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Modelling *Salmonella* transmission among pigs from farm to slaughterhouse: interplay between management variability and epidemiological uncertainty

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Abstract

Salmonella carriage and cutaneous contamination of pigs at slaughter are a major risk for carcass contamination. They depend on *Salmonella* prevalence at farm, but also on transmission and skin soiling among pigs during their journey from farm to slaughterhouse. To better understand and potentially control what influences *Salmonella* transmission within a pig batch during this transport and lairage step, we proposed a compartmental, discrete-time and stochastic model. We calibrated the model using pork chain data from Brittany. We carried out a sensitivity analysis to evaluate the impact of the variability in management protocols and of the uncertainty in epidemiological parameters on three model outcomes: prevalence of infection, average cutaneous contamination and number of new infections at slaughter. Each outcome is mainly influenced by a single management factor: prevalence at slaughter mainly depends on the prevalence at farm, cutaneous contamination on the contamination of lairage pens and new infections on the total duration of transport and lairage. However, these results are strongly affected by the uncertainty in epidemiological parameters. Re-excretion of carriers due to stress does not have a major impact on the number

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of new infections.

Key words: stochastic model, sensitivity analysis, transport and lairage, salmonellosis, swine

1. Introduction

Human salmonellosis is the second most common foodborne zoonosis in the European Union (EU) and it is frequently attributed to the consumption of pork products (Hald et al., 2003; Pires and Hald, 2010). According to surveillance estimates by the European Food Safety Authority (EFSA), *Salmonella* is endemic in the pig population: around 10% of pigs at slaughter have infected lymph nodes and around 8% of the processed carcasses are contaminated (European Food Safety Authority, 2008). In order to reduce the incidence of human salmonellosis, EU states are required to monitor each stage of the pork supply chain and urged to adopt control strategies ensuring pig health and welfare, depending on their country-specific pig industry, herd statuses, slaughterhouse structures and compliance with hygienic measures (EFSA Panel on Biological Hazards (BIOHAZ), 2010).

Non-negligible transmission rates during pig transport and lairage were reported, both among animals belonging to the same herd and across herds (Hurd et al., 2001a,b). Transmission occurs primarily via the fecal–oral route: a healthy pig gets infected after ingesting a large number of microorganisms. In turn, infected individuals intermittently excrete large numbers of the bacteria in their faeces, contaminating their local environment. Stress imposed by food withdrawal, transportation, or lairage can significantly increase the number of shedding pigs, as well as the amount of both excreted and ingested *Salmonella* (Scherer et al., 2008).

There is strong evidence showing that prevalence at slaughter depends on (i) the proportion of animals shedding at departure from farm (Boughton et al., 2007), (ii) the degree of environmental contamination and (iii) the duration of exposure to this contamination (Hurd et al., 2001a; Mannion et al., 2012). How-

ever, there is a wide diversity in the epidemiological status of pigs departing from farms (European Food Safety Authority, 2008), in the transport conditions and in the exposure to environmental contamination (Rostagno et al., 2003), both
30 at an individual level and at a batch level (Hernández et al., 2013). Moreover, many epidemiological characteristics of *Salmonella* spread remain highly uncertain, for instance: the dose–response relationship between the environmental contamination and the infection probability of healthy pigs (Boughton et al., 2007; Loynachan and Harris, 2005), the excretion rate of shedders (Ivanek et al.,
35 2012; Martín-Peláez et al., 2009; Tanaka et al., 2014), or the rate of stressed non-shedding carriers reverting to excretion (Scherer et al., 2008). This prevents drawing definite conclusions regarding the relative impact of factors on the risk of carcass contamination (Rostagno and Callaway, 2012).

The complex interplay between biological and management processes affecting
40 *Salmonella* transmission appeals for a modelling approach to evaluate the impact of different factors at different levels of the production chain (Hotes et al., 2012; Smid et al., 2012). Mechanistic models have been successfully implemented at a farm level (Berriman et al., 2013; Hill et al., 2015; Lurette et al., 2008). Transport and lairage are usually not represented, with the notable
45 exception of an EFSA scientific report (VLA, DTU, RIVM, 2010), which performed a quantitative microbial risk analysis of the pork production chain to investigate the effect of interventions at different points of the food chain (Schaffner and Doyle, 2008). It resulted in a hierarchy of control measures and an estimation of their impact on the public health risk of salmonellosis. The
50 model describing transmission during transport and lairage was recently detailed (Simons et al., 2015).

In line with these communications, an exhaustive exploration of the interactions between management conditions and epidemiological settings in a batch during transport and lairage was carried out. The aim of this study was to
55 assess their relative impact on the epidemiological status of pigs at slaughter under different transmission regimes, while considering the internal carriage and cutaneous contamination, as both can lead to (cross-)contamination during

slaughter. The impact of the slaughter processes on carcass contamination is outside the scope of this study.

60 2. Material and methods

2.1. Model description

This work presents a discrete-time stochastic epidemiological model that follows a single batch from farm to slaughter considering three stages: waiting at farm (stage F), transport by truck (stage T) and waiting at lairage (stage 65 A), as shown in Figure 1a. The time spent in each stage is given by the stage duration t_X . The batch is characterised by its epidemiological state $B(t)$ at time t , describing the number of healthy (S), latent (E), actively shedding (I) and non-shedding carrier (R) pigs (Figure 1b), and by its average cutaneous contamination $C(t)$, which represents the average skin soiling of a pig. Its 70 initial state at time $t_0 = 0$ is defined by three parameters: batch size $b_0 = S(t_0) + E(t_0) + I(t_0) + R(t_0)$, which remains constant over time, *Salmonella* prevalence at farm $p_0 = \frac{I(t_0)+R(t_0)}{b_0}$ and initial cutaneous contamination c_0 . At each stage $X \in \{F, T, A\}$, pens are characterised by their final environmental contamination Q_X , initialised by q_X .

