



## A Stochastic Model to Assess the Effect of Meat Inspection Practices on the Contamination of the Pig Carcasses

de Freitas Costa, Eduardo; Corbellini, Luis Gustavo; da Silva, Ana Paula Serafini Poeta; Nauta, Maarten

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

3

4 **Eduardo de Freitas Costa\*<sup>1</sup>; Luis Gustavo Corbellini<sup>1</sup>; Ana Paula Serafini Poeta da Silva<sup>1</sup>;**  
5 **Maarten Nauta<sup>2</sup>**

6 1: Laboratory of Veterinary Epidemiology (Epilab), Department of Preventive Veterinary  
7 Medicine, Federal University of Rio Grande do Sul, Brazil

8 Address: Avenida Bento Gonçalves, n° 9090, zip code 91540-000, Porto Alegre, RS, Brazil;  
9 phone: +55 51 3308 8025

10 2: Technical University of Denmark – National Food Institute

11 Mørkhøj Bygade, 19, Building G, DK-2860 Søborg, Denmark

12 **Abstract**

13 The objective of meat inspection is to promote animal and public health, by preventing, detecting  
14 and controlling hazards originating from animals. With the improvements of sanitary level in pig  
15 herds the hazards profile has shifting and the inspection procedures have no longer targeting  
16 major foodborne pathogens (i.e., not risk-based). Additionally carcass manipulations performed  
17 when searching for macroscopic lesions can lead to cross-contamination. We therefore  
18 developed a stochastic model to quantitatively describe cross-contamination when consecutive  
19 carcasses are submitted to classic inspection procedures. The microbial hazard used to illustrate  
20 the model was *Salmonella*, the data set was obtained from Brazilian slaughterhouses and some  
21 simplifying assumptions were made. The model predicted that, due to cross-contamination  
22 during inspection, the prevalence of contaminated carcass surfaces increased from 1.2% to  
23 95.7%, whereas the mean contamination on contaminated surfaces decreased from 1 to -0.87  
24 logCFU/cm<sup>2</sup>, and the standard deviations decreased from 0.65 to 0.19. These results are  
25 explained by the fact that, due to carcass manipulations with hands, knives and hooks, including  
26 the cutting of contaminated lymph nodes, *Salmonella* is transferred to previously uncontaminated  
27 carcasses, but in small quantities. These small quantities can easily go undetected during  
28 sampling. Sensitivity analyses gave insight in the model performance and showed that the  
29 touching and cutting of lymph nodes during inspection can be an important source of carcass  
30 contamination. The model can serve as a tool to support discussions on the modernization of pig  
31 carcass inspection.

32 **Keywords:** Mathematical modeling, cross-contamination, carcass inspection

## 34 1. INTRODUCTION

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

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

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

54 Nowadays, farms adhere to specific management requirements like all-in-all-out production,  
55 controlled feed sources, indoor production, and a traceability system from the farm to the  
56 slaughterhouse.<sup>(8)</sup> As a consequence, hazards like parasites are getting rare in the industrial pork

57 production chain.<sup>(8-10)</sup> On the other hand, the intensification of the production brings changes in  
58 the epidemiology and other microbial pathogens are emerging.<sup>(11)</sup> *Salmonella* spp., *Yersinia*  
59 *enterocolitica*, *Toxoplasma gondii* and *Trichinella* spp. are identified as the most important  
60 hazards to be covered by the meat inspection of swine carcasses.<sup>(5)</sup>

61 The interaction of these pathogens with the host and the environment raises some concerns about  
62 the suitability of the classic inspection procedures. It demands structured control using all food  
63 chain information to reach a risk-based inspection system.<sup>(12)</sup> The modernization of meat  
64 inspection has been extensively studied in Europe and since 2014, according to EC Regulation  
65 219/2014, the inspection of pig carcasses is visual-only for pig herds that have been reared in  
66 integrated farm systems, doing palpation and incision when a lesion has been found after visual-  
67 only inspection.<sup>(13)</sup>

