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A stochastic model to assess the effect of meat inspection practices on the contamination of the pig carcasses

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Abstract

The objective of meat inspection is to promote animal and public health, by preventing, detecting and controlling hazards originating from animals. With the improvements of sanitary level in pig herds the hazards profile has shifting and the inspection procedures have no longer targeting major foodborne pathogens (i.e., not risk-based). Additionally carcass manipulations performed when searching for macroscopic lesions can lead to cross-contamination. We therefore developed a stochastic model to quantitatively describe cross-contamination when consecutive carcasses are submitted to classic inspection procedures. The microbial hazard used to illustrate the model was *Salmonella*, the data set was obtained from Brazilian slaughterhouses and some simplifying assumptions were made. The model predicted that, due to cross-contamination during inspection, the prevalence of contaminated carcass surfaces increased from 1.2% to 95.7%, whereas the mean contamination on contaminated surfaces decreased from 1 to -0.87 logCFU/cm², and the standard deviations decreased from 0.65 to 0.19. These results are explained by the fact that, due to carcass manipulations with hands, knives and hooks, including the cutting of contaminated lymph nodes, *Salmonella* is transferred to previously uncontaminated carcasses, but in small quantities. These small quantities can easily go undetected during sampling. Sensitivity analyses gave insight in the model performance and showed that the touching and cutting of lymph nodes during inspection can be an important source of carcass contamination. The model can serve as a tool to support discussions on the modernization of pig carcass inspection.

Keywords: Mathematical modeling, cross-contamination, carcass inspection

1. INTRODUCTION

The main objective of meat inspection is to promote both animal and public health, by preventing, detecting and controlling microbial hazards originating from animals.[1] Although no precise definition about meat inspection procedures has been proposed, the *Codex Alimentarius* refers to two types of inspection. *Ante-mortem* examination consists of a clinical examination aimed at detection of non-healthy animals. *Post-mortem* examination consists of a pathological examination to identify potential hazards for human or animal health.[2] Classically the inspection of pigs is done at all carcasses and the procedures are based on physical examinations, like incisions, palpation and observation of the carcass, organs and lymph nodes, searching for macroscopic lesions, typical for classical zoonotic diseases.[3]

Although the recognition of animals as a source of pathogens to humans dates from prehistoric times, the current procedures were developed in Europe by Robert von Ostertag in 1900.[4] They have an important role in controlling zoonotic diseases, mainly in places, where the production is not done in an intensive production chain[5] and, consequently, classic zoonosis are endemic.

The global livestock production systems have undergone an industrial revolution and the production has shifted increasingly from smallholders to large-scale, industrial production chains. An increasing share of production comes from pigs and chickens that are more easily adapted to large-scale industrial production than ruminants.[6] In 2010, even in developing countries, at least 50% of the herds in pork production are processed in integrated productions systems.[7]

Nowadays, farms adhere to specific management requirements like all-in-all-out production, controlled feed sources, indoor production, and a traceability system from the farm to the slaughterhouse.[8] As a consequence, hazards like parasites are getting rare in the industrial pork

production chain.[8–10] On the other hand, the intensification of the production brings changes in the epidemiology and other microbial pathogens are emerging.[11] *Salmonella* spp., *Yersinia enterocolitica*, *Toxoplasma gondii* and *Trichinella* spp. are identified as the most important hazards to be covered by the meat inspection of swine carcasses.[5]

The interaction of these pathogens with the host and the environment raises some concerns about the suitability of the classic inspection procedures. It demands structured control using all food chain information to reach a risk-based inspection system.[12] The modernization of meat inspection has been extensively studied in Europe and since 2014, according to EC Regulation 219/2014, the inspection of pig carcasses is visual-only for pig herds that have been reared in integrated farm systems, doing palpation and incision when a lesion has been found after visual-only inspection. [13]

In 2011 the European Food Safety Authority (EFSA) discussed the limitations of the meat inspection system procedures, such as lymph node incision, in terms of consumer health protection and stated that the classic procedures could increase the level of cross-contamination, also for zoonotic pathogens. However, quantitative data on the impact of the inspection procedures on cross-contamination are lacking and Hill *et al*.[14] highlighted the need of studies regarding the cross-contamination during the inspection procedures to support a risk-based approach to meat inspection, which could improve the efficiency in dealing with public health issues related to animal slaughter.

In this paper we describe a modelling approach to study the impact of meat inspection practices on cross-contamination between pig carcasses and to provide insight in the potential effect of these practices on the prevalence and concentration of pathogens on pig carcasses. Using methods applied in quantitative microbiological risk assessment (e.g. Nauta *et al*.)[15], we aim to

quantify the cross-contamination during meat inspection of pig carcasses via specific transfer routes and to assess their relevance for the contamination of the carcasses. The model is set up as a generic model for cross-contamination during meat inspection and is applied to *Salmonella* transfer during inspection of pig carcasses in Brazil, because there is relevant data available from some large slaughterhouses in Brazil, and transfer of this pathogen from lymph nodes to the carcass surface has been considered a potential hazard.[16,17] To illustrate the model, we focus on the point of the meat inspection identified as "*CARCASS"* by the Food and Agriculture Organization of the United Nations (FAO).[18] This point of inspection is not the same in all countries. In Brazil this inspection occurs after the carcass splitting and refers to inspection of specific parts of the pig carcass by looking, cutting and touching the skin, musculature, exposed bones, joints, tendon sheaths and serous membrane. It also includes several cuttings and palpation of the following lymph nodes: superficial inguinal, supramammary, external and internal iliac, according to ordinance 711/1995. [19]

2. MATERIALS AND METHODS

2.1 Conceptual model

The basic structure of the model and the transfer routes considered are shown in Fig. 1. The model has a similar structure as the one developed by Nauta *et al*.[15] for broiler processing and is based on classic meat inspection procedures, where a series of consecutively slaughtered carcasses are submitted to several manipulations, and cross-contamination between carcasses may occur via equipment (like cutting knives and hooks used to hang up the carcasses) and hands. Therefore, the *knife, hands and hook* were considered as the relevant components of the slaughter environment. As both the surface and organs of the pig may get in contact with hands and equipment, the carcass was separated in two components: the *carcass surface* and the

possible *organs* evaluated during the inspection. Contact between the carcass (carcass surface and organs) and the environmental components occurs on specific areas of the carcass surface and the organs. The transfer of bacteria can happen from the environmental components to carcass and from the carcass to environmental components.