75 The model considers three stochastic processes governing the evolution of the variables from the beginning ($t_{b,X}$) to the end ($t_{e,X} = t_{b,X} + t_X$) of each stage: excretion, transmission and skin soiling (Figure 1b). Note that the beginning of the waiting at farm corresponds to the initial time ($t_{b,F} = t_0 = 0$) and that stages are connected without lapses (for instance, $t_{e,F} = t_{b,T}$).

80 To determine the final environmental contamination Q_X , the amount of *Salmonella* shed by active shedders using random samples taken from a normal distribution (\mathcal{N}) was computed with:

$$Q_X = q_X + \mathcal{N}\left(\varepsilon, \frac{\varepsilon}{10}\right) I(t_{b,X}) t_X \quad (1)$$

where ε is the excretion rate. The standard deviation of the excretion rate is set arbitrarily to $\varepsilon/10$, so that the different levels of ε explored in the sensitivity

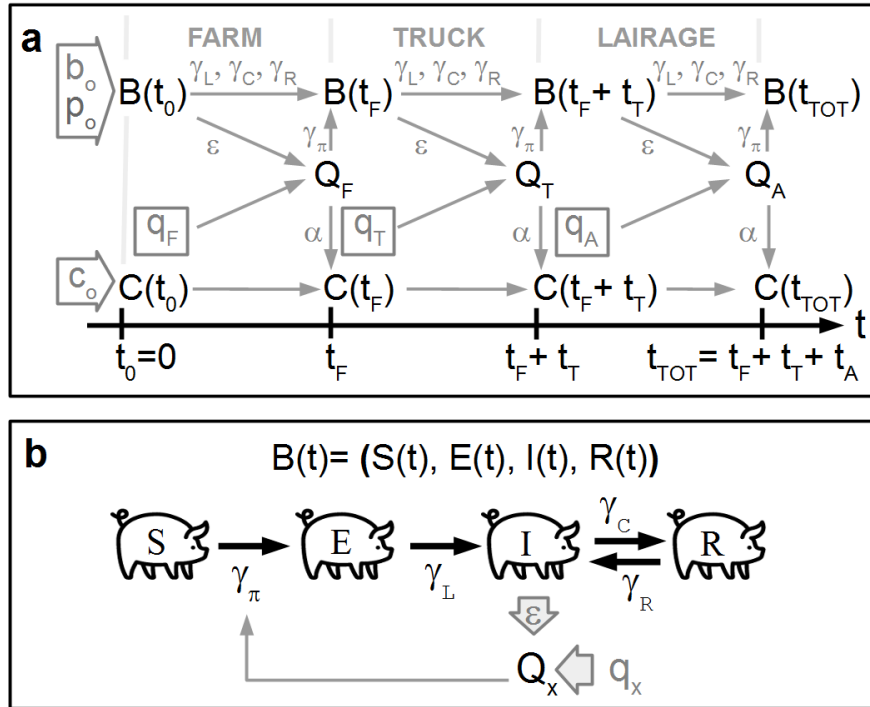


Figure 1: Outline of the stochastic compartmental model representing *Salmonella* dynamics in a batch from farm to slaughter. **(a)** The model derives the environmental contamination (Q_X), the epidemic state (B) and the average cutaneous contamination (C) of the batch at each stage (X : F = farm, T = transport and A = lairage), from the batch size (b_0), prevalence at farm (p_0), initial cutaneous contamination (c_0) and environmental contaminations (q_X); it considers *Salmonella* excretion (ϵ), contamination (γ_π), infection dynamics (γ_L , γ_C and γ_R) and skin soiling (α). **(b)** The epidemic model considers four infection states (S = healthy, L = latent, I = active shedder and C = non-shedder carrier); fecal-oral transmission (γ_π) is driven by environmental contamination (Q_X), resulting from the initial contamination (q_X) and from shedding (ϵ); the other transition rates between states (γ_L , γ_C and γ_R) only depend on the stage duration (t_X).

85 analysis do not overlap. Occasional samples of negative numbers, which very seldom occurred, were replaced by zero.

In the following equations, we dropped the stage subscript X for $t_{X,b}$ and $t_{X,e}$, as there was no possible ambiguity. For each stage, the model updates the number of pigs in each epidemiological state (Figure 1b) as follows:

$$\begin{cases} S(t_e) = S(t_b) - N_{S \rightarrow E}(t_b, t_e) \\ E(t_e) = E(t_b) + N_{S \rightarrow E}(t_b, t_e) - N_{E \rightarrow I}(t_b, t_e) \\ I(t_e) = I(t_b) + N_{E \rightarrow I}(t_b, t_e) - N_{I \rightarrow R}(t_b, t_e) + N_{R \rightarrow I}(t_b, t_e) \\ R(t_e) = R(t_b) + N_{I \rightarrow R}(t_b, t_e) - N_{R \rightarrow I}(t_b, t_e) \end{cases} \quad (2)$$

90 Each $N_{Y \rightarrow Z}$ corresponds to the number of pigs transiting from epidemiological state Y to state Z and was determined by a random sample drawn from a binomial distribution (\mathcal{B}):

$$\begin{cases} N_{S \rightarrow E} = \mathcal{B}(S(t_b), \gamma_\pi(t_e - t_b)) \\ N_{E \rightarrow I} = \mathcal{B}(E(t_b), \gamma_L(t_e - t_b)) \\ N_{I \rightarrow R} = \mathcal{B}(I(t_b), \gamma_C(t_e - t_b)) \\ N_{R \rightarrow I} = \mathcal{B}(R(t_b), \gamma_R(t_e - t_b)) \end{cases} \quad (3)$$

where γ_π , γ_L , γ_C and γ_R are the transition probabilities from S to E , from E to I , from I to R and from R to I , respectively; they depend on the stage duration
95 $(t_e - t_b)$.