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

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

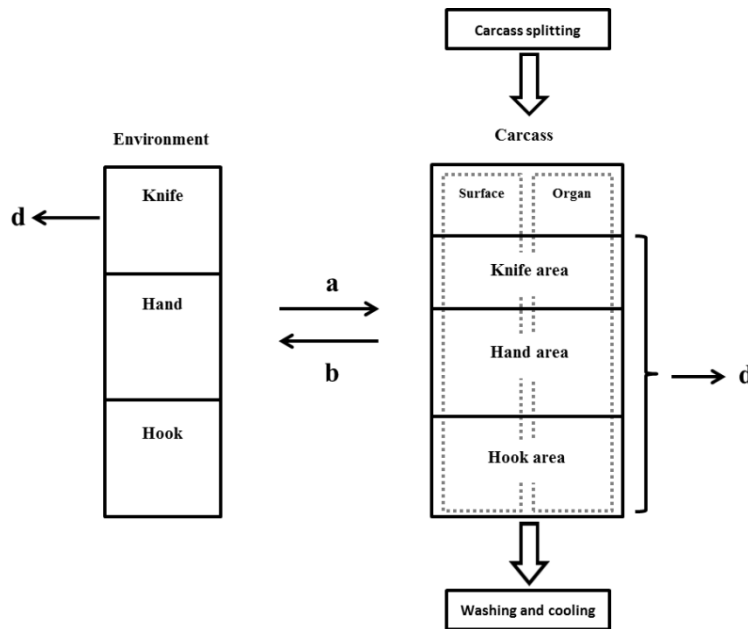
80 quantify the cross-contamination during meat inspection of pig carcasses via specific transfer  
81 routes and to assess their relevance for the contamination of the carcasses. The model is set up as  
82 a generic model for cross-contamination during meat inspection and is applied to *Salmonella*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

## 93 **2. MATERIALS AND METHODS**

### 94 **2.1 Conceptual model**

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

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



107  
108 **Fig. 1.** Schematic representation of the pig carcass inspection. Consecutive carcasses pass  
109 through the point of inspection and get in contact with the environmental components, which can  
110 lead to cross-contamination via bacterial transfer from the environment to the carcass (arrow a)  
111 or from the carcass to the environment (arrow b). The arrows d represent the reduction in the  
112 concentration of the bacteria due to inactivation or removal.

113  
114 The model only considers the carcass and the predefined environmental components as sources  
115 of bacteria, the influence of the air, carcass to carcass contact or other external factors are  
116 ignored. Also, bacterial growth during the inspection is excluded from the model. Removal of  
117 the bacteria from the carcass (surface or organs) can only occur by the inspection activities that  
118 are included in the model. Bacteria on the knife are frequently inactivated by putting the knife in

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

## 121 2.2 Mathematical model

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

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

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



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

145

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

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

$G_i$	Amount of <i>Salmonella</i> on hook by the carcass $i$ after inspection	Model	CFU	Calculation
150	# Distribution expressing variability between carcasses $i$ ; $\diamond$ Parameters ( $\mu$ , $\sigma$ and $Prev_{C_{i(S-1)}}$ ) were			
151	fitted according a zero inflated normal distribution by Maximum Log likelihood estimation			
152	method (Appendix B); $\#\#$ CFU per inspected area.			
153				
154	The model was implemented as a Monte Carlo simulation model. Transfers were described as			
155	binomial processes taking into account the successive contacts between environment and carcass,			
156	as explained in appendix C. For example, in the first term in the equations considering the			
157	carcass, $(1 - d_C)(1 - b_{C,Ae})^{J_{i,C,Ae}}$ is the fraction of the number of <i>Salmonella</i> that are not lost by			
158	removal ( $d$ ) and not transferred from the carcass, to the environment on different areas, indicated			
159	by the index $Ae$ (knife area, hand area, and hook area). The second term is $(1 - (1 - a_{Ae,C})^{J_{i,C,Ae}})$ ,			
160	the fraction of the number of <i>Salmonella</i> received from the environment indicated by the index			
161	$Ae$ (knife, hand and hook) to the carcass and can be derived as explained in appendix C.			
162	In the last three equations, modeling the environmental components, using the knife as example,			
163	the first term: $K_{(i-1)} \prod_{Ac \in \{C,O\}} (1 - a_{K,Ac})^{J_{(i-1),Ac,K}}$ concerns the <i>Salmonella</i> that are not transferred			
164	from the knife to the carcass ( $i-1$ ) on different compartments indexed by $Ac$ (surface and lymph			
165	nodes). The second term $(1 - d_{Ac})(1 - (1 - b_{Ac,K})^{J_{i,Ac,K}})$ indicates the <i>Salmonella</i> received from the			
166	carcass indicated by the index $Ac$ (surface or lymph nodes). The variables $C_{i,(S-1)}$ and			
167	$O_{i,(S-1)}$ represent the counts of <i>Salmonella</i> before the inspection and describe the variability			
168	between inspected carcass surfaces and organs respectively. To account for the random spatial			
169	distribution of cells over the inspected area, a Poisson distribution was used. In order to assess			
170	the true prevalence, the variables $C_{(i-1),S}$ and $O_{(i-1),S}$ were multiplied by the positive/negative			

