamination
ML – Moderate level of contamination
[a] Simulations obtained in three independent validation tests (E1, E2 and E3)
[b, c] Alternative simulations of ASZ
[d] Percentage of data in the ASZ zone

a)



b)



c)

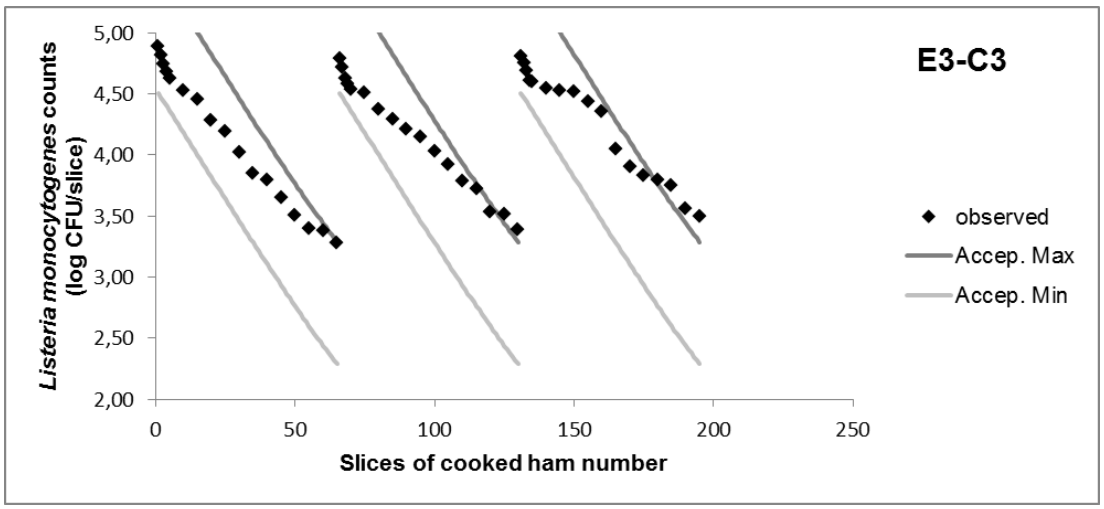


Figure 3- Performance of the predicted data generated by the transfer model 4p-2se, considering an ASZ of ±0.5 log CFU/slice, for challenge test C3. a) Validation E1, b) Validation E2 and c) Validation E3.

3.4 Performance comparison of 4p-2se, 4p-1se and 5p-2se transfer models

The performance of the 4p-2se model was compared to the 4p-1se model developed by Nauta et al. (2005) and to the 5p-2se model developed by Moller et al. (2012), using F-tests (Zwietering et al., 1990) (Table 8). The performance evaluations considered the Residual Sum of Squares (RSS) of the models, the number of observations and the number of parameters of each model. The root mean sum of squared error (RMSE) (Ratkowsky, 2004) was obtained by measurement of the calculated best fit (Hurvich and Tsai, 1989).

The 4p-2se model presented the best performance as it resulted in the lowest RSS values (Table 8), which varied from 0.7504 (B1) to 5.8672 (D3). For 4p-1se and 5p-2se models, the variations were from 1.1795 (C2) to 27.1682 (A3) and 1.1795 (C2) to 13.7237 (D3), respectively. The best performance of the 4p-2se model was also supported by significant F-tests.

Table 8- Performance comparison of 4p-2se, 4p-1se and 5p-2se transfer models, by F-tests (Zwietering et al., 1990).

Trials	Model								
	4p-2se			4p-1se			5p-2se		
	Number of observations	Parameters	RSS	RSS	Values *f*	Values *p*	RSS	Values *f*	Values *p*
A1	33	4	3.2951	21.3204	39.6595	8.28E-17	3.7611	1.0252	0.475816
A2	33	4	2.8858	25.0210	55.6098	7.38E-19	4.3822	3.7594	0.000254
A3	33	4	2.1253	27.1682	85.4297	1.71E-21	3.3579	4.2048	8.94E-05
B1	33	4	0.7504	12.6777	115.2421	2.40E-23	1.2283	4.6180	3.59E-05
B2	33	4	0.7869	12.6777	109.5547	4.95E-23	1.2283	4.0669	0.000123
B3	33	4	2.4208	18.2301	47.3469	7.03E-18	3.0903	2.0052	0.030247
C1	33	4	2.0638	9.8210	27.2500	1.44E-14	2.2441	0.6333	0.897519
C2	33	4	0.9337	1.1795	1.9081	4.04E-02	1.1795	1.9081	0.040407
C3	33	4	1.9335	8.8049	25.7650	3.08E-14	2.8826	3.5588	0.000416
D1	36	4	5.6037	22.4945	24.1138	4.26E-15	9.8705	6.0914	6.43E-07
D2	36	4	2.8186	13.9927	31.7155	6.99E-17	9.9214	20.1600	6E-14
D3	36	4	5.8672	22.2775	22.3757	1.29E-14	13.7237	10.7124	4.63E-10

4. Conclusions

The proposed model was able to satisfactorily describe the obtained data, and the tailing phenomenon of transfer of *Listeria monocytogenes* during slicing of cooked ham. Several models are available in the scientific literature describing the transfer of pathogens in food processing with relatively good performance. However, most of them are empirical and cannot explain the meaning behind the model parameters. Furthermore, some models require input values to obtain predicted data. The pathogen transfer model proposed in this overcome these requirements and is transferable to other cross-contamination scenarios.