The probabilities $\gamma_L = 1 - e^{-\frac{(t_e - t_b)}{\tau_L}}$ and $\gamma_C = 1 - e^{-\frac{(t_e - t_b)}{\tau_C}}$ were computed using the average durations of the latent period (τ_L) and of the shedding phase (τ_C) of *Salmonella* infection in pigs.

The probabilities $\gamma_\pi = 1 - (1 - \pi)^{(t_e - t_b)}$ and $\gamma_R = 1 - (1 - \rho)^{(t_e - t_b)}$ were
100 computed by cumulating the infection (π) and re-excretion (ρ) rates over the stage duration (Figure 2a). The infection and re-excretion rates are defined the probability for a pig to become infected and to start re-excreting, respectively, during a unit of time. Indeed, these individual probabilities are integrated values that would be easier to measure than proper continuous rates and that can well

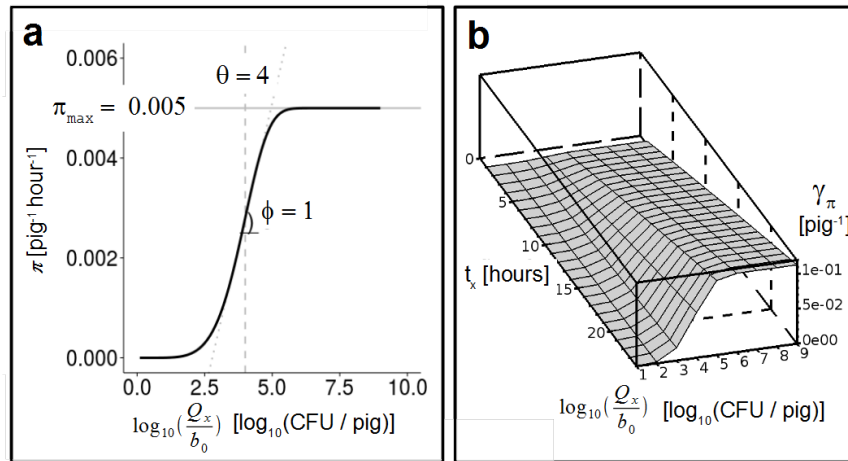


Figure 2: Representation of the functions implemented for the fecal-oral transmission at each stage (X : F = farm , T = transport and A = lairage). **(a)** The dose–response function computes the infection rate (π), defined as the probability for a pig to become infected during a unit of time, as a function of the environmental contamination per pig (Q_X/b_0); its parameters are the maximum probability at saturation (π_{\max}), the contamination inflection point (θ) and the steepness of the curve (ϕ). **(b)** The probability of infection (γ_π) cumulates the rate (π) over the stage duration t_X .

105 be used in a discrete-time model.

The infection rate π is given by the dose–response function, a Weibull cumulative distribution function of the logarithm of the environmental contamination Q_X divided by the batch size b_0 (Figure 2b). This particular shape was chosen for its flexibility and efficiency in parameter exploration (Fekedulegn et al.,
110 1999). Similar simulation results were obtained when testing other types of S-shaped curves, characterised by the same parameters: probability of infection under saturated environments (π_{\max}), environmental contamination corresponding to an inflection point of the infection probability (θ) and steepness of the curve (ϕ). Pigs are not individualised in the model, which follows the number
115 of pigs in each epidemiological state. So assuming that each susceptible pig was exposed to the same average environmental contamination ($\pi = \pi(Q_X/b_0)$) is adequate in such a context. The re-excretion rate, which depends on the stress of pigs, was assumed constant from farm to slaughter.

Finally, the average cutaneous contamination of a pig $C(t)$ was computed
120 from Q_X using a fixed rate of skin soiling α :

$$C(t_e) = C(t_b) + \alpha \frac{Q_X}{b_0}. \quad (4)$$

Individual contaminations can be drawn from a probability distribution using this average cutaneous contamination as parameter.

We chose to concentrate on four model outcomes: the prevalence at slaughter $p_s = \frac{I(t_{\text{TOT}}) + R(t_{\text{TOT}})}{b_0}$, the average cutaneous contamination at slaughter
125 $c_s = C(t_{\text{TOT}})$, the number of new infections during transport and lairage $N_I = S(t_0) - S(t_{\text{TOT}})$ and the number of pigs that revert from carriers to active shedders during transport and lairage $N_R = R(t_0) - R(t_{\text{TOT}})$.

2.2. Model parameters

To simulate the model, its parameters were either set to fixed values or
130 they varied to take into account the variability, *i.e.* the “natural” variations observed among individuals or in the processes represented in the model, and the uncertainty, *i.e.* the lack of knowledge, in real data (Table 1).