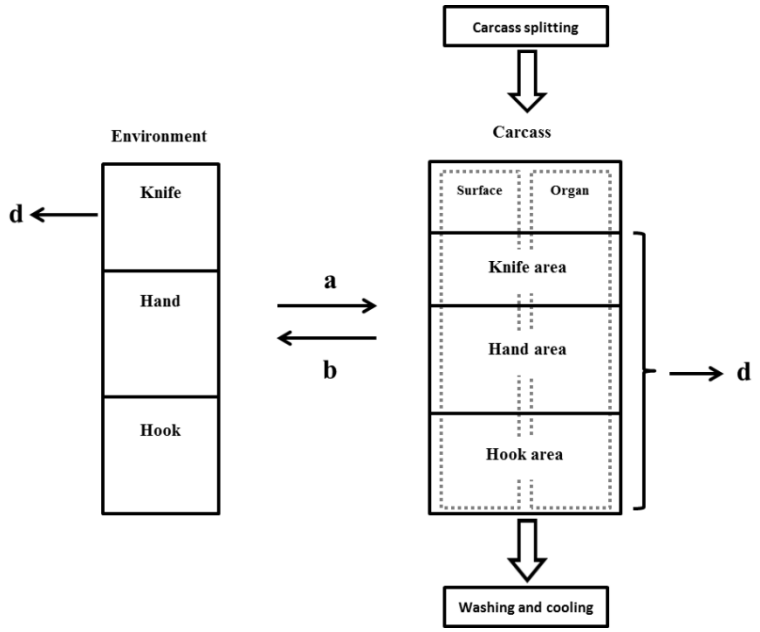

Fig. 1. Schematic representation of the pig carcass inspection. Consecutive carcasses pass through the point of inspection and get in contact with the environmental components, which can lead to cross-contamination via bacterial transfer from the environment to the carcass (arrow a) or from the carcass to the environment (arrow b). The arrows d represent the reduction in the concentration of the bacteria due to inactivation or removal.

The model only considers the carcass and the predefined environmental components as sources of bacteria, the influence of the air, carcass to carcass contact or other external factors are ignored. Also, bacterial growth during the inspection is excluded from the model. Removal of the bacteria from the carcass (surface or organs) can only occur by the inspection activities that are included in the model. Bacteria on the knife are frequently inactivated by putting the knife in

hot water (i. e. 83 °C). Washing of hands and cleaning of the hooks are unusual or don't follow a clear rule during meat inspection and have therefore not been considered.

2.2 Mathematical model

The model can be written as a system of five difference equations as given below (1). It describes the changes in the concentrations in the five components for consecutively slaughtered carcasses *i*, before inspection (stage S-1) and after inspection (stage S). Variables are listed in Table 1. The upper cases letters represent variables, and lower case letters represent model parameters. *Ae* and *Ac* are used as index and refer to the different areas on carcass (*Ae* = knife (K), hand (H), hook (G)) and different compartments of carcass (*Ac* = surface (C) or organs (O)).

$$\begin{cases} C_{i,S} = \sum_{Ae\in\{K,H,G\}} (1-d_C)(1-b_{C,Ae})^{J_{i,C,Ae}} C_{i,(S-1),Ae} + E_{(i-1),Ae}\left(1-\left(1-a_{Ae,C}\right)^{J_{i,C,Ae}}\right) \\ O_{i,S} = \sum_{Ae\in\{K,H,G\}} (1-d_O)(1-b_{O,Ae,})^{J_{i,O,Ae}} O_{i,(S-1),Ae} + E_{(i-1),Ae}\left(1-\left(1-a_{Ae,O}\right)^{J_{i,O,Ae}}\right) \\ K_i = K_{(i-1)} \prod_{Ac\in\{C,O\}}(1-a_{K,Ac})^{J_{(i-1),Ac,K}} + \sum_{Ac\in\{C,O\}} N_{i,Ac}(1-d_{Ac})\left(1-(1-b_{Ac,K})^{J_{i,Ac,K}}\right) \\ H_i = H_{(i-1)} \prod_{Ac\in\{C,O\}}(1-a_{H,Ac})^{J_{(i-1),Ac,H}} + \sum_{Ac\in\{C,O\}} N_{i,Ac}(1-d_{Ac})\left(1-(1-b_{Ac,H})^{J_{i,Ac,H}}\right) \\ G_i = G_{(i-1)} \prod_{Ac\in\{C,O\}}(1-a_{G,Ac})^{J_{(i-1),Ac,G}} + \sum_{Ac\in\{C,O\}} N_{i,Ac}(1-d_{Ac})\left(1-(1-b_{Ac,G})^{J_{i,Ac,G}}\right) \end{cases} \quad (1)$$

The variable $E_{i,Ae}$ is the generic term to refer to the environment and the value of *Ae* for knife, hands or hook will be used according to the area modeled. Similarly, the variable $N_{i,Ac}$ is a generic term to refer to the carcass surface or organs, according to the component *Ac* modeled. Organs will be referred to from here onward, as lymph nodes, because that is the most relevant organ evaluated during this specific inspection point. The variables are explained in the Table I. The numbers of contacts between the environmental components and the carcasses compartments are represented generically by $J_{i,Ae,Ac}$. When *Ae*= K, H, G it refers to the number of contacts between the carcass and the knife, hand and hook respectively. These values are sampled from empirical distributions (see appendix A) and are assumed to affect either the

surface or lymph nodes (*Ac*=C, O) with equal probability (50%). Also, the three areas on carcass or lymph nodes are considered mutually exclusive: the worker does not touch the same carcass area with his hands as the worker cuts with a knife or holds the carcass with the hook.

Table I. Overview of model variables (eq. 1): Each variable describes a quantity that is changing for consecutive carcasses (with rank number *i*) and over the process steps S-1 (before) or S (after inspection). Values before inspection are sampled from the indicated distributions and values after inspection are calculated by the model

Variable	Description	Distribution/function	Unit	Source
$J_{i,Ac,Ae}$	Number of cuts, touches or hooking (*Ae*) in the surface or organs (*Ac*) of the carcass *i*	Empirical[#]	Count	Appendix A
$log_{10}[C_{i,(S-1)}]$	*Salmonella* concentration on the carcass surface *i* before inspection (S-1) on contaminated carcass.	Normal(-5.4;2.2)[◊ #]	$\log_{10}$CFU/cm^2	[20] Appendix B
$PrevC_{i\,(S-1)}$	Status of carcass surface contamination on the carcass *i* before inspection (*S*-1)	100%	Positive/Negative	[20] Appendix B
$C_{i,(S-1)}$	*Salmonella* counts on the carcass surface *i* before inspection (S-1) on contaminated carcass.	$Poisson([C_{i\,(S-1)}]ea_{Ae}\,J_{i,C,Ae})$	CFU[##]	Calculation, see Table 2
$[O_{i,(S-1)}]$	*Salmonella* concentration in organs (lymph nodes) *i* before inspection *(S-1)* in contaminated lymph nodes.	Triangular(0.1;1;100) [#]	CFU/cm^2	Assumption
$O_{i,(S-1)}$	*Salmonella* counts in organs (lymph nodes) *i* before inspection *(S-1)* in contaminated lymph nodes.	$Poisson([O_{i\,(S-1)}]ea_{Ae}\,J_{i,O,Ae})$	CFU[##]	Calculation, see Table 2
$PrevO_{i,(S-1)}$	Status of organs contamination in the carcass *i* before inspection (*S*-1) (i.e., carrying *Salmonella* in lymph nodes)	Bernoulli(14.1%)	Positive/Negative	[11]
K_i	Amount of *Salmonella* on knife by the carcass *i* after inspection	Model	CFU	Calculation
H_i	Amount of *Salmonella* on hands by the carcass *i*	Model	CFU	Calculation

	after inspection			
G_i	Amount of *Salmonella* on hook by the carcass *i* after inspection	Model	CFU	Calculation

[#] Distribution expressing variability between carcasses *i*; [◊]Parameters (μ, σ and $PrevC_{i\,(S-1)}$) were fitted according a zero inflated normal distribution by Maximum Log likelihood estimation method (Appendix B); [##]CFU per inspected area.

