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Characterization of the transfer probability of *Salmonella* ser. Typhimurium between pork and a cutting knife in an experimental model

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ABSTRACT

Cross-contamination is an important event for bacterial transfer throughout the pork production chain. In Brazil, *Salmonella* sp. is the most relevant hazard in the pork industry, and further knowledge concerning its contamination is essential for in-depth risk assessments. Thus, we aimed to assess the transfer probability of *Salmonella* sp. between a knife and pork in a domestic kitchen scenario to provide parametrization for incorporating transfer of *Salmonella* sp. in risk assessment models. To estimate *Salmonella* Typhimuium transfer rates between contaminated pork and a knife blade during cutting, 23 independent experiments were performed. A Bayesian inference was utilized to determine the transfer probability, capturing the uncertainty generated in the transfer probability experiments. The mean transfer probability was 0.03 for knife to pork [0.029; 0.032] 95% credible interval (CrI) and 0.0042 for pork to knife [0.0041; 0.0043] 95% CrI. The probabilistic estimate of the transfer probability of *Salmonella* sp. during pork cutting gives insights on a relevant parameter for the consumer phase of the pork production industry in Brazil, allowing for enhanced risk assessment models.

1. Introduction

In Brazil, pork is the third most consumed type of meat (ABPA, 2021), with sausages (fresh, cooked, and dried) being the most purchased pork product. However, fresh cuts of pork are also consummed in Brazilian households, especially as barbecues. *Salmonella enterica* (hereafter *Salmonella*) causes food poisoning via pork (WHO, 2015) and was deemed the highest risk for consumers in the Brazilian pork production chain (de Freitas Costa et al., 2020). The prevalence of *Salmonella* in pig carcasses has been estimated between 8 and 10% (Brasil, 2019; Corbellini et al., 2016); however, higher isolation frequencies have also been reported in some cases (Pissetti et al., 2012; Kich et al., 2020).

In Brazil, processed pork products have been quantitatively assessed regarding exposure to *Salmonella* (Mürmann et al., 2011; Werlang et al., 2021), yet there is a lack of models to characterize the exposure through fresh pork cuts. Although Brazilian consumers prefer to eat well-done pork, the hazard of alternative exposure routes, such as cross-contamination from other raw or cooked foodstuffs, surfaces, and knives used during pork preparation at home, remains. Similar to other countries, the majority of foodborne illness cases in Brazil occur in private households and may be related to failures in food preparation practices and hygiene leading to cross-contamination events (Brasil, 2019; EFSA, 2009).

Cross-contamination is a major cause of bacterial transfer throughout the pork supply chain (Nauta, 2008; Pérez-Rodríguez et al., 2008), involving both the slaughterhouse (Snary et al., 2016; Swart et al., 2016a) and food preparation at home (Iulietto and Evers, 2020; Kennedy et al., 2011; Swart et al., 2016b). Transfer probability plays a crucial role in dynamic quantitative microbiological risk assessment (QMRA), and thus its evaluation is key in this analysis (Iulietto and Evers, 2020).

To estimate the bacterial transfer probability, experiments are customarily performed under laboratory conditions and the bacterial

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transfer is calculated as the ratio of the observed cells recovered from the recipient surface divided by the number of observed cells recovered from the donor surface (Chen et al., 2001). In frequentist statistical approaches, the transfer probability is reported as a parameter and its confidence intervals, and it is not meaningful to talk about the probability distribution of the parameter. In contrast, in Bayesian inference the posterior probability distribution is the state of belief or knowledge of the parameter (Morey et al., 2016; Schervish, 1995).

Therefore, we applied the Bayesian inference model proposed by Smid et al. (2013) to assess uncertainty around the transfer probability using data from experiments on *Salmonella* transfer between a cutting knife and pork using a strain of *Salmonella enterica* ser. Typhimurium isolated from pig carcasses in Brazil. We aimed to mimic a domestic cutting practice to further enhance consumer phase models in risk assessments.