	Parameter	Value(s)	Unit	Source
b_0	Batch size	100	pigs	(BDPORC, 2010)
c_0	Initial cutaneous contamination	10^1	$\frac{\text{CFU}}{\text{pig}}$	*
α	Proportion of skin soiling	0.1	—	*
τ_L	Average latent period	0.5	hours	(Hurd et al., 2002)
τ_C	Average shedding duration	5×10^4	hours	(Boughton et al., 2007)
Management factors				
p_0	Prevalence at farm	0, 5, 10, 20	hours	(VLA, DTU, RIVM, 2010)
t_{TOT}	Total duration	8, 16, 24	hours	(BDPORC, 2010)
t_F	Waiting time at farm	2.5, 5.5, 9	hours	(BDPORC, 2010)
t_T	Transport duration	0.5, 1.5, 3	hours	(BDPORC, 2010)
t_A	Waiting time at lairage	5, 9, 12	hours	(BDPORC, 2010)
q_F	Farm initial contamination	$0, 10^5, 10^6, 10^7, 10^9$	CFU	(Frotin et al., 2007) †
q_T	Truck initial contamination	$0, 10^5, 10^6, 10^7, 10^9$	CFU	(Frotin et al., 2007) †
q_A	Lairage initial contamination	$0, 10^5, 10^6, 10^7, 10^9$	CFU	(Frotin et al., 2007) †
Epidemiological factors				
ε	Excretion rate	$10, 10^2, 10^3, 10^4, 10^5$	$\frac{\text{CFU}}{\text{pig}\cdot\text{hour}}$	‡
π_{max}	Infection rate at saturation	0.002, 0.005, 0.01	$\frac{1}{\text{pig}\cdot\text{hour}}$	‡
θ	Threshold contamination	3, 4, 5	$\log_{10}\left(\frac{\text{CFU}}{\text{pig}}\right)$	‡
ϕ	Steepness of the dose–response curve	0.1, 0.5, 1	$\frac{\text{pig}^{-1}\text{hour}^{-1}}{\log_{10}\left(\frac{\text{CFU}}{\text{pig}}\right)}$	‡
ρ	Re-excretion rate	0.005, 0.01, 0.05, 0.1, 0.5	$\frac{1}{\text{pig}\cdot\text{hour}}$	‡

Table 1: Parameters of the model. The waiting at farm (t_F), transport in truck (t_T) and waiting at lairage (t_A) durations were set from real data in Brittany (BDPORC, 2010). The total duration was computed as follows: $t_{\text{TOT}} = t_F + t_T + t_A$. Initial prevalence (c_0) of batches were set according to surveillance data (VLA, DTU, RIVM, 2010). * Initial cutaneous contamination (c_0) and proportion of skin soiling (α) were fixed to arbitrary values due to lack of experimental data. † Reference values for the range of contaminations that can be found in slaughterhouses in Brittany. They are consistent with other measurements found in the literature (Small et al., 2006; Swanenburg et al., 2001). ‡ Epidemiological factors were chosen to obtain model outcomes that were consistent with the literature (Boughton et al., 2007; Hurd et al., 2001a,b): π_{max} , θ and ϕ are the parameters of the dose-response function, τ_L is the lag before an infected pig starts shedding and τ_C is the shedding duration.

The batch size (b_0) was set to a fixed value representing an average size of batches. This choice does not affect our results because transmission is not modeled at an individual level. The initial cutaneous contamination (c_0) and proportion of skin soiling (α) were fixed to arbitrary values due to lack of information. In particular, we considered that all pigs were initially clean. The relative impact of management and epidemiological parameters on skin soiling does not depend on these parameter values because, they affect neither excretion nor transmission. The latent period (τ_L) and the shedding duration (τ_C) were fixed because their variations had no impact on the outcomes: the former is much shorter and the latter much longer than the transport and lairage durations.

To deal with the management factor variability, we chose three levels of total duration ($t_{\text{TOT}} = t_F + t_T + t_A$), corresponding to the 5th percentile, median and 95th percentile of the distribution of the waiting at farm (t_F), transport (t_T) and lairage (t_A) times provided by the professional union BDPORC (BDPORC, 2010). We explored four levels of prevalence at farm (p_0) and five levels of initial environmental contamination (q_X) at each stage, extracted from data describing the pork industry in Brittany (Frotin et al., 2007).

Because of the uncertainty on the epidemiological factors, we first screened 2500 combinations of epidemiological parameters and pre-selected the parameters generating outcomes in accordance with the literature (Hurd et al., 2001a,b). This ensured that our model could reproduce realistic patterns. We then selected 27 discrete combinations of dose–response parameters ($3 \pi_{\text{max}} \times 3 \theta \times 3 \phi$), 5 levels of the excretion rates (ε) and 5 levels of the re-excretion rate (ρ).

2.3. Model exploration and sensitivity analyses

Our model considers three sources of randomness. Firstly, transmission is a stochastic process as pigs under same transport and lairage conditions may or may not become contaminated. Secondly, management contexts (initial status of batches and transport conditions) are diverse. Thirdly, parameters describing *Salmonella* spread are highly uncertain. From now on, we use the term scenario

to refer to a combination of management contexts (q_X at each stage, p_0 and $t_{\text{TOT}} = t_F + t_T + t_A$) and epidemiological settings (π_{max} , θ , ϕ , ρ and ε).

165 To determine the number of repetitions of each scenario that capture stochasticity in transmission, we carried out a preliminary analysis varying this number in $\{51, 101, 201, 501, 801, 1001, 5001\}$. We found that $N_{\text{reps}} = 501$ repetitions were sufficient to ensure that model outcomes do not differ from their asymptotic distributions: the variance of the mean of each model outcome remained
170 fixed at around 1% when 500 or more repetitions were considered. The mean of each model outcome was calculated by averaging its value among the repetitions. The variance of the mean was computed by repeating 100 times the calculation of the mean of the model outcome (100 different samples).

Intra-scenario and inter-scenario variabilities were compared, revealing that
175 the former was significantly smaller than the latter for all three outcomes. Therefore, we decided to examine inter-scenario effects on the model outcomes averaged over intra-scenario repetitions.

Two numerical experiments were carried out to systematically explore the impact of the variability of management contexts and of the uncertainty in epidemiological parameters on the model outcomes (p_s , c , N_I and N_R), averaged
180 over $N_{\text{reps}} = 501$ repetitions. Firstly, all pigs initially infected at farm were considered as active shedders, by setting $I(0) = p_0 b_0$ and hence $R(0) = 0$. As the average shedding duration τ_C is very long compared to the total duration t_{TOT} (Table 1), the impact of the re-excretion rate ρ was ignored, hence obtaining a
185 simplified $S \rightarrow E \rightarrow I$ model. This entailed exploring 202500 scenarios combining 1500 management contexts and 135 epidemiological settings. Secondly, all pigs initially infected at farm were considered as non-shedding carriers, by setting $R(0) = p_0 b_0$ and hence $I(0) = 0$. Even with a initial population consisting of non-shedding carriers, the impact of ρ could not be neglected, so the original
190 $S \rightarrow E \rightarrow I \leftrightarrow R$ model was maintained. However, the impact of the steepness of the dose-response curve ϕ was now overlooked, as it turned out to be the least significant factor in the previous experiment. As a result, 337500 scenarios, combining 1500 management contexts and 225 epidemiological settings were

explored in this second set of simulations.