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

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

180 Counts of *Salmonella* were expressed in CFU and the outputs were calculated for the inspected  
181 areas (CFU/cm<sup>2</sup>) and then transformed to natural logarithm (presented here as “log”),  
182 considering only the contaminated carcasses (because log (0) is not defined). When a carcass has  
183 not been submitted to any contact with the environment by hands, knife or hook, the carcass was  
184 considered as not inspected and, consequently, the concentration on inspected area is assumed to  
185 be the same as before (S-1). Also the probability of inactivation or removal on carcass or in  
186 lymph nodes ( $d_C$  or  $d_O$ ) are underlying assumed to be zero. The analyses were done using  
187 @Risk 6.2.1 (Palisade) for Excel with 10000 iterations using 500 and 100 consecutively  
188 processed carcasses in two separate simulations. These numbers were chosen to approximate  
189 realistic numbers of pigs slaughtered in a slaughter line per shift of two hours (i.e. 350  
190 carcasses/hour), whilst keeping the model manageable and restricting the running time.

191

192 **Table II.** Parameters used to illustrate the dynamics of the model. The indices  $Ae$  and  $Ac$  are  
193 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 (cm <sup>2</sup> )	cm <sup>2</sup>	150	Assumption*
$Ae=G$	Area of hook (cm <sup>2</sup> )	cm <sup>2</sup>	1	Assumption*
$Ae=K$	Area of cut (cm <sup>2</sup> )	cm <sup>2</sup>	10	Assumption*
ck	Probability of changing the knife	%	90	Assumption (based on observations)

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

### 195 **2.3 Sensitivity analysis**

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

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

$$202 \quad f(x; y, y^-, y^+) = \begin{cases} y + (y - y^-)x, & \text{for } x < 0 \\ y - (y - y^+)x, & \text{for } x \geq 0 \end{cases} \quad (2)$$

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

## 207 **2.4 Data sources**

208 The data on the carcass surface contamination were obtained from da Silva *et al.*<sup>(20)</sup> These  
209 authors collected carcass surface swabs in three Brazilian commercial slaughterhouses. Data  
210 regarding the lymph nodes prevalence where obtained from 12 cohorts representing finishing  
211 herds located in the state of Santa Catarina, Brazil.<sup>(11)</sup> These herds belong to an integrated system  
212 responsible for approximately 7% of all Brazilian pork production in 2007. Manipulation data  
213 were observed during two weeks in March 2015 in a large Brazilian pig slaughterhouse  
214 dedicated to exportation. 778 inspection procedures were counted during this period, of which  
215 290 in the inspection point “CARCASS”. The numbers of manipulations were recorded in a  
216 database. Although no data regarding transfer probability in slaughterhouse environment could  
217 be found, results from Kim *et al.*<sup>(21)</sup> and Hong and Bahk<sup>(22)</sup>, provide transfer probabilities  
218 between hands and pork and knife and pork, respectively. We have not found suitable  
219 concentration data for *Salmonella* in lymph nodes, the values used were based on estimates of  
220 the authors.

## 221 **3. RESULTS**

### 222 **3.1 Baseline and distributions**

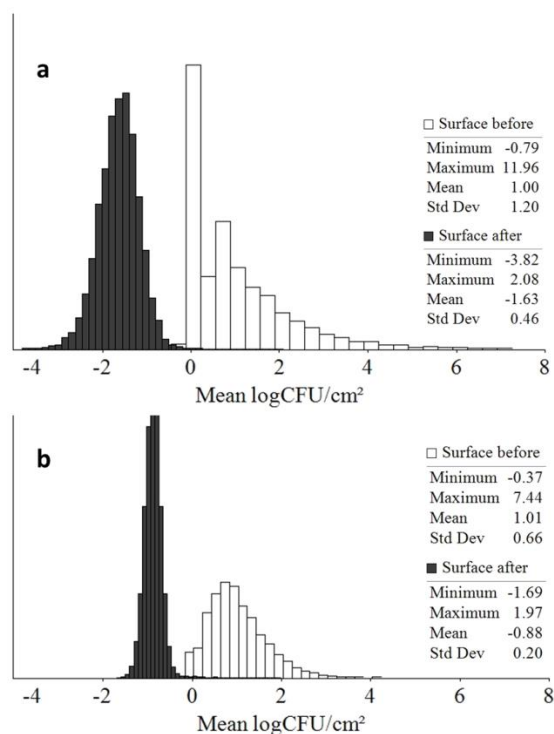
223 In the baseline model, the mean of the mean concentrations ( $\mu$ ) and the mean prevalence were  
 224 determined for two independent simulations with 500 and 100 consecutive carcasses, over 10000  
 225 iterations. As the mean concentration is the mean of logs, only contaminated carcasses are  
 226 included in the calculations. The results are summarized and given in Table III. The mean  
 227 concentration on inspected areas of the contaminated carcass surfaces is decreasing after  
 228 inspection procedures, from 1 to -0.87 logCFU/cm<sup>2</sup>. Standard deviations of the means decrease  
 229 as well, from 0.65 to 0.2. The reason is that many more carcass surfaces are getting contaminated  
 230 by cross-contamination, resulting in a large number of carcasses (i. e. a prevalence difference of  
 231 94.6 percentage points) contaminated with lower concentrations. Consequently the variability is  
 232 decreasing (See Fig. 2).