The model was implemented as a Monte Carlo simulation model. Transfers were described as binomial processes taking into account the successive contacts between environment and carcass, as explained in appendix C. For example, in the first term in the equations considering the carcass, $(1-d_C)(1-b_{C,Ae})^{J_{i,C,A_e}}$ is the fraction of the number of *Salmonella* that are not lost by removal (*d*) and not transferred from the carcass, to the environment on different areas, indicated by the index *Ae* (knife area, hand area, and hook area). The second term is $\left(1-\left(1-a_{Ae,C}\right)^{J_{i,C,Ae}}\right)$, the fraction of the number of *Salmonella* received from the environment indicated by the index *Ae* (knife, hand and hook) to the carcass and can be derived as explained in appendix C.

In the last three equations, modeling the environmental components, using the knife as example, the first term: $K_{(i-1)}\ \prod_{Ac\in\{C,O\}}(1-a_{K,Ac})^{J_{(i-1),Ac,K}}$ concerns the *Salmonella* that are not transferred from the knife to the carcass (*i*-1) on different compartments indexed by *Ac* (surface and lymph nodes). The second term $(1-d_{Ac})(1-(1-b_{Ac,K})^{J_{i,Ac,K}}$ indicates the *Salmonella* received from the carcass indicated by the index *Ac* (surface or lymph nodes). The variables $C_{i,\,(S-1)}$ and $O_{\,i,(S-1)}$ represent the counts of *Salmonella* before the inspection and describe the variability between inspected carcass surfaces and organs respectively. To account for the random spatial distribution of cells over the inspected area, a Poisson distribution was used. In order to assess the true prevalence, the variables $C_{(i\,-1),S}$ and $O_{\,(i-1),S}$ were multiplied by the positive/negative

status (1 or 0) of carcass surface contamination $PrevC_{i,(S-1)}$ and lymph nodes $PrevO_{i,(S-1)}$, both sampled from Bernoulli distributions.

The transfer parameters *a*, *b* are used in combination with the index *Ae* or *Ac* according to the area on the carcass or the environmental components modeled. For instance when the parameter *a* is used with index *Ae*, it refers to the probability of transfer of a CFU from the environment according index *Ae* used (knife, hand or hook) to the carcass (C) or lymph nodes (O). The removal parameter *d*, is indexed by *Ac* because detection or reduction are accounted only on carcass surface and lymph nodes. Table II provides an overview of the parameters used in the model.

Counts of *Salmonella* were expressed in CFU and the outputs were calculated for the inspected areas (CFU/cm²) and then transformed to natural logarithm (presented here as "*log*"), considering only the contaminated carcasses (because log (0) is not defined). When a carcass has not been submitted to any contact with the environment by hands, knife or hook, the carcass was considered as not inspected and, consequently, the concentration on inspected area is assumed to be the same as before (S-1). Also the probability of inactivation or removal on carcass or in lymph nodes (d_C *or* d_O) are underlying assumed to be zero. The analyses were done using @Risk 6.2.1 (Palisade) for Excel with 10000 iterations using 500 and 100 consecutively processed carcasses in two separate simulations. These numbers were chosen to approximate realistic numbers of pigs slaughtered in a slaughter line per shift of two hours (i.e. 350 carcasses/hour), whilst keeping the model manageable and restricting the running time.

Table II. Parameters used to illustrate the dynamics of the model. The indices *Ae* and *Ac* are given by the initials of environment and carcass compartments respectively

Parameters	Description	Unit	Value	Source
$a_{K,C}$	Transfer probability knife-carcass	%	0. 17	(21)
$a_{K,O}$	Transfer probability knife-lymph nodes	%	0. 17	(21)
$a_{H,C}$	Transfer probability hand-carcass	%	0. 21	(22)
$a_{H,O}$	Transfer probability hand-lymph nodes	%	0. 21	(22)
$a_{G,C}$	Transfer probability hook-carcass	%	0. 17	(21)
$a_{G,O}$	Transfer probability hook-lymph nodes	%	0. 17	(21)
$b_{C,K}$	Transfer probability carcass-knife	%	0. 17	(21)
$b_{C,H}$	Transfer probability carcass-hand	%	3.1	(22)
$b_{C,G}$	Transfer probability carcass-hook	%	0. 17	(21)
$b_{O,K}$	Transfer probability lymph nodes-knife	%	0. 17	(21)
$b_{O,H}$	Transfer probability lymph nodes-hand	%	0. 21	(22)
$b_{O,G}$	Transfer probability lymph nodes-hook	%	0. 17	(21)
ea_{Ae}	Environmental components area			
Ae=H	Area of touch (cm²)	cm²	150	Assumption*
Ae=G	Area of hook (cm²)	cm²	1	Assumption*
Ae=K	Area of cut (cm²)	cm²	10	Assumption*
ck	Probability of changing the knife	%	90	Assumption (based on observations)

* Estimates for the medium size of these areas, author's best guess.

2.3 Sensitivity analysis

First, the baseline model was built with the parameter values indicated in Tables 1 and 2. Next, two types of sensitivity analyses were performed. First, several *univariate* analyses were done to assess the impact of parameters on the model outputs. To avoid unrealistic values we used a range of values between each parameter baseline value (*y*) and realistic minimum and maximum

values of the parameter considered (y^-) and (y^+) respectively (Appendix D). To assess the impact of ranges of input values, above and below the baseline we applied:

$$f(x; y, y^-, y^+) = \begin{cases} y + (y - y^-)x, & for\ x < 0 \\ y - (y - y^+)x, & for\ x \geq 0 \end{cases} \quad (2)$$

witch runs from minimum to maximum when *x* runs from -1 to 1 and meets the baseline when *x*=0. The univariate analyses were ran with 10000 iterations using 100 carcasses. Based on the *univariate* results, nine scenarios were submitted to *multivariate* analyses (Appendix E) and simulated with 10000 iterations using 500 carcasses.

2.4 Data sources

The data on the carcass surface contamination were obtained from da Silva *et al*.[20] These authors collected carcass surface swabs in three Brazilian commercial slaughterhouses. Data regarding the lymph nodes prevalence where obtained from 12 cohorts representing finishing herds located in the state of Santa Catarina, Brazil.[11] These herds belong to an integrated system responsible for approximately 7% of all Brazilian pork production in 2007. Manipulation data were observed during two weeks in March 2015 in a large Brazilian pig slaughterhouse dedicated to exportation. 778 inspection procedures were counted during this period, of which 290 in the inspection point "*CARCASS"*. The numbers of manipulations were recorded in a database. Although no data regarding transfer probability in slaughterhouse environment could be found, results from Kim *et al*.[21] and Hong and Bahk[22], provide transfer probabilities between hands and pork and knife and pork, respectively. We have not found suitable concentration data for *Salmonella* in lymph nodes, the values used were based on estimates of the authors.