2. Material and methods

2.1. Outline of the transfer experiments

The transfer experiments were devised to reproduce a household scenario where pork is cut or chopped before being cooked. We aimed to incorporate the transfer of *Salmonella* on one single cut to estimate the transfer probability in a manner that could be used in further implementations of consumer phase models in risk assessments.

Salmonella enterica subsp. *enterica* serovar Typhimurium phage type DT177 (resistant to ampicillin) was utilized in the transfer experiments; the strain (PV32 from the culture collection of the Preventive Veterinary Laboratory, UFRGS) was isolated from the mesenteric lymph nodes of a slaughtered pig in Brazil. The strain was kept frozen (−20 °C) and was recovered in Brain Heart Infusion broth (37 °C, 18–24 h) followed by isolation in Tryptic Soy Agar and confirmation of identity before performing the experiments.

Refrigerated pork belly cuts (the food matrix used in the experiments) were obtained from a slaughterhouse in southern Brazil. A portion of each pork batch was tested for *Salmonella* presence according to ISO 6579–1:2017. *Salmonella*-negative pork batches were cut into 17 × 15 cm chops and frozen. Prior to the transfer experiments, pork chop units were thawed overnight under refrigeration. The knives used (Tramontina®, Brazil) were equipped with stainless steel blades (7.5 cm length × 0.5 cm width) and polypropylene handles. Knives were sterilized by autoclaving before the transfer experiments.

2.2. Experiments on Salmonella transfer from pork to knife

To estimate *Salmonella* transfer rates from contaminated pork to a knife blade during cutting, 23 independent experiments were performed as follows: a 3 mL aliquot of Buffered Peptone Water 1% (BPW) containing 10^8 cfu.mL^{-1} of *Salmonella* PV32 was spread homogeneously over the surface (255 cm^2) of a pork chop to produce an inoculated donor surface. After 30 min, a sterilized knife was used to make a single cut (0.5 cm deep and 8 cm long) in the contaminated chop. The knife blade was then placed in 5 mL of BPW in a sterile plastic bag and the plastic bag was rubbed to release the bacteria attached to the blade. After 15 min of contact, a 1 mL aliquot of the suspension was serially diluted in 9 mL sterile 0.85% NaCl solution to produce 10^{-1}, 10^{-2}, 10^{-3} concentrations. From each dilution, three aliquots of 0.1 mL were individually spread onto xylose-lysine-deoxycholate agar (XLD, Becton Dickinson, Heidelberg, Germany), added to 32 μg.mL^{-1} of ampicillin (Sigma, Belgium) (XLD/Amp), and incubated at 35 °C (± 2°) for 48 h. Typical colonies in XLD/Amp agar plates were manually counted by the same operator for each dilution.

2.3. Experiments of Salmonella transfer from knife to pork

First, the number of *Salmonella* adhering to a contaminated knife blade (after contamination by immersion in a *Salmonella* suspension) was determined. A set of 20 independent experiments were conducted as follows: the blade of a sterile knife was immersed in a suspension of 10^8 cfu.mL^{-1} of *Salmonella* PV-32 for 1 h. Following this contact period, 1 mL of the BPW was diluted in 9 mL sterile 0.85% NaCl solution (for 10^{-1} dilution), and again for serial dilution (10^{-2}, 10^{-3}). From each dilution, three aliquots of 0.1 mL were spread onto XLD/Amp agar plates and incubated at 35 °C (± 2°) for 48 h. Typical colonies were visually counted on each plate.

To estimate the *Salmonella* transfer rate from a contaminated knife to pork, 23 independent experiments were performed. A sterile knife blade was immersed in 5 mL BPW 1% containing 10^8 cfu.mL^{-1} of *Salmonella* PV-32. After 1 h of contact, the knife was removed from the suspension; after allowing the excess liquid to drip, one cut was made on the surface of the pork chop. A 25 g sample was taken from the cut area and suspended in 225 mL BPW 1%. After homogenization, serial dilutions (10^{-2} and 10^{-3}) were performed. From each dilution, three aliquots of 0.1 mL were each spread onto an XLD/Amp agar plate and incubated at 35 °C (± 2°) for 48 h. Typical colonies in the XLD/Amp plates were manually counted by the same operator.