195 A global sensitivity analysis to explore the influence of the scenario factors on the model outcomes (Saltelli et al., 2000) was performed by carrying out out a standard ANOVA for each outcome considering up to second-order interactions. The sensitivity index associated with each term was evaluated, split into main effect of a factor and two-factor interactions, as the ratio between the
200 sum of squares corresponding to that term and the total sum of squares. The total sensitivity index was computed for each factor as the sum of the sensitivity indices corresponding to this factor (main effect plus interactions involving the factor). The sensitivity indices obtained quantify the fraction of outcome variance among simulations explained by the variation of each factor within its
205 value range.

All simulations and analyses were performed using R and the multisensi package (Lamboni et al., 2011).

3. Results

3.1. Inter-scenario sensitivity analysis without carriers (SEI model)

210 The relative impact of management and epidemiological factors on batches initially composed of healthy pigs and active shedders is assessed in Figures 3 and 4.

Outcomes of the scenarios explored in this first experiment exhibit wide asymmetric distributions (Figure 3a–c) that aggregate overlapping distributions even
215 when results are split by the factors that most impact each variable (Figure 3d–f).

There are generally new infections during transport and lairage: the 95% confidence interval of this outcome is $CI_{0.95}(N_I) = [0, 22]$ pigs (Figure 3c,f). Prevalence may increase from its initial value $p_0 \in [0, 20]\%$ to its value at slaughter
220 $CI_{0.95}(p_s) = [0, 32.7]\%$ (Figure 3d). The average cutaneous contamination saturates under highly contaminated environments, regardless of the infection dynamics (Figure 3e).

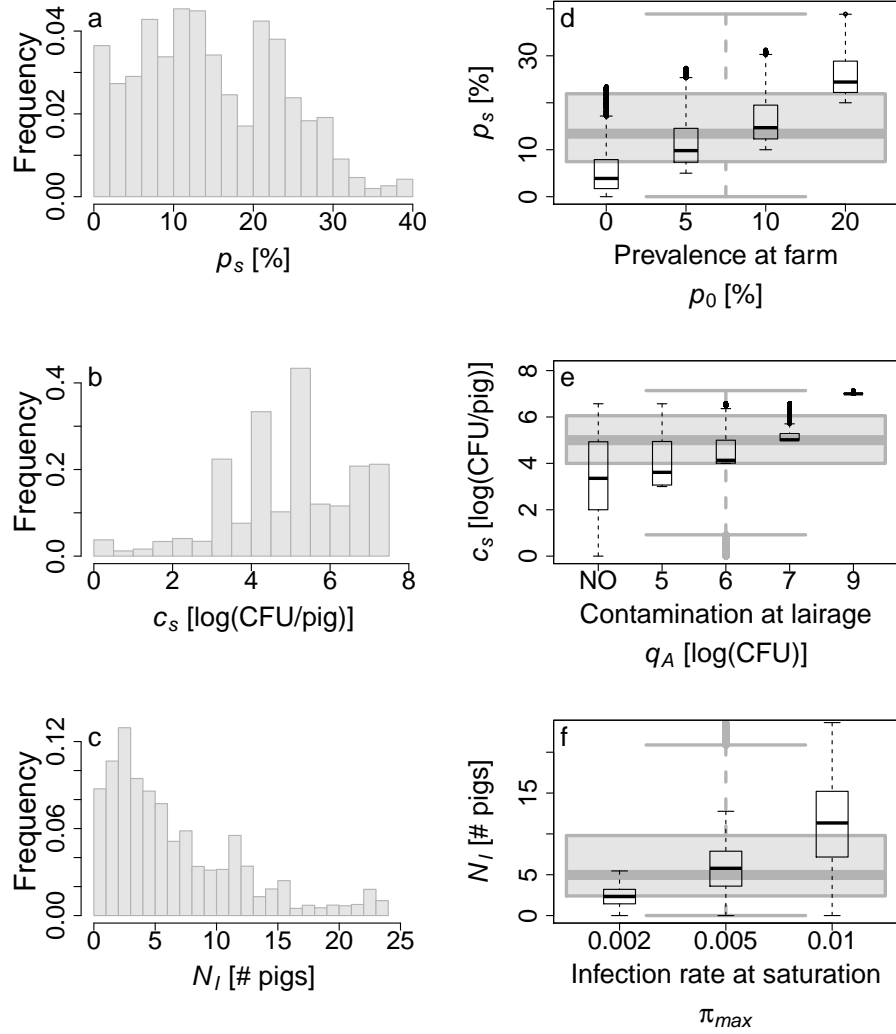


Figure 3: Inter-scenario distributions of the *SEI* model outcomes: (a,d) prevalence at slaughter, (b,e) average cutaneous contamination at slaughter and (c,f) new infections from farm to slaughter. Histograms (a–c) present the model outcomes averaged over 501 repetitions for 202500 scenarios. Scenarios cross 27 dose–response functions with 5 excretion rates and 1500 management contexts. Corresponding boxplots (d–f) are presented alongside (grey), as well as boxplots split by the values of the factor that most affects the outcome considered (white): (d) initial prevalence at farm, (e) environmental contamination at lairage and (f) maximum infection probability at saturation (parameter of the dose–response function).