233 **Table III.** Outputs for inspected areas of carcass surface and lymph nodes before and after  
 234 inspection procedures in model simulations with 500 and 100 carcasses. The mean ( $\mu$ ) and  
 235 standard deviation ( $\sigma$ ) are those of the mean values for the contaminated inspected carcass areas  
 236 among the 500 or 100 simulated carcasses; the prevalence is the mean prevalence after 10000  
 237 iterations

	500 carcasses			100 Carcasses		
	$\mu$ ( $\sigma$ ) logCFU/cm <sup>2</sup>	Prevalence % <sup>#</sup>	CFU in the system <sup>##</sup>	$\mu$ ( $\sigma$ ) logCFU/cm <sup>2</sup>	Prevalence % <sup>#</sup>	CFU in the system <sup>##</sup>
Surface before	1 (0.65)	1.2	2.6*10 <sup>5</sup>	1 (1.22)	1.2	4.2*10 <sup>4</sup>
Surface after	-0.87 (0.2)	95.8	3.7*10 <sup>5</sup>	-1.6 (0.47)	92	5.2*10 <sup>4</sup>
LN before	3.17 (0.13)	22.2	2.3*10 <sup>6</sup>	3.17 (0.3)	23.8	4.6*10 <sup>5</sup>
LN after	0.08 (0.2)	96.7	2.1*10 <sup>6</sup>	-0.41 (0.51)	93.9	4.1*10 <sup>5</sup>

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

241



242

243 **Fig. 2.** Distribution of mean carcasses surface contamination (logCFU/cm<sup>2</sup>) considering only  
244 contaminated carcasses before and after inspection in two separated simulations with 100 (a) and  
245 500 (b) carcasses.

246

247 For the lymph nodes the effects of the inspection procedures on the mean and prevalence are  
248 similar to those observed in the carcass surface (i.e. decrease and increase respectively), but the  
249 standard deviation increases after inspection. Although the results suggest a reduction in mean  
250 carcass surface contamination, after inspection, the sum of the numbers of *Salmonella* (“CFU in  
251 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.

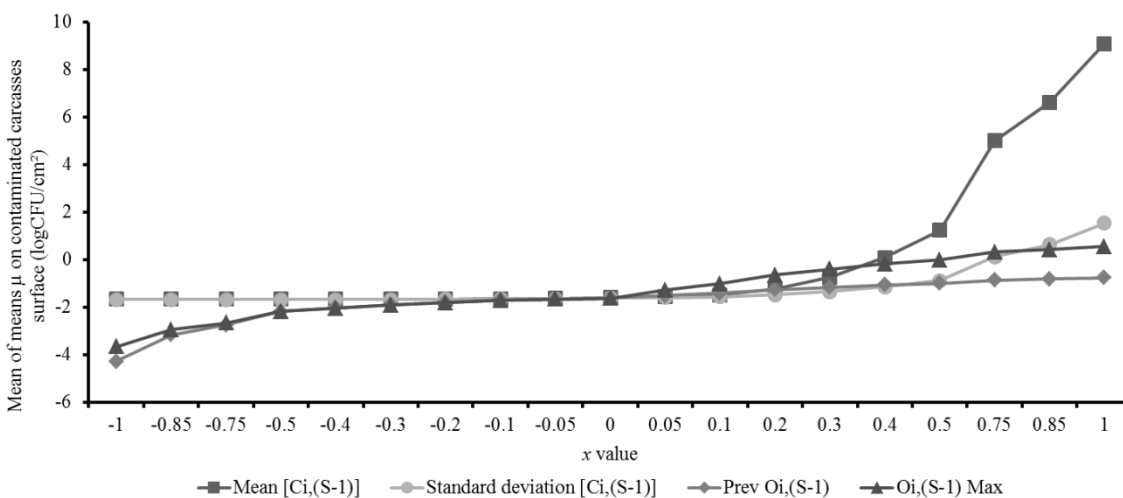
257 Results differ depending on the number of simulated carcasses. With a lower number of  
258 carcasses, the variation in mean concentrations sampled from the zero inflated Poisson  
259 Lognormal is larger. For example, with the prevalence 1.2%, the probability that the  
260 concentrations in all 100 carcass surfaces are zero is  $(1-0.012)^{100} = 30\%$ , for 500 carcass surfaces  
261 it is  $(1-0.012)^{500} = 0.24\%$  (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 ( $\mu$ ) 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<sup>th</sup> and 97.5<sup>th</sup> percentiles of the distribution describing the variability between model  
272 iterations in the mean  $\mu$  from -2.57 to -0.83 logCFU/cm<sup>2</sup> 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