3. RESULTS

3.1 Baseline and distributions

In the baseline model, the mean of the mean concentrations (μ) and the mean prevalence were determined for two independent simulations with 500 and 100 consecutive carcasses, over 10000 iterations. As the mean concentration is the mean of logs, only contaminated carcasses are included in the calculations. The results are summarized and given in Table III. The mean concentration on inspected areas of the contaminated carcass surfaces is decreasing after inspection procedures, from 1 to -0.87 logCFU/cm². Standard deviations of the means decrease as well, from 0.65 to 0.2. The reason is that many more carcass surfaces are getting contaminated by cross-contamination, resulting in a large number of carcasses (i. e. a prevalence difference of 94.6 percentage points) contaminated with lower concentrations. Consequently the variability is decreasing (See Fig. 2).

Table III. Outputs for inspected areas of carcass surface and lymph nodes before and after inspection procedures in model simulations with 500 and 100 carcasses. The mean (μ) and standard deviation (σ) are those of the mean values for the contaminated inspected carcass areas among the 500 or 100 simulated carcasses; the prevalence is the mean prevalence after 10000 iterations

	500 carcasses			100 Carcasses		
	μ (σ) logCFU/cm²	Prevalence %[#]	CFU in the system[##]	μ (σ) logCFU/cm²	Prevalence %[#]	CFU in the system[##]
Surface before	1 (0.65)	1.2	$2.6*10^5$	1 (1.22)	1.2	$4.2*10^4$
Surface after	-0.87 (0.2)	95.8	$3.7*10^5$	-1.6 (0.47)	92	$5.2*10^4$
LN before	3.17 (0.13)	22.2	$2.3*10^6$	3.17 (0.3)	23.8	$4.6*10^5$
LN after	0.08 (0.2)	96.7	$2.1*10^6$	-0.41 (0.51)	93.9	$4.1*10^5$

LN=Lymph node; [#] The prevalence refers to at least one cell on inspected area; [##] arithmetic mean (over 10000 iterations) of the total number of *Sallmonella* on carcasses surface and lymph nodes (i. e. whole carcass) in all simulated areas per iteration.

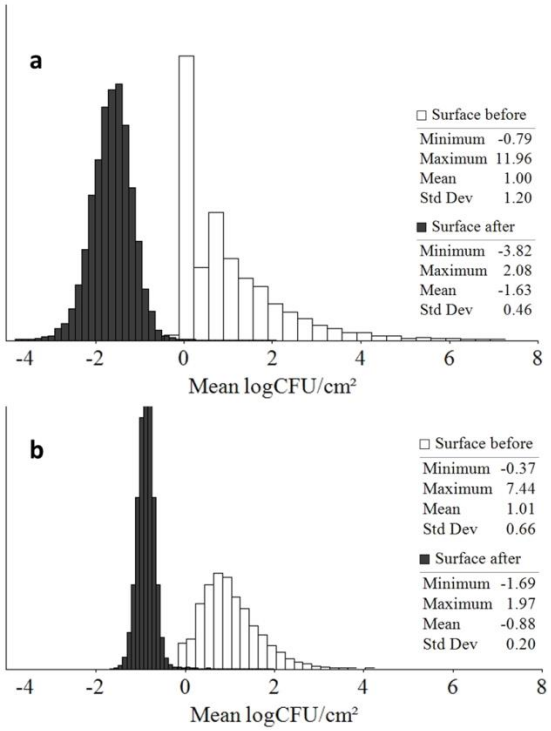

Fig. 2. Distribution of mean carcasses surface contamination (logCFU/cm^2) considering only contaminated carcasses before and after inspection in two separated simulations with 100 (a) and 500 (b) carcasses.

For the lymph nodes the effects of the inspection procedures on the mean and prevalence are similar to those observed in the carcass surface (i.e. decrease and increase respectively), but the standard deviation increases after inspection. Although the results suggest a reduction in mean carcass surface contamination, after inspection, the sum of the numbers of *Salmonella* ("CFU in the system") is increasing on the carcass surface and decreasing in the lymph nodes. The reason

252 is that the geometric mean (mean of logCFU), which only can includes values larger than zero
253 (i.e. contaminated carcasses), should not be interpreted as an arithmetic mean. As the model does
254 not assume any growth, the only sources of contamination are the carcasses entering into the
255 slaughterhouse and therefore the results indicate a flow of contamination from the lymphatic
256 tissue to the surface by inspection procedures.
257 Results differ depending on the number of simulated carcasses. With a lower number of
258 carcasses, the variation in mean concentrations sampled from the zero inflated Poisson
259 Lognormal is larger. For example, with the prevalence 1.2%, the probability that the
260 concentrations in all 100 carcass surfaces are zero is $(1\text{-}0.012)^{100}$ =30%, for 500 carcass surfaces
261 it is $(1\text{-}0.012)^{500}$ = 0.24% (compare Fig 2a and 2b). The peaks in figure 2a before inspection
262 reflect the sampling of 1 and 2 positive carcasses, with the variability in concentrations around it.
263 Differences between distributions are smaller when considered after inspection, because more
264 carcass surfaces are contaminated. Hence, the number of carcasses used in the analysis is
265 relevant and the number of carcasses used to run the model should be realistic. Still, very large
266 numbers of carcasses slow down the calculations considerably.

267 **3.2 Univariate sensitivity analyses**

268 Of the 23 parameters analyzed (see appendix D) seven had a significant impact on the output
269 mean of the means (μ) and four on the mean prevalence. Here, the impact is considered
270 significant if the mean output values in the sensitivity analysis fall out of the range correspondent
271 to 2.5^{th} and 97.5^{th} percentiles of the distribution describing the variability between model
272 iterations in the mean μ from -2.57 to -0.83 logCFU/cm^2 and prevalence 81-98%. As shown in
273 Fig. 3 the mean concentration of carcass surface contamination before inspection $[C_{i,(S-1)}]$ and
274 its standard deviation had an important effect on the surface contamination after inspection

procedures. The mean after inspection increased from -1.6 to 9.07 logCFU/cm^2 on the inspection area when the load of *Salmonella* on carcass surfaces before inspection approaches the maximum value (2 $\log_{10}$CFU/cm^2 compared to -5.4 in the baseline). The same effect cannot be seen when mean and standard deviation are decreased below the baseline. Changes in lymph nodes contamination, by changing the maximum value of the triangular distribution used in $[O_{i,(S-1)}]$ from 100 to 1000 CFU/cm^2, increased the mean to 0.54 logCFU/cm^2, and decreases it to -3.6 logCFU/cm^2 when the parameter is reduced to 10 CFU/cm^2. Also the prevalence of animals carrying *Salmonella* in lymph nodes had an important effect by reducing the mean contamination to -4.3 logCFU/cm^2 and increasing it to -0.7 logCFU/cm^2 compared to the baseline (-1.6 logCFU/cm^2).