2.4. Bayesian model

The reasoning behind the Bayesian model relies on the Smid et al. (2013) publication assuming that the number of bacteria transferred between two surfaces is described by a binomial distribution. Experiment here refers to a cut in pork meat made by a single operator. Therefore, in each independent experiment, the pork or knife are inoculated and used as the donor surface. After a cut the bacterial cells retained on the recipient surface are counted. Bacterial count is the countable bacteria on the recipient surfaces estimated by a sequence of dilutions, replications, and colony counts. The dilutions are serial steps of ten-fold dilutions of a sample homogenate until the counting on agar plates is possible. The range of countable cells is from 30 to 300 cfu per agar plate. If the count exceeds 300 cfu, more dilutions should be done. Replicates are one or more aliquots that are sampled to be plated within the same dilution, and refer to replicates of the same experiment.

Considering the transfer probability as the ratio of cells recovered from a recipient surface divided by the number of cells recovered from a donor surface (Chen et al., 2001), we assume that the total count of *Salmonella* on the recipient surface for the *i*th experiment (*ni*) can be described by a binomial distribution $n_i \sim Binomial(T, \theta_i)$. The θ_i is the probability of *Salmonella* transfer between surfaces given a single cut in the *i*th experiment (parameter of interest), and T is the total number of cells on the donor surface that could be transferred, assumed to be homogeneously distributed; therefore $T \sim Poisson(\lambda_T)$. When pork was the donor surface, λ_T was calculated from the inoculate concentration, the volume spread on the pork surface, and the area of the cut. In all experiments of transfer from the pork to the knife, 3 mL of 10^8 cfu.mL^{-1} of *Salmonella* was spread evenly over the surface, and a cut of 8 cm^2 was made, availing 9411,765 cfu per donor surface. When the knife was the donor surface, λ_T was estimated using the mean cfu count on 20 inoculated knives by immersing each blade into a solution of 5 mL of 10^8 cfu.mL^{-1} of *Salmonella*, availing 568,492 cfu per donor surface.

Note that n_i is not accessible in the model yet, since we have only the plate counts. Thus, we assumed that the *Salmonella* distribution on every individual plate ($y_{i,j,k}$) (i.e., *i*th experiment, *j*th dilution, and *k*th replicate) as a realization of a Poisson distribution $y_{i,j,k}|\lambda_{ij} \sim Poisson(\lambda_{ij})$; therefore λ_{ij} is the average concentration of cfu per 0.1 mL (plated volume in the counting protocol) in each *j*th dilution and each *i*th experiment. According to Smid et al. (2013) $\lambda_{ij} \sim \text{Gamma}(5, 0.05)$ as prior for λ_{ij} accounts for a bacterial count in a plate ranging from 30 to 300 cfu. According to Clough et al. (2005) the Poisson likelihood can be inverted by conjugacy with gamma distribution resulting in $\lambda_{i,j} \sim \text{Gamma}(5 + n_i,\ 0.05 + v_i * d_i^{-1})$ where n_i is the total counts on all

plates in the ith experiment, d_i^{-1} is the first dilution factor providing countable bacterial numbers on the plate on the ith experiment, and v_i is the fraction of total sample volume used in the ith experiment (Clough et al., 2005). To calculate v_i, the samples were first converted to mL. The knife surface was considered equivalent to 5 mL of homogenate (1 knife/5 mL), of which one tenth was plated. In this case, $v_i = 1/50mL$ (i.e., one fiftieth of the sample). For the pork, 25 g sample of pork chop was processed into 250 mL of homogenate (10 g pork per mL), of which one tenth was plated. In this case, $v_i = 1/100mL$ (i.e., one hundredth of a sample). Note that by conjugating Poisson and gamma distributions, n_i is now accessible in the model, and finally, the parameter θ_i is assumed to be beta distributed vague priors chosen to a and b (Table 1).