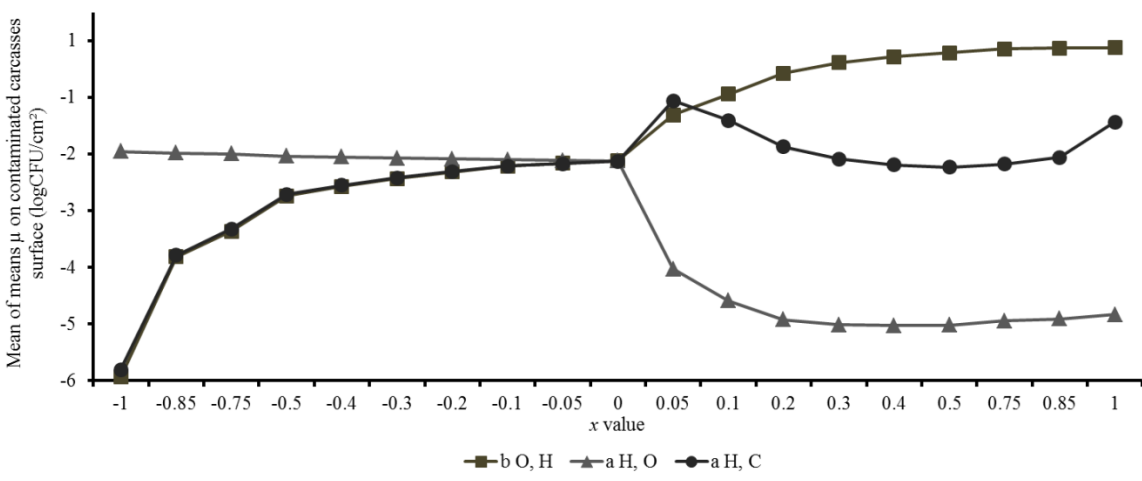
275 procedures. The mean after inspection increased from -1.6 to 9.07 logCFU/cm<sup>2</sup> on the inspection  
 276 area when the load of *Salmonella* on carcass surfaces before inspection approaches the maximum  
 277 value (2 log<sub>10</sub>CFU/cm<sup>2</sup> compared to -5.4 in the baseline). The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



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

288  
 289 Fig. 4 shows how changes in transfer probabilities affect the mean contamination on the carcass  
 290 surface. If the transfer probability from hands to lymph nodes ( $a_{H,O}$ ) decreases to 0% using  $x = -$   
 291 1, the lack of bacterial transfer from hands to lymph nodes leads to an increase of the amount on