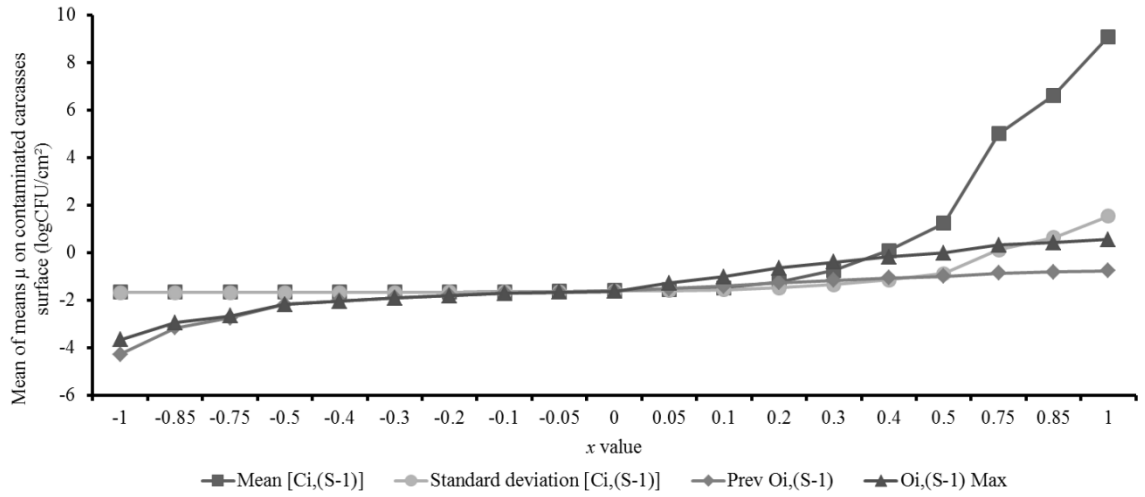

Fig. 3. Mean of the means (μ) logCFU/cm^2 in in function of different *x* values regarding the variables mean [$C_{i,(S-1)}$], standard deviation of [$C_{i,(S-1)}$], Prev $O_{i,(S-1)}$ and maximum [$O_{i,(S-1)}$].

Fig. 4 shows how changes in transfer probabilities affect the mean contamination on the carcass surface. If the transfer probability from hands to lymph nodes $(a_{H,O})$ decreases to 0% using *x* = -1, the lack of bacterial transfer from hands to lymph nodes leads to an increase of the amount on

the hands and a subsequent increase of transfer to the carcass surface, leading to a small increase of the mean to approximately -1.45 logCFU/cm^2. But when the same parameter is increased, the mean decreases because the cells transferred to the lymph nodes can no longer be transferred to the carcass surface.

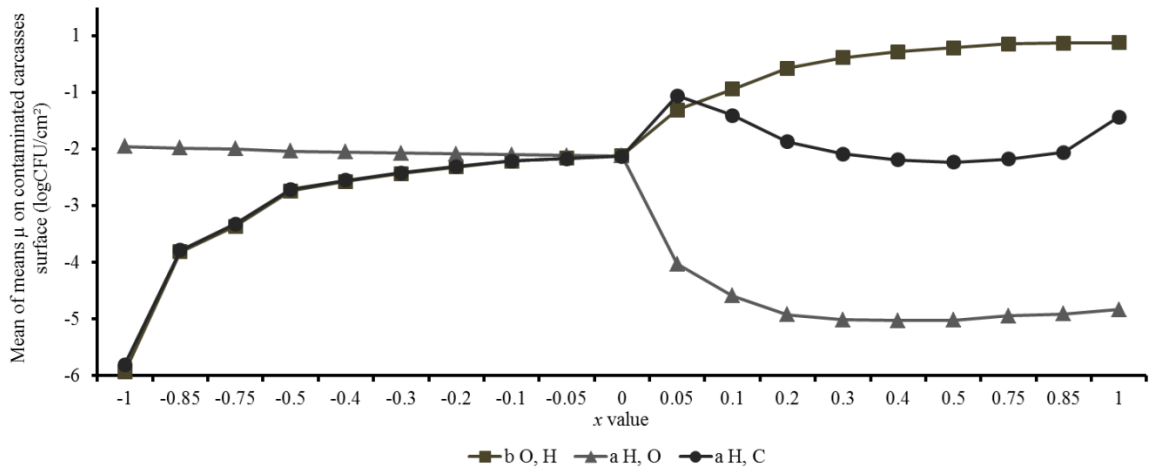

Fig. 4. Mean of the means (μ) logCFU/cm^2 as a function of different *x* values regarding the parameters ($b_{O,H}$), ($a_{H,O}$) and ($a_{H,C}$).

Both transfer probabilities from the lymph node to hand $(b_{O,H})$ and to carcass by the hand $(a_{H,C})$, show similar results below the baseline, but $b_{O,H}$ keeps increasing the mean until *x* approaches 1 ($b_{O,H}$= 100%). On the other hand, $a_{H,C}$ has a peak when *x* is close to 0.05. There is a peak because, at some point, the transfer from hand to carcass gets so large that the concentration on the hands gets too low. Once a large number of bacteria are transferred to the first carcasses only a few bacteria are transferred to the subsequent carcasses, reducing the mean concentration without relevant effects on prevalence (Fig. 5). As the $a_{H,C}$ keeps increasing, bacteria get even more concentrated on the first carcasses after hands contamination, reducing

prevalence compared to the situation with a lower $a_{H,C}$ (Fig. 5). As the mean log can be calculated only for contaminated carcasses (i. e. one or more CFU), the reduction of contaminated carcasses leads to increases of the mean (logCFU/cm²) when the *x* increases for the variables $a_{H,C}$ and $a_{H,O}$.

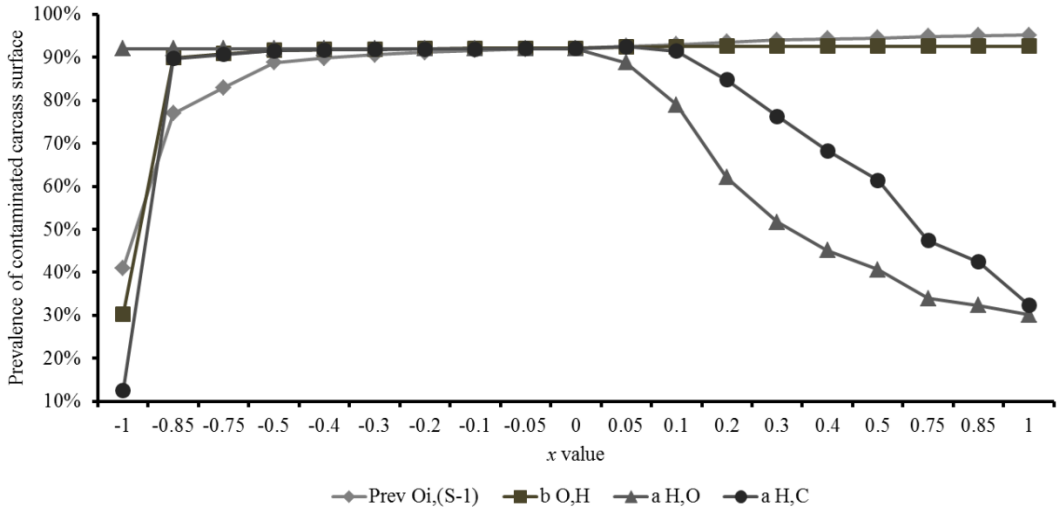

Fig. 5. Prevalence of carcass surface contamination as a function of different *x* values regarding the variable Prev $O_{,i\,(S-1)}$ and parameters ($b_{O,H}$), ($a_{H,O}$) and ($a_{H,C}$).