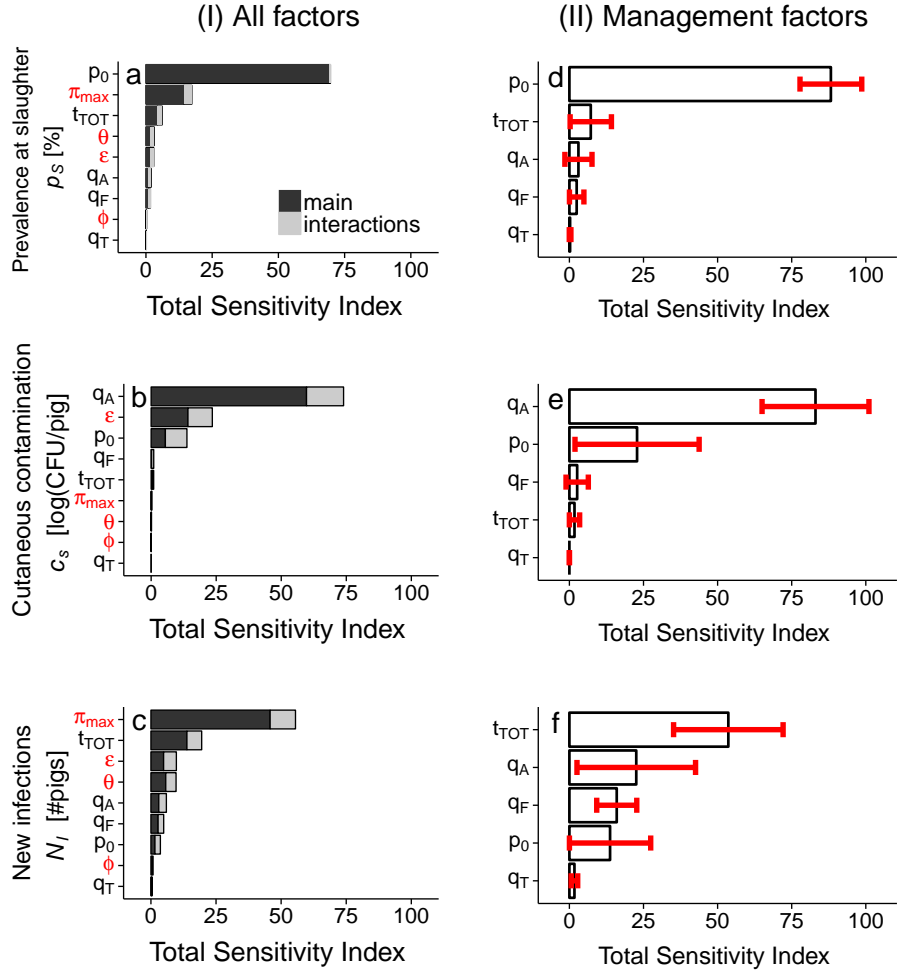


Figure 4: Inter-scenario sensitivity analysis of the *SEI* model outcomes: **(a,d)** prevalence at slaughter, **(b,e)** average cutaneous contamination at slaughter and **(c,f)** new infections from farm to slaughter. Outcomes are averaged over 501 repetitions for 202500 scenarios that cross 27 dose–response functions with 5 excretion rates and 1500 management contexts. Column **(I)**: total sensitivity indices for all factors, split into main effect (dark grey) and interactions (light grey). Column **(II)**: total sensitivity indices for the management factors alone, averaged over all scenarios sharing the same management constraints; so the associated errorbars represent the variability arising from the uncertainty over the epidemiological parameters. Factors are described in Table 1.

For all outcomes, there is always a factor explaining more than 50% of the inter-scenario variability, whereas the interactions between factors explain less than 20% of the variability (Figure 4a–c). The most important factor determining the prevalence at slaughter (p_s) is the prevalence at the farm of origin (p_0); the factor mainly affecting the average cutaneous contamination (c_s) is the initial environmental contamination at lairage (q_A); and *Salmonella* transmission is mostly affected by the transmission rate at saturation (π_{max}). The environmental contamination of trucks (q_T) and the steepness of the dose–response curve (ϕ) were found to be the least influential factors for all outcomes.

Epidemiological factors have a significant impact on the model outcomes. However, they are both difficult to assess and hardly affected by prevention measures and control interventions. Therefore, the impact of management factors alone was further explored in detail. The total sensitivity indices of the management factors were computed averaging over all the scenarios that share the same management factors, while varying epidemiological factors. Results are shown in Figure 4d–f; errorbars depict the variability associated with the uncertainty over the epidemiological parameters. We found that the order of importance of management factors for each outcome is generally maintained. However, the relative impact of management factors on the average cutaneous contamination and on the number of new infections is strongly influenced by epidemiological factors. In order to explore this dependency, a detailed analysis with three contrasted sets of epidemiological factors was carried out.

3.2. Detailed analysis of three contrasted epidemiological settings

Specific values of epidemiological parameters were selected to display circulation settings with low, moderate or high transmissibility (Table 2).

Transmissibility does not modify the relative impact of management factors on the prevalence at slaughter (Figure 5).