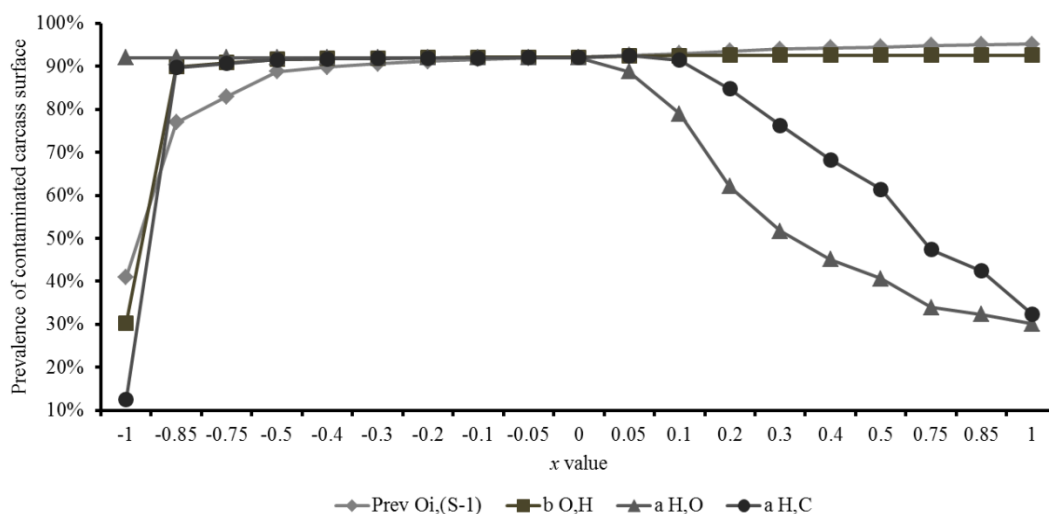
292 the hands and a subsequent increase of transfer to the carcass surface, leading to a small increase  
 293 of the mean to approximately  $-1.45 \log\text{CFU}/\text{cm}^2$ . But when the same parameter is increased, the  
 294 mean decreases because the cells transferred to the lymph nodes can no longer be transferred to  
 295 the carcass surface.



296  
 297 **Fig. 4.** Mean of the means ( $\mu$ )  $\log\text{CFU}/\text{cm}^2$  as a function of different  $x$  values regarding the  
 298 parameters ( $b_{O,H}$ ), ( $a_{H,O}$ ) and ( $a_{H,C}$ ).

299  
 300 Both transfer probabilities from the lymph node to hand ( $b_{O,H}$ ) and to carcass by the hand  
 301 ( $a_{H,C}$ ), show similar results below the baseline, but  $b_{O,H}$  keeps increasing the mean until  $x$   
 302 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  
 303 a peak because, at some point, the transfer from hand to carcass gets so large that the  
 304 concentration on the hands gets too low. Once a large number of bacteria are transferred to the  
 305 first carcasses only a few bacteria are transferred to the subsequent carcasses, reducing the mean  
 306 concentration without relevant effects on prevalence (Fig. 5). As the  $a_{H,C}$  keeps increasing,  
 307 bacteria get even more concentrated on the first carcasses after hands contamination, reducing

308 prevalence compared to the situation with a lower  $a_{H,C}$  (Fig. 5). As the mean log can be  
 309 calculated only for contaminated carcasses (i. e. one or more CFU), the reduction of  
 310 contaminated carcasses leads to increases of the mean ( $\log\text{CFU}/\text{cm}^2$ ) when the  $x$  increases for the  
 311 variables  $a_{H,C}$  and  $a_{H,O}$ .



312  
 313 **Fig. 5.** Prevalence of carcass surface contamination as a function of different  $x$  values regarding  
 314 the variable  $Prev O_{i,(S-1)}$  and parameters ( $b_{O,H}$ ), ( $a_{H,O}$ ) and ( $a_{H,C}$ ).

315  
 316 Fig. 5 shows the effects of tested parameters on prevalence after inspection. The effect of  
 317 reduction of  $Prev O_{i,(S-1)}$ ,  $b_{O,H}$  and  $a_{H,C}$  to zero (minimum values, when  $x=-1$ ) leads to a reduction  
 318 of the surface prevalence to approximately 40%, 29% and 12% respectively, whereas reductions  
 319 in  $a_{H,O}$  do not seem to affect the surface prevalence. When the values of the transfer parameters  
 320  $a_{H,O}$  and  $a_{H,C}$  are increased, a reduction of the prevalence is observed. The reduction in the  
 321 number of positive carcasses leads to an increase of the mean log surface contamination as  
 322 observed in Fig. 4, as this can be calculated for positive carcasses only.

323 **3.3 Multivariate sensitivity analyses**

324 Table IV shows the mean of the means ( $\mu$ ) logCFU/cm<sup>2</sup>, its standard deviation ( $\sigma$ ) and mean  
 325 prevalence on carcass surface ‘before’ and ‘after’ inspection in the multivariate sensitivity  
 326 analyses. The first scenario shows the baseline for comparison proposes. The second and third  
 327 scenarios present a stress analysis to verify the model performance. As expected, when transfer  
 328 probabilities are set to zero, the outputs ‘before’ and ‘after’ were the same. Also, the absence of  
 329 sources of contamination results in a completely uncontaminated scenario after inspection,  
 330 meeting the null contamination set by the parameters.