Fig. 5 shows the effects of tested parameters on prevalence after inspection. The effect of reduction of $PrevO_{i,(S-1)}$, $b_{O,H}$ and $a_{H,C}$ to zero (minimum values, when x=-1) leads to a reduction of the surface prevalence to approximately 40%, 29% and 12% respectively, whereas reductions in $a_{H,O}$ do not seem to affect the surface prevalence. When the values of the transfer parameters $a_{H,O}$ and $a_{H,C}$ are increased, a reduction of the prevalence is observed. The reduction in the number of positive carcasses leads to an increase of the mean log surface contamination as observed in Fig. 4, as this can be calculated for positive carcasses only.

3.3 Multivariate sensitivity analyses

Table IV shows the mean of the means (μ) logCFU/cm^2, its standard deviation (σ) and mean prevalence on carcass surface 'before' and 'after' inspection in the multivariate sensitivity analyses. The first scenario shows the baseline for comparison proposes. The second and third scenarios present a stress analysis to verify the model performance. As expected, when transfer probabilities are set to zero, the outputs 'before' and 'after' were the same. Also, the absence of sources of contamination results in a completely uncontaminated scenario after inspection, meeting the null contamination set by the parameters.

Table IV. Scenarios used in multivariate analyses to test the effect of different variables combination on mean of the means (μ) logCFU/cm^2 its standard deviation (σ) and mean prevalence of contaminated carcass surface before and after inspection

	μ (σ) logCFU/cm²		Prevalence %	
Scenario	before	after	before	after
Baseline	1 (0.65)	-0.87 (0.2)	1.2	95.8
No transfer	1 (0.65)	1 (0.65)	1.2	1.2
No contamination (S-1)	-	-	0	0
Only carcass (S-1)	1 (0.65)	-5.08 (0.89)	1.2	43.9
Only LN (S-1)	-	-0.93 (0.18)	0	95.7
Hand influence high mean on $log_{10}[C_{i,(S-1)}]$	1.8 (0.28)	0.64 (0.82)	11.3	96.7
Hand influence high standard deviation on $log_{10}[C_{i,(S-1)}]$	2.6 (0.52)	0.12 (1.56)	6.2	96.4

Hand Influence high $[O_{i,(S-1)}]$	1 (0.65)	1.31 (0.18)	1.2	97.3
Hand Influence high $PrevO_{i,(S-1)}$	1 (1.65)	0.5 (0.11)	1.2	97.4

When only the carcass surface was included as the source of *Salmonella*, an important difference could be found as both the level of surface contamination and prevalence after inspection were drastically reduced compared to the baseline. The influence of high transfer probability involving the hands and carcass, by increasing the parameters $a_{H,C}$, $b_{C,H}$ (appendix E) tested together with a higher initial concentration on contaminated carcass surfaces ($log_{10}[C_{i,(S-1)}]$ = -3 $\log_{10}$ CFU/cm^2), increased the mean from -1.6 to 0.64 logCFU/cm^2 and the mean prevalence to 96.7%. The influence of high transfer probability involving the hands and carcass was also tested with an increase of variability of contamination on carcass surface. It resulted in an increase of carcass surface contamination because surface contamination (S-1) is entered in the model as $\log_{10}$, so increases in variability affect the expected value, as the arithmetic mean of C_{iS} equals $10^{(\mu+(\frac{1}{2}\log(10)\sigma^2)}$ and the transfer of bacteria acts as a factor of quantity and not of the log-quantity.

Also, the influence of high transfer probability involving hands and carcass was tested with a higher concentration of *Salmonella* in lymph nodes (mean=337 CFU/cm^2) and a high frequency of animals carrying *Salmonella* in lymph nodes (100%). The increase in lymph nodes contamination $O_{i,(S-1)}$ had an important effect on the mean, changing it from -0.88 to 1.31 logCFU/cm². Changings in Prev $O_{i,(S-1)}$ also increased the surface contamination and prevalence after inspection. The mean on surface contamination increased to 0.55 logCFU/cm^2 and the prevalence to 97.4%.

4. DISCUSSION

We developed a generic mechanistic model to assess the effect of cross-contamination during pig carcass inspection, which can be applied to different hazards for different inspection practices. Its performance has been studied for one inspection step, using a Brazilian data set on *Salmonella* contamination and some parameters assumptions. The results allow us to draw conclusions on the potential impact of the cross-contamination during meat inspection, but are not necessarily considered representative for the impact of the whole inspection process of pig carcasses and the related policies in Brazil, since it deals with only one point of inspection. To do so, all the three points in Brazilian inspection of pig carcasses should be included and data about the contamination in lymph nodes should be also used as an input. Corbellini *et al*. [(23)] have reported the importance of variability between different days and slaughterhouses on *Salmonella* contamination in Brazil and this information is essential for a realistic assessment of the impact of meat inspections practices in the country.

With the inputs used, the model showed that the meat inspection leads to a redistribution of *Salmonella* over the carcasses, which implies that many more carcasses become contaminated, but with (very) low numbers of bacteria. In terms of prevalence and concentrations we found an increase in the surface contamination prevalence with more than 90 percentage points through the inspection process and, due to the increase in the number of contaminated carcasses, a decrease in the mean of the mean log concentrations in contaminated carcasses. The cutting of the lymph nodes during inspection plays an important role, as it adds *Salmonella* to the carcass surface areas that were not present on carcass surfaces before inspection. Overall, the model shows that the conduction of meat inspection can lead to a spread of *Salmonella* from the lymphatic tissue to carcass surface, decreasing the differences between the surface contamination

of different carcasses.

Note that the baseline depicts a scenario of high lymph node contamination, and although we have no data about lymph nodes contamination, prevalence studies have shown that this is not always realistic.[24–26] The phenomena described here meet results from previous research on the effect of carcass manipulation on carcass surface contamination by *Salmonella*, where the importance of lymphatic tissue manipulation has been observed in herds with a high number of pigs harboring the bacteria in lymph nodes.[17,24] If the model would be applied to obtain realistic estimates, the user should adjust parameter values and distributions to their observations. For example, variation in the prevalence of contaminated pigs or contaminated lymph nodes entering in slaughterhouse can be found as a function of season, slaughterhouse and slaughter day.[27–29]

In the sensitivity analyses, equation (2), used to standardize the domain, can result in a sudden changing on the value of the parameters (i.e. $f(x)$) when x=0 (e.g. Fig 4.). It occurs because the derivative $f'(x)$ is $(y - y^-)$ for x<0 and $(y - y^+)$ for x≥0, so when $(y - y^-) \neq (y - y^+)$ the equation has two different slopes below and above the baseline x = 0. As the distances in the image (i.e. $\Delta f(x)$) are not the same, this can give the impression that the effect is stronger on one side than the other (Fig. 4 and 5). As an example when the bacterial transfer baseline is 0.17%, (i. e. far from the 50%, center of this domain) with minimum and maximum values as 0 and 1 respectively, applying equation (2) we obtain a transfer value of approximately 11% when x=0.1 and 0.1% when x=-0.1.