Figure 6 displays two remarkable results regarding the effect of management factors on the average cutaneous contamination at slaughter. Firstly, *Salmonella* transmissibility affects the relative impact of the initial contamination of lairage

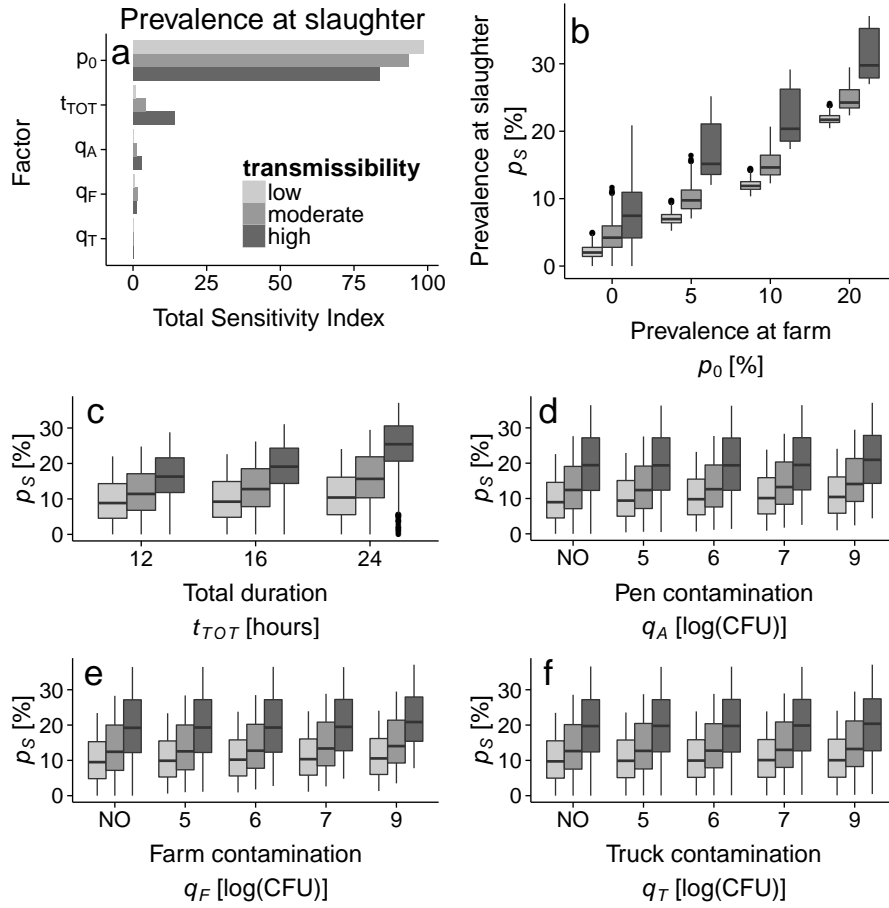


Figure 5: Detailed analysis of the prevalence at slaughter (p_s) for three epidemiological settings: low (light grey), moderate (medium grey) and high (dark grey) transmissibilities. p_s is averaged over 501 repetitions for 1500 scenarios (management contexts) of the *SEI* model. (a) Total sensitivity indices for the management factors. (b-f) Boxplots split by factor values, in decreasing order of importance. Factors are described in Tables 1 and 2.

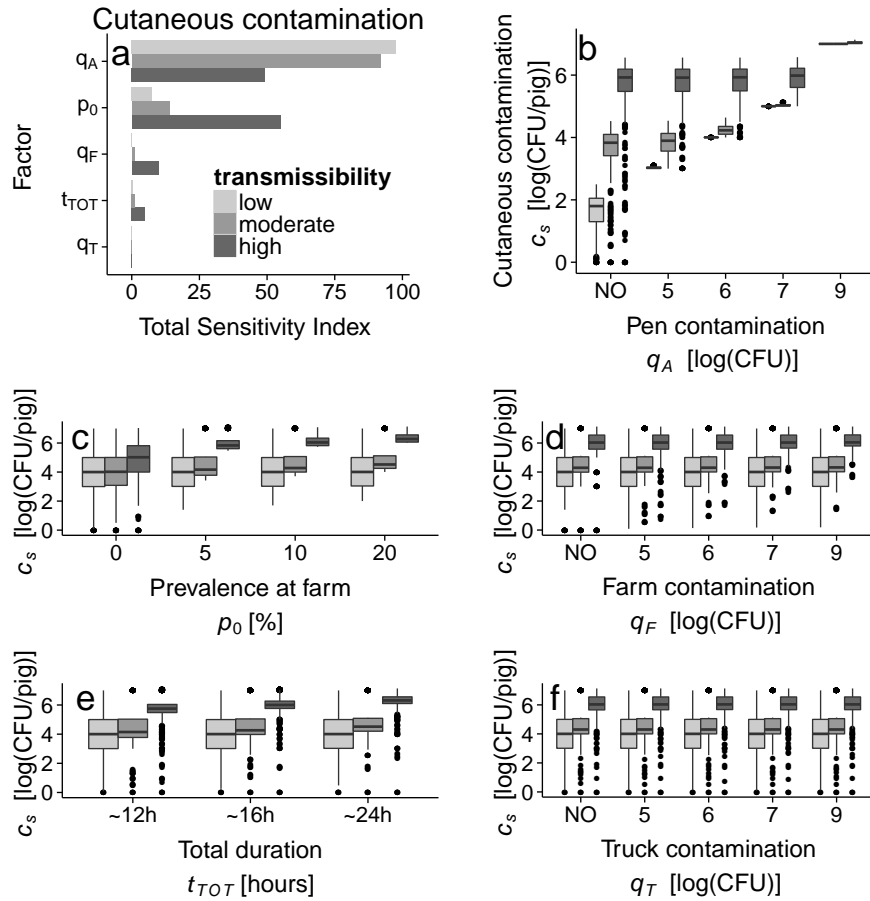


Figure 6: Detailed analysis of the average cutaneous contamination at slaughter (c_s) for three epidemiological settings: low (light grey), moderate (medium grey) and (high (dark grey) transmissibilities. c_s is averaged over 501 repetitions for 1500 scenarios (management contexts) of the *SEI* model. **(a)** Total sensitivity indices for the management factors. **(b-f)** Boxplots split by factor values, in decreasing order of importance. Factors are described in Tables 1 and 2.