331 **Table IV.** Scenarios used in multivariate analyses to test the effect of different variables  
 332 combination on mean of the means ( $\mu$ ) logCFU/cm<sup>2</sup> its standard deviation ( $\sigma$ ) and mean  
 333 prevalence of contaminated carcass surface before and after inspection

Scenario	$\mu$ ( $\sigma$ ) logCFU/cm <sup>2</sup>		Prevalence %	
	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

---

334

335 When only the carcass surface was included as the source of *Salmonella*, an important difference  
336 could be found as both the level of surface contamination and prevalence after inspection were  
337 drastically reduced compared to the baseline. The influence of high transfer probability involving  
338 the hands and carcass, by increasing the parameters  $a_{H,C}$ ,  $b_{C,H}$  (appendix E) tested together with a  
339 higher initial concentration on contaminated carcass surfaces ( $\log_{10}[C_{i,(S-1)}] = -3 \log_{10} \text{CFU/cm}^2$ ),  
340 increased the mean from -1.6 to 0.64 logCFU/cm<sup>2</sup> and the mean prevalence to 96.7%. The  
341 influence of high transfer probability involving the hands and carcass was also tested with an  
342 increase of variability of contamination on carcass surface. It resulted in an increase of carcass  
343 surface contamination because surface contamination (S-1) is entered in the model as log<sub>10</sub>, so  
344 increases in variability affect the expected value, as the arithmetic mean of C<sub>iS</sub> equals  
345  $10^{(\mu + \frac{1}{2} \log(10) \sigma^2)}$  and the transfer of bacteria acts as a factor of quantity and not of the log-  
346 quantity.

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

354 **4. DISCUSSION**

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

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

377 of different carcasses.

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

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

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

400 would be below the limit of detection ( $-4 \log\text{CFU}/\text{cm}^2$ )<sup>(20)</sup>, so the observable prevalence would  
401 only be 5% instead of 44%. On the other hand, when only the lymph nodes are considered as  
402 source of bacteria, the results were kept similar to the baseline, indicating that the effect of  
403 lymph nodes inspection dominated the surface carcass contamination.

404 When only considering the prevalence, the results obtained here may seem to be alarming and  
405 unrealistic, because the increase in more than 90 percentage points is very large and it is not  
406 observed in prevalence studies, which give values like 24%<sup>(11)</sup> and 14%.<sup>(20)</sup> Although these  
407 prevalence results were obtained in Brazilian slaughterhouses before chilling, no inactivation  
408 step is used in Brazil between the carcass inspection and the chilling. A reason that observed  
409 prevalences are so much lower than predicted by the model may be the localization of the  
410 contaminating bacteria, which is restricted to areas manipulated by the inspection workers. These  
411 may not correspond with areas sampled when these prevalence studies were performed.

412 According to Jongenburger *et al.*<sup>(30)</sup> batches with localized bacterial concentration reduce the  
413 observed prevalence with a factor  $l$ , derived from the relative size of the contaminated areas  
414 compared to the whole surface (see Appendix F). Another issue could be related with the  
415 difference between the measured prevalence (observed frequency of carcasses positive for  
416 *Salmonella* in the microbial test) and the modeled prevalence, which refers to the true  
417 prevalence, that is carcasses with one or more CFU. Although this difference must to be taken  
418 into account, in our simulations the mean (SD) contamination on carcass surfaces after  
419 inspection was  $-0.8$  ( $0.2$ )  $\log\text{CFU}/\text{cm}^2$  (Figure 2) which assuming a normal distribution, means  
420 that none carcasses will have a level of contamination below the limit of detection ( $\text{LOD}=-4$   
421  $\log\text{CFU}/\text{cm}^2$ )<sup>(20)</sup> (calculations not shown). Hence, it is expected that the modeled and measured  
422 prevalences are expected to be the same in our simulations.



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

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

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

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

446 in food products<sup>(36-41)</sup>, and a particularly interesting approach is proposed by Smid *et al.*<sup>(42)</sup>,  
447 taking into account the uncertainty generated in transfer experiments. Here, we preferred to use  
448 a binomial process, assuming a mechanistic approach regarding the transfer of cells<sup>(38)</sup>, but the  
449 model can be updated in order to consider new evidence about the transfer of different hazards in  
450 pork.