The multivariate sensitivity analysis (table IV) showed that, when the lymph nodes were considered to be uncontaminated (i.e only the carcass surface was a source of contamination), the surface contamination after inspection was much lower than in the baseline. Also, the mean (SD) logCFU/cm^2 decreased to -5 (0.89) and in such a scenario a large number of positive carcasses

would be below the limit of detection (-4 logCFU/cm^2)[20], so the observable prevalence would only be 5% instead of 44%. On the other hand, when only the lymph nodes are considered as source of bacteria, the results were kept similar to the baseline, indicating that the effect of lymph nodes inspection dominated the surface carcass contamination.

When only considering the prevalence, the results obtained here may seem to be alarming and unrealistic, because the increase in more than 90 percentage points is very large and it is not observed in prevalence studies, which give values like 24%[11] and 14%.[20] Although these prevalence results were obtained in Brazilian slaughterhouses before chilling, no inactivation step is used in Brazil between the carcass inspection and the chilling. A reason that observed prevalences are so much lower than predicted by the model may be the localization of the contaminating bacteria, which is restricted to areas manipulated by the inspection workers. These may not correspond with areas sampled when these prevalence studies were performed. According to Jongenburger *et al*.[30] batches with localized bacterial concentration reduce the observed prevalence with a factor *l*, derived from the relative size of the contaminated areas compared to the whole surface (see Appendix F). Another issue could be related with the difference between the measured prevalence (observed frequency of carcasses positive for *Salmonella* in the microbial test) and the modeled prevalence, which refers to the true prevalence, that is carcasses with one or more CFU. Although this difference must to be taken into account, in our simulations the mean (SD) contamination on carcass surfaces after inspection was -0.8 (0.2) logCFU/cm^2 (Figure 2) which assuming a normal distribution, means that none carcasses will have a level of contamination below the limit of detection (LOD=-4 logCFU/cm^2)[20] (calculations not shown). Hence, it is expected that the modeled and measured prevalences are expected to be the same in our simulations.

The carcass inspection is one of the many activities in the whole pork production and the model does not allow us to access the impact of the inspection procedures when compared to more extensive dressing activities. In this sense studies as conducted by Swart *et al*. [31] describing *Salmonella* concentrations at different stages of the slaughterhouse process should be conducted. Also no direct conclusion can be drawn regarding the impact of inspection procedures on the number of human salmonellosis cases. A quantitative microbial risk assessment (QMRA) could help to answer such question, since the outputs of this model can be applied to assess the impact of the cross-contamination on human exposure. [32]

The present model is a tool to account for cross-contamination during the carcass inspection. The purpose of the model is to capture the essential dynamics, and therefore the right balance between reality/complexity and simplicity is important.[33] Simplifying assumptions about the inspection process are for example that we choose to give an equal probability of handling the carcass surface and lymph nodes and that the inspection workers are equally capable to run the inspections and treat each carcass randomly. Only knife, hand and hook are modelled, because procedures regarding the use of these three components are more standardized and easier to quantify. In general, the effect of direct contact (hand, knife) is more important to carcass contamination than other potential sources of contamination.[34,35]

Also, in this model, only carcass surface and lymph nodes are considered as sources of contamination. Regarding the meat inspection procedures, this can be considered a realistic approach because viscera, like intestines, are cut and manipulated, usually, in another step of the slaughter process[2]. Although some adaptations of the model may be necessary, the model is generic enough to deal with different hazards and inspection processes.

Several authors have reported different approaches to deal with cross-contamination and transfer

in food products[36–41], and a particularly interesting approach is proposed by Smid *et al*.[42], taking into account the uncertainty generated in transfer experiments. Here, we preferred to use a binomial process, assuming a mechanistic approach regarding the transfer of cells[38], but the model can be updated in order to consider new evidence about the transfer of different hazards in pork.

The model applied to *Salmonella* has shown that the manipulation parameters and the initial contamination of the carcass surfaces and the lymph nodes are the most important for the surface contamination after inspection. Although some studies have reported bacterial quantification on carcass surfaces[43,44], these studies are scarce and they do not always account for quantification immediately before the inspection point. To our knowledge, no data are available on *Salmonella* concentrations in the lymph nodes of pigs, whereas the model shows that this information is essential to assess the impact of cross-contamination during meat inspection.

Although the traditional meat inspection procedures aim to protect human health, our results show that the cutting and handling of the carcasses and organs during inspection may also have the opposite effect. According to Hill *et al*.[14] modernization of meat inspection towards to visual-only approach does not seem to be a threat to public health. However, these authors also identify a lack of knowledge regarding cross-contamination during the traditional pig carcass inspection and indicate that this information is needed. Furthermore, Ravel *et al*.[45] discuss that during the traditional inspection system, cross-contamination can occur between the lymph nodes and other parts of the same carcass or even between consecutive carcasses, but the cross-contamination level has not been described so far.[45]

The results, also, highlight the importance of bacterial transfer between carcass surface, hands and lymph nodes if a high number of animals carrying *Salmonella* in lymph nodes are expected.

Furthermore it sheds some light on the potential inadequacy of classic pig carcass inspection and therefore it can be considered as a tool to quantify the effects of cross-contamination and answer questions about the modernization of the classic carcass inspection system for the implementation of risk-based approaches in meat-inspection.[46]

5. CONCLUSIONS

In the classic veterinary meat inspection of pig carcasses, the effect of cross-contamination may not be negligible. The model presented in this paper offers a tool to quantify these effects. Our analyses how that, especially when animals that carry high concentrations of *Salmonella* in lymph nodes are entering the slaughterhouse, bacteria will be spread to many previously uncontaminated carcasses. The model had not been validated, so far, and this step is important to figure out the suitability of this model in describe cross-contamination during classic inspection procedures and support the modernization of inspection of pig carcass.

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Appendix A. Values used in the empirical distribution that is applied to sample the number of interactions between carcass/organ with hands, knife and hook using the index Ae= (H, K, G). Probabilities in columns sum up to 1

Counts	$J_{i,Ac,H}$	$J_{i,Ac,G}$	$J_{i,Ac,K}$
0	0.009	0	0
1	0.009	0.017	0.008
2	0.018	0.483	0.051

3	0.027	0.433	0.034
4	0.152	0.033	0.144
5	0.116	0.017	0.161
6	0.143	0.017	0.169
7	0.134	0	0.161
8	0.161	0	0.059
9	0.045	0	0.068
10	0.098	0	0.051
11	0.009	0	0.000
12	0.018	0	0.034
13	0	0	0
14	0.018	0	0.017
15	0.009	0	0.025
16	0.018	0	0
17	0	0	0.008
18	0	0	0

19	0.009	0	0.008
20	0.009	0	0
21	0	0	0
23	0	0	0

637

638 **Appendix B**

639 It is assumed that the concentrations on the carcass surfaces before inspection $[C_{i\,(S-1)}]$ can be
640 described by a zero inflated lognormal distribution with prevalence *p* and thus a probability of an
641 uncontaminated carcasses 1-*p*. Let $u = Log_{10}(x)$ and the probability density function $f(u)$ for
642 concentration $[C_{i\,(S-1)}]$, can be defined by:

643 $$f(u) = \begin{cases} p * \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{u-\mu}{\sigma}\right)^2} & u > LOQ \\ p * \int_{LOD}^{LOQ} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{u-\mu}{\sigma}\right)^2} du & LOD < u < LOQ \\ (1-p) + \left(p * \int_{0}^{LOD} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{u-\mu}{\sigma}\right)^2} du\right) & u < LOD \end{cases}$$