All analyses were conducted using R statistical software (R Core Team, 2019). Bayesian models were compiled in OpenBUGS (Lunn et al., 2000) using the packages rjags (Plummer, 2019) and coda (Plummer et al., 2006). The data and the syntax used are available online: https://github.com/eduardodefreitascosta/Project_cross_cont.

3. Results

The results for the frequentist approach using the first dilution with countable bacteria between 30 and 300 cfu, resulted in the transfer probability from the knife to pork ranging from 0.005 to 0.068, in the 95% quantile interval (Fig. 1A), with mean 0.0278 (0.021–0.0346) 95% confidence interval (CI). The transfer probability from pork to knife ranged from 0.0015 to 0.007 in the 95% quantile interval (Fig. 1B), with mean 0.0045 (0.0033–0.005) 95% CI.

The mean results were obtained in the posterior distribution for the transfer probabilities using Bayesian inference and were similar using the frequentist approach. The mean transfer probability was 0.03 for knife to pork [0.029; 0.032] 95% credible interval (CrI) and 0.0042 for pork to knife [0.0041; 0.0043] 95% CrI. Statistics for the posterior distribution of the transfer probabilities and the a and b parameters for the beta distributions are shown in Table 2.

Table 1
Variables and parameters used in the Bayesian model to estimate the uncertainty of the *Salmonella* transfer probability between pork and knife.

Variable/ Parameter	Description	Distribution/ function	Units/value
$y_{i,j,k}$	Observed cfu on the plate from experiment i, dilution j, and replication k	$Poisson(\lambda_{i,j})$	cfu
$\lambda_{i,j}$	The concentration of cells in each experiment and each dilution	$gamma(5 + n_i, 0.05 + d_i * v_i^{-1})$	cfu/0.1 mL
v_i	The fraction of the sample used to a single count on the plate converted to mL	1/50 for the knife surface 1/100 for the pork surface	mL
n_i	Expected number of bacteria on the recipient surface after a single cut	$binomial(T, \theta_i)$	cfu
T	The total amount of *Salmonella* in the donor surface before the cut	$Poisson(\lambda_T)$	cfu
λ_T	Mean *Salmonella* on the total donor surface area	9411,765 cfu for the pork surface 568,492 cfu for the knife surface	cfu/surface
θ_i	Probability of *Salmonella* transfer between surfaces by a single cut in the ith experiment	$beta(a, b)$	Percentage
a	The mean of the Parameter of beta distribution	$uniform(0, 1000)$*	Dimensionless
b	Parameter of beta distribution	$uniform(0, 1000)$*	Dimensionless

*Priors for a and b were chosen in a way to be vague priors.

4. Discussion

In this study, we used a Bayesian inference approach to account for uncertainty in the transfer of *Salmonella* between pork and a knife in a household kitchen scenario. It gives insights on a relevant parameter for consumer phase models. The frequentist model provides similar point estimates when compared to the Bayesian model; however, frequentist models outcomes are not considered suitable for implementation of a probabilistic interpretation on the transfer probability (Schervish, 1995). According to Morey et al. (2016), using confidence intervals as a probabilistic measure of certainty for the parameter estimate should be avoided. In the context of risk assessment, the outcome from the Bayesian model is the parametrization for a probabilistic approach (Albert et al., 2008) quantifying the uncertainty of the transfer probability of *Salmonella*.