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

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

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

469 Furthermore it sheds some light on the potential inadequacy of classic pig carcass inspection and  
470 therefore it can be considered as a tool to quantify the effects of cross-contamination and answer  
471 questions about the modernization of the classic carcass inspection system for the  
472 implementation of risk-based approaches in meat-inspection.<sup>(46)</sup>

## 473 **5. CONCLUSIONS**

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

481

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632

633

634 **Appendix A.** Values used in the empirical distribution that is applied to sample the number of  
635 interactions between carcass/organ with hands, knife and hook using the index  $A_e = (H, K,$   
636  $G)$ . 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  $u = \text{Log}_{10}(x)$  and the probability density function  $f(u)$  for  
642 concentration  $[C_{i(S-1)}]$ , can be defined by:

$$643 \quad f(u) = \begin{cases} p * \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{u-\mu}{\sigma}\right)^2} & u > LOQ \\ p * \int_{LOD}^{LOQ} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{u-\mu}{\sigma}\right)^2} du & LOD < u < LOQ \\ (1-p) + \left( p * \int_0^{LOD} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{u-\mu}{\sigma}\right)^2} du \right) & u < LOD \end{cases}$$

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.*<sup>(20)</sup>, the parameters ( $\mu$ ,  $\sigma$  and  $p$ ) were assessed by the Maximum Log

647 likelihood estimation using the Solver function in Excel, i.e. maximizing

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

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  $J \in \mathbb{N}$  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:

$$656 E_0 = 100 \text{ CFU}$$

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.

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

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

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

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

674 
$$C_J = E_0 - (E_0 * (1 - q)^J)$$

675 so

676 
$$C_J = E_0 * (1 - (1 - q)^J)$$

677

678

679

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

Parameters	Baseline (y)	Maximum (y <sup>+</sup> )	Minimum (y <sup>-</sup> )	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 <sup>2</sup>
ha	150	200	100	cm <sup>2</sup>
ga	1	3	0.5	cm <sup>2</sup>
$\mu \log_{10}[C_{i(S-1)}]$	-5.2	2	-15	log <sub>10</sub> CFU/cm <sup>2</sup>
$\sigma \log_{10}[C_{i(S-1)}]$	2.2	4	0	log <sub>10</sub> CFU/cm <sup>2</sup>
$PrevO_{i(S-1)}$	14.1	100	0	%
$PrevC_{i(S-1)}$	100	100	0	%
$[O_{i(S-1)}]$ Min	0.01	0.1	0.0001	CFU/cm <sup>2</sup>
$[O_{i(S-1)}]$ MP	1	100	0.01	CFU/cm <sup>2</sup>
$[O_{i(S-1)}]$ Max	100	1000	10	CFU/cm <sup>2</sup>

682  $\mu$ = mean;  $\sigma$  =standard deviation; Min= minimum; MP=most likely; Max=maximum values in  
683 triangular distribution

684

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

Parameters	Scenarios						
	Baseline	Only carcass (S-1)	Only LN (S-1)	Hand influence	Hand influence	Hand Influence high [ $O_{i(S-1)}$ ]	Hand Influence high $PrevO_{i(S-1)}$
				high	high		
				$\log_{10}[C_{i(S-1)}]$	$\sigma \log_{10}[C_{i(S-1)}]$		
$PrevC_{i(S-1)}$	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

687  $\mu$ = mean;  $\sigma$  =standard deviation; LN=Lymph nodes; Min=minimum; MP= most probable; Max=  
688 maximum values in triangular distribution

## 689 **Appendix F**

690 Adopting Jongenburger's approach<sup>(30)</sup> the observed prevalence will be a product of the factor  $l$   
691 and the modeled prevalence. The value of  $l$  can be considered as a probability that at least one  
692 sample unit (CFU) is drawn from an inspected area:  $l = P(x \geq 0)$ . This probability follows as a  
693 hypergeometric distribution. Consider four swabs taken with a sponge in fixed and mutually  
694 exclusive areas of  $100\text{cm}^2$  on carcass surface ( $n=4$ ). Next, consider the whole carcass area as  
695  $14000\text{cm}^2$ <sup>(47)</sup> represented as a rectangle composed by  $N = 14000/100 = 140$  different  $N$  areas that  
696 can potentially be sampled.

697 According to the model the total area inspected per carcass during meat inspection is on average  
698  $700\text{cm}^2$  (data not shown), and the number of possible inspected areas sampled is  $K = 700/100 = 7$   
699 different areas. The probability that at least one sample is drawn from the inspected areas is (1-



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

$$701 \quad P(x = 0) = \frac{\binom{K}{x} \binom{N-K}{n-x}}{\binom{N}{n}} = \frac{\binom{7}{0} \binom{140-7}{4-0}}{\binom{140}{4}} \approx 81\%$$

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