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Attachment

Table a- Counts of *Listeria monocytogenes* (log CFU/slice) in challenge test (Trials 1, 2 and 3)

	Slice number	Trial			Mean±SD
		1	2	3	
Slices of cooked ham used for experimental contamination of the slicer	C1	5.5	5.6	5.7	5.6±0.1
	C2	5.4	5.5	5.6	5.5±0.1
	C3	5.3	5.4	5.4	5.4±0.0
	C4	5.2	5.2	5.3	5.2±0.1
	C5	5.1	5.2	5.3	5.2±0.1
Slices of cooked ham obtained after experimental contamination of the slicer	1	4.8	4.8	4.9	4.8±0.0
	2	4.8	4.7	4.8	4.8±0.0
	3	4.7	4.7	4.7	4.7±0.0
	4	4.6	4.7	4.7	4.6±0.1
	5	4.5	4.6	4.6	4.6±0.1
	10	4.2	4.4	4.5	4.4±0.1
	15	4.2	4.3	4.5	4.3±0.1
	20	4.1	4.3	4.3	4.2±0.1
	25	4.0	4.1	4.2	4.1±0.1
	30	3.9	3.9	4.0	3.9±0.1
	35	3.7	3.9	3.9	3.8±0.1
	40	3.6	3.8	3.8	3.7±0.1
	45	3.5	3.7	3.6	3.6±0.1
	50	3.4	3.6	3.5	3.5±0.1
	55	3.3	3.5	3.4	3.4±0.1
	60	3.1	3.4	3.4	3.3±0.2
	65	3.3	3.3	3.3	3.3±0.0

Table b- Counts of *Listeria monocytogenes* (log CFU/slice) in challenge test (Trials 1, 2 and 3)

	Slice number	Trial			Mean±SD
		1	2	3	
Slices of cooked ham used for experimental contamination of the slicer	C1	5.5	5.7	5.6	5.6±0.1
	C2	5.4	5.5	5.5	5.5±0.1
	C3	5.5	5.5	5.5	5.5±0.0
	C4	5.4	5.5	5.4	5.4±0.1
	C5	5.4	5.4	5.5	5.4±0.0
Slices of cooked ham obtained after experimental recontamination of the slicer	66	4.8	4.7	4.8	4.8±0.0
	67	4.7	4.7	4.7	4.7±0.0
	68	4.6	4.6	4.6	4.6±0.0
	69	4.5	4.6	4.6	4.6±0.0
	70	4.5	4.5	4.5	4.5±0.0
	75	4.3	4.5	4.5	4.4±0.1
	80	4.2	4.3	4.4	4.3±0.1
	85	4.1	4.2	4.3	4.2±0.1
	90	4.1	4.1	4.2	4.2±0.1
	95	3.9	4.0	4.2	4.0±0.1
	100	3.9	3.9	4.0	3.9±0.1
	105	3.8	3.9	3.9	3.9±0.1
	110	3.7	3.7	3.8	3.7±0.1
	115	3.6	3.7	3.7	3.7±0.1
	120	3.2	3.5	3.5	3.4±0.2
	125	3.0	3.4	3.5	3.3±0.2
	130	2.8	3.1	3.4	3.1±0.3

Table- Counts of *Listeria monocytogenes* (log CFU/slice) in challenge test (Trials 1, 2 and 3)

	Slice number	Trial			Mean±SD
		1	2	3	
Slices of cooked ham used for experimental contamination of the slicer	C1	5.7	5.7	5.6	5.7±0.0
	C2	5.7	5.6	5.7	5.7±0.0
	C3	5.6	5.6	5.7	5.6±0.0
	C4	5.6	5.4	5.7	5.6±0.1
	C5	5.5	5.4	5.6	5.5±0.1
Slices of cooked ham obtained after experimental recontamination of the slicer	131	4.8	4.7	4.8	4.8±0.0
	132	4.7	4.7	4.8	4.7±0.0
	133	4.6	4.7	4.7	4.7±0.0
	134	4.5	4.6	4.6	4.6±0.1
	135	4.4	4.6	4.6	4.5±0.0
	140	4.5	4.5	4.5	4.5±0.0
	145	4.5	4.5	4.5	4.5±0.1
	150	4.2	4.4	4.5	4.4±0.1
	155	4.3	4.3	4.4	4.4±0.1
	160	4.2	4.3	4.4	4.3±0.1
	165	3.9	4.0	4.0	4.0±0.1
	170	3.7	3.9	3.9	3.8±0.1
	175	3.7	3.8	3.8	3.8±0.1
	180	3.5	3.6	3.8	3.6±0.2
	185	3.4	3.6	3.7	3.6±0.2
	190	3.3	3.5	3.6	3.4±0.2
	195	3.0	3.4	3.5	3.3±0.3

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