The point estimates for transfer probabilities observed here were low for pork to knife and knife to pork. Moreover, the mean transfer probabilities in this study were lower than those estimated by Smid et al. (2013) using a similar estimation approach. While we found a mean value of 0.03 for transfer from knife to pork and 0.0042 from pork to knife, Smid et al. (2013) reported, mean values of 0.19 and 0.58, respectively. In the latter study, a scenario of *Salmonella* fecal contamination of the knife and pork was devised, which reproduced a frequent occurrence at slaughterhouses. By doing so, pig feces may have played a role in facilitating the transfer and contributed to the higher probability estimation. However, fecal contamination is not likely during food preparation at home, where the organic matter involved in the transfer would be solely the meat protein and fat. Thus, our results may be more suitable for a consumer phase model, and the adoption of estimations based on slaughterhouse practices (Smid et al., 2013) may lead to an overestimation of the exposure caused by cross-contamination during at-home food preparation.

Even in the different scenarios of slaughter (Smid et al., 2013) and pork preparation at home, a similar phenomenon of higher transfer from knife to pork than from pork to knife was observed. The higher transfer probabilities from knife to pork than in the reverse may be explained by environmental factors (such as moisture and fat content) contributing to bacterial transfer during contact between surfaces (Pérez-Rodríguez et al., 2008). For instance, high-fat content and moisture may allow more bacteria to adhere firmly to the pork surface (Wang et al., 2015) and in turn, may contribute to making pork-to-knife transfer more difficult. On the contrary, smoothness of surfaces is identified as an important factor that interferes with bacterial attachment: the smoother a surface, the fewer bacteria remain attached (Flint et al., 2000). For instance, the use of steel utensils is favored in good food preparation practices because bacteria are more readily removed from them during cleaning (Gkana et al., 2016). In addition, the temperature of pork manipulation could be hypothesized as a factor interfering in the higher *Salmonella* adhesion on pork observed. We conducted the transfer experiments at room temperature (around 27 °C), and the pork chops were around the same temperature since we wanted to mimic the manipulation routine in the kitchen environment. However, Møller et al. (2016) observed that *Salmonella* cross-contamination is virtually the same considering pork grinded at room temperature 22 °C or 4 °C; therefore, we believe that the temperature has not played an important role in the transfer results.

Our results presented another aspect of this scenario: the smooth, stainless steel surface of the knife's blade may have hindered cell adherence and facilitated cell transfer to pork. Thus, once the knife surface is contaminated, the possibility of cross-contamination to other cooked foods or salads is likely. Gkana et al. (2016) demonstrated that cleaning knives reduces cross-contamination in the kitchen; conversely, it was observed that people in households may fail to thoroughly wash knives used to cut meat before preparing other foods (Kennedy et al.,