644 Where *LOQ* and *LOD* are limits of quantification and detection of *Salmonella*, respectively.
645 Applying this probability density function to each sample x_i for carcass surfaces *i* in the study
646 from da Silva *et al*.[20], the parameters (μ, σ and *p*) were assessed by the Maximum Log
647 likelihood estimation using the Solver function in Excel, i.e. maximizing

648 $$\sum_{i=1}^{n} \text{Log}\, f(x_i; \mu, \sigma, p)$$

649 Where, *i* is the carcass number and *n* is the last carcass sampled.

650

651 **Appendix C**

652 Consider a system with two compartments *E* and *C* in which a sequence of events (in this case
653 the manipulations: cuts, touches or hookings) is defined by the index *J*, with $J \in \mathrm{N}$ indicating the
654 number of manipulations. Also consider no loss and no increase of units, only transfer is
655 modeled with a given initial condition:

656 $E_0 = 100$ CFU

657 The transfer probability from the compartment *E* to the *C* is called *q*, and it is assumed to be
658 constant through the sequential steps *J*.

659 So, the amount on the *E* compartment after *J* steps is the amount before the step *J* minus a
660 fraction *q*:

661 $$E_J = E_{J-1} - (E_{J-1} * q)$$

662 so

663 $$E_J = E_{J-1} * (1 - q)$$

664 This applies to *J* subsequent steps, so.

665 $$\begin{cases} E_1 = E_0 * (1 - q) \\ E_2 = E_0 * (1 - q) * (1 - q) \\ E_3 = E_0 * (1 - q) * (1 - q) * (1 - q) \ldots \\ \boldsymbol{E_J = E_0 * (1 - q)^J} \end{cases}$$

666 In the stochastic model this is interpreted as a Binomial process, with N = E_0 and p = $(1\text{-}q)^J$. For
667 example in equation (1), considering the term $(1 - b_{C,Ae})^{J_{i,C,A_e}}$. It was implemented by sampling

values from a Binomial distribution as: ~ Binomial $(C_{i,(S-1),Ae}; (1 - b_{C,Ae})^{J_{i,C,Ae}})$ describing the number of cells not transferred to from carcass to environment after *J* interactions.

Using the previous equation (Appendix C) we can derive the amount of *Salmonella* in compartment *C*. Considering the fact that only transfer between two compartments is possible (no die off or growth), the amount in *C* is the difference between the E_0 and the E_J. Applying it in the previous equations:

$$C_J = E_0 - (E_0 * (1 - q)^J)$$

so

$$C_J = E_0 * (1 - (1 - q)^J)$$

Appendix D. Parameters, baseline**,** minimum and maximum values used during the univariate sensitivity analyses. See tables I and II for an explanation of the parameter symbols and indices.

Parameters	Baseline (y)	Maximum (y^+)	Minimum (y^-)	Unit
$a_{K,C}$	0.17	100	0	%
$a_{K,O}$	0.17	100	0	%
$b_{C,K}$	0.17	100	0	%
$b_{O,K}$	0.17	100	0	%
$a_{H,C}$	0.21	100	0	%

$a_{H,O}$	0.21	100	0	%
$b_{C,H}$	3.1	100	0	%
$b_{O,H}$	3.1	100	0	%
$a_{G,C}$	0.17	100	0	%
$a_{G,O}$	0.17	100	0	%
$b_{C,G}$	0.17	100	0	%
$b_{O,G}$	0.17	100	0	%
d_C	0	100	0	%
d_O	0	100	0	%
ka	10	15	5	cm^2
ha	150	200	100	cm^2
ga	1	3	0.5	cm^2
$\mu\ log_{10}[C_{i\,(S-1)}]$	-5.2	2	-15	$log_{10}CFU/cm^2$
$\sigma\ log_{10}[C_{i\,(S-1)}]$	2.2	4	0	$log_{10}CFU/cm^2$
$PrevO_{i\,(S-1)}$	14.1	100	0	%
$PrevC_{i\,(S-1)}$	100	100	0	%
$[O_{i\,(S-1)}]$ Min	0.01	0.1	0.0001	CFU/cm^2
$[O_{i\,(S-1)}]$ MP	1	100	0.01	CFU/cm^2
$[O_{i\,(S-1)}]$ Max	100	1000	10	CFU/cm^2

682 μ= mean; σ =standard deviation; Min= minimum; MP=most likely; Max=maximum values in
683 triangular distribution

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Appendix E. Parameter values for scenarios used in multivariate sensitivity analyses. The scenarios: no transfer and no contamination were omitted

	Scenarios						
Parameters	Baseline	Only carcass (S-1)	Only LN (S-1)	Hand influence high $log_{10}[C_{i\,(S-1)}]$	Hand influence high $\sigma\ log_{10}[C_{i\,(S-1)}]$	Hand Influence high $[O_{i\,(S-1)}]$	Hand Influence high $PrevO_{i\,(S-1)}$
$PrevC_{i\,(S-1)}$	100%	100%	0%	100%	100%	100%	100%
$PrevO_{i\,(S-1)}$	14.4%	0%	14.1%	14.1%	14.1%	14.1%	100%
$a_{H,C}$	0.21%	0.21%	0.21%	10%	10%	10%	10%
$b_{C,H}$	3.1%	3.1%	3.1%	10%	10%	10%	10%
$\mu\ log_{10}[C_{i\,(S-1)}]$	-5.4	-5.4	0	-3	-5.4	-5.4	-5.4
$\sigma\ log_{10}[C_{i\,(S-1)}]$	2.2	2.2	0	2.2	3.3	2.2	2.2
$[O_{i\,(S-1)}]$ Min	0.01	0	0.01	0.01	0.01	0.1	0.01
$[O_{i\,(S-1)}]$ MP	1	0	1	1	1	10	1
$[O_{i\,(S-1)}]$ Max	100	0	100	100	100	1000	100

μ= mean; σ =standard deviation; LN=Lymph nodes; Min=minimum; MP= most probable; Max= maximum values in triangular distribution

Appendix F

Adopting Jongenburger's approach[30] the observed prevalence will be a product of the factor *l* and the modeled prevalence. The value of *l* can be considered as a probability that at least one sample unit (CFU) is drawn from an inspected area: $l = P(x \geq 0)$. This probability follows as a hypergeometric distribution. Consider four swabs taken with a sponge in fixed and mutually exclusive areas of 100cm^2 on carcass surface (n=4). Next, consider the whole carcass area as 14000 cm^2[47] represented as a rectangle composed by N =14000/100=140 different N areas that can potentially be sampled.

According to the model the total area inspected per carcass during meat inspection is on average 700 cm^2 (data not shown), and the number of possible inspected areas sampled is K=700/100=7 different areas. The probability that at least one sample is drawn from the inspected areas is (1-

P(x=0)) has hypergeometric distribution according:

$$P(x = 0) = \frac{\binom{K}{x}\binom{N-K}{n-x}}{\binom{N}{n}} = \frac{\binom{7}{0}\binom{140-7}{4-0}}{\binom{140}{4}} \approx 81\%$$

So, 1-P(x=0) = 19% is the *l* factor.