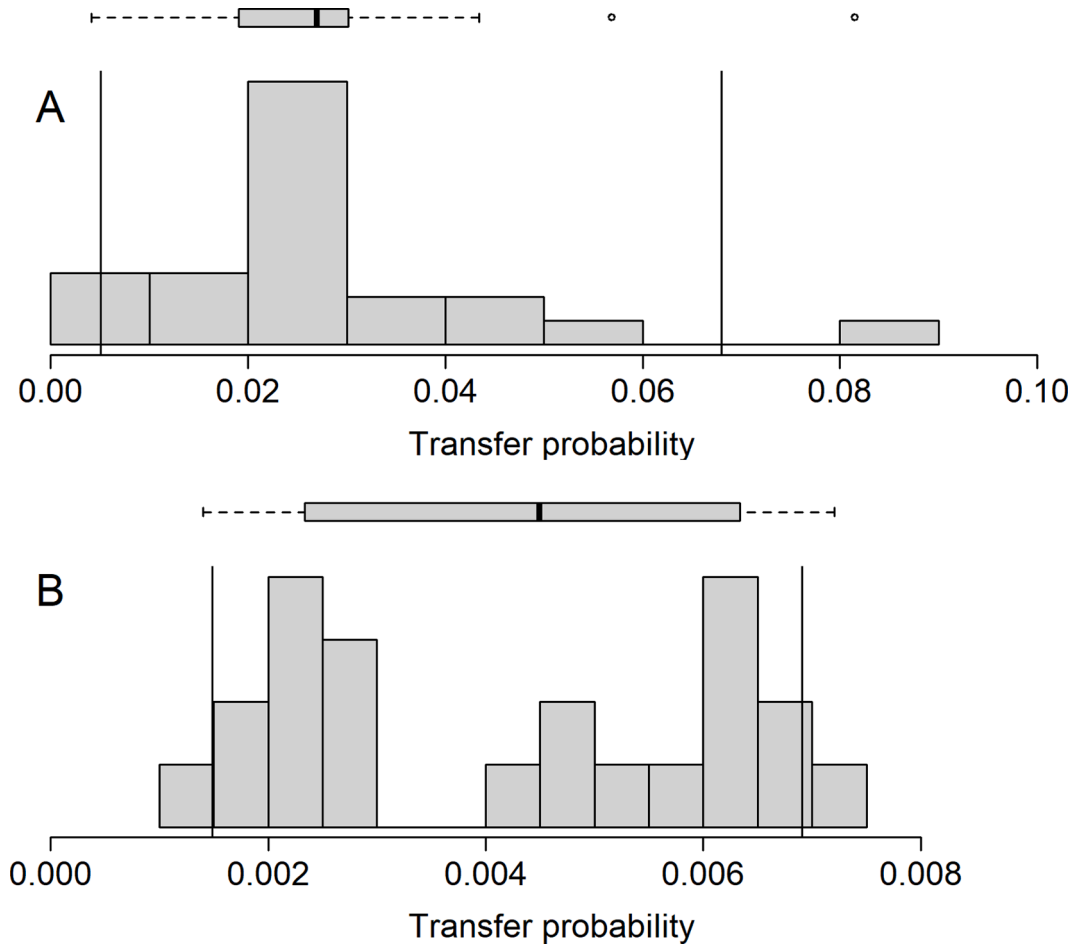

Fig. 1. Distribution of the transfer probabilities from knife to pork (A) and from pork to knife (B) considering the frequentist approach using the first dilution with bacterial counts ranging from 30 to 300. Vertical lines represent the 95% quantile interval.

Table 2
Summary for the posterior distribution of the transfer probability and mean values for *a* and *b* parameters from beta distribution used to describe the uncertainty around the transfer probability of *Salmonella* between pork and knife.

	Mean and percentiles for the posterior transfer probability						Beta distribution	
	Mean	2.5%	25%	50%	75%	97.5%	*a* [95%CrI]*	*b* [95%CrI]
Knife to pork	0.03	0.02916	0.03021	0.03078	0.03134	0.03243	22.6 [5.17; 38.71]	712.2 [163; 987]
Pork to knife	0.0042	0.00412	0.00419	0.00423	0.00427	0.00434	3.5 [0.62; 8.4]	702 [142; 988]

*CrI=credible interval.

2011). Thus, the estimated transfer probability of *Salmonella* sp. during pork preparation in household kitchens contributes to in-depth consumer phase risk assessment implementation. Further studies considering other important routes of cross-contamination, such as cutting boards and hands, as well as the interaction of all these factors and hygienic practices should be considered.

5. Conclusions

We assessed the transfer probability of *Salmonella* between pork and a knife surface in a Brazilian household scenario. The transfer probability from knife to pork is higher than the transfer probability from pork to knife. The Bayesian inference allows researchers to account for uncertainty in transfer probability for further implementations in risk assessment models.

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CRediT authorship contribution statement

Eduardo de Freitas Costa: Conceptualization, Data curation, Methodology, Writing – original draft. **Claudia Navarrete Rivas:** Conceptualization, Methodology, Writing – original draft. **Vanessa Bielefeld Leotti:** Methodology, Writing – original draft. **Marisa Cardoso:** Conceptualization, Supervision, Writing – original draft. **Luis Gustavo Corbellini:** Conceptualization, Supervision, Writing – original draft.

Declaration of Competing Interest

The authors declare no conflict of interest.

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