Parameter	Low	Moderate	High	Unit
ε	10^2	10^4	10^6	$\frac{\text{CFU}}{\text{pig}\cdot\text{hour}}$
π_{\max}	0.002	0.005	0.01	$\frac{1}{\text{pig}\cdot\text{hour}}$
θ	5	4	3	$\log_{10}\left(\frac{\text{CFU}}{\text{pig}}\right)$
ϕ	0.1	0.5	1	$\frac{\text{pig}^{-1}\text{hour}^{-1}}{\log_{10}\left(\frac{\text{CFU}}{\text{pig}}\right)}$

Table 2: Epidemiological parameters selected for the low, moderate and high transmissibilities. π_{\max} : infection probability at saturation, θ : threshold contamination triggering infection, ϕ : steepness of the dose–response curve and ε : excretion rate.

pens (q_A): this factor is determining under low transmissibility regimes but has less impact for higher transmissibility regimes (Figure 6a). In contrast, the relative impact of the initial prevalence at farm (p_0) is more important under high transmissibility regimes. Secondly, there is a threshold for q_A above which the average cutaneous contamination saturates and is no longer affected by other factors (Figure 6b). This threshold increases with transmissibility: for the low, moderate and high transmissibility regimes, saturation occurs for $q_A \geq 10^5 \frac{\text{CFU}}{\text{pig}}$, $q_A \geq 10^7 \frac{\text{CFU}}{\text{pig}}$ and $q_A \geq 10^9 \frac{\text{CFU}}{\text{pig}}$, respectively.

Figure 7 shows that, even if the total duration (t_{TOT} is the most important factor determining the number of new infections from farm to slaughter, the relative impact of the other factors is probably affected by the transmissibility regime. Under a high transmissibility, the second most important factor is the initial prevalence at farm (p_0), while under low and moderate transmissibility regimes it is the initial contamination of lairage pens (q_A).

3.3. Impact of re-excretion (SEIR model)

To explore the impact of re-excretion, all initial infected pigs were considered as non-shedding carriers. We analysed how the re-excretion rate (ρ) alters the relative impact of management and epidemiological factors on the three previous model outcomes (p_s , c_s and N_I) and on the number of pigs reverting to excretion (N_R).

This numerical experiment showed that the outcome distributions are more

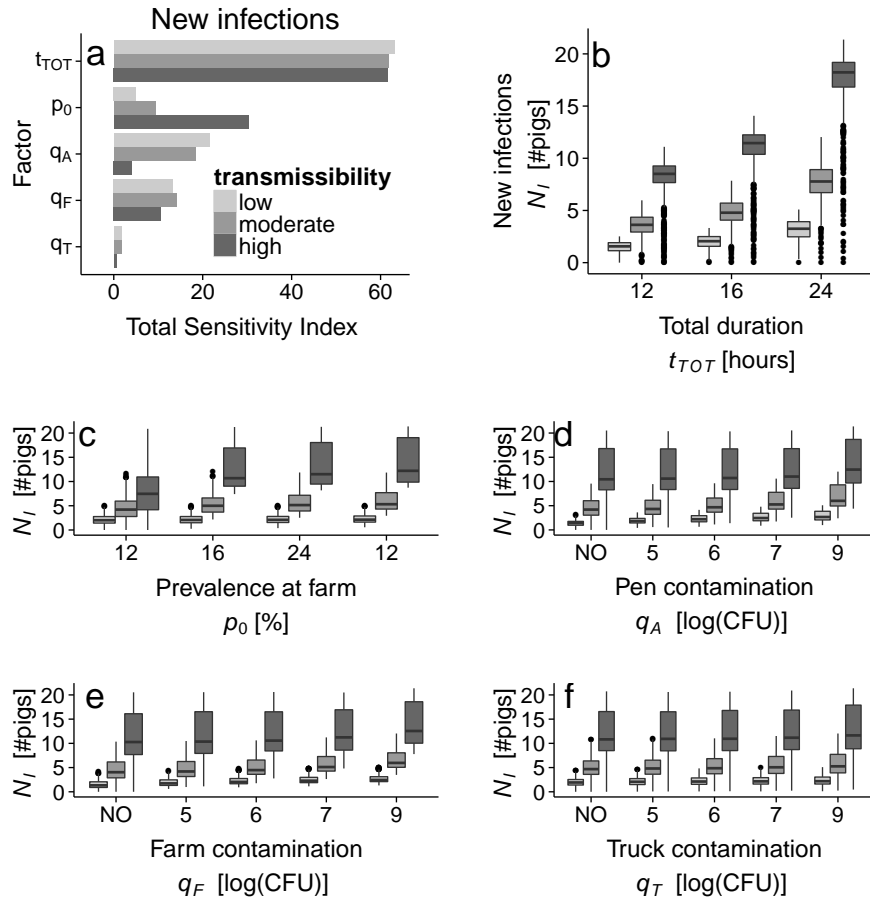


Figure 7: Detailed analysis of the new infections from farm to slaughter (N_I) for three epidemiological settings: low (light grey), moderate (medium grey) and high (dark grey) transmissibilities. N_I is averaged over 501 repetitions for 1500 scenarios (management contexts). (a) Total sensitivity indices for the management factors. (b-f) Boxplots split by factor values, in decreasing order of importance. Factors are described in Tables 1 and 2.

strongly shaped by the initial conditions. For instance, the distribution of the
 275 prevalence at slaughter (p_s) clearly exhibits four peaks, corresponding to the
 four initial values of the prevalence at farm (p_0) (Figure 8a,e).

Not surprisingly, we found that the outcomes of the *SEIR* model are lower
 than those of the *SEI* model (Table 3). Indeed, all initially infected pigs in the
SEIR model were non-shedding carriers. Not all became shedders and when
 280 they did, they globally started shedding later than in the *SEI* model, in which
 all initially infected pigs were active shedders.

Outcome	$CI_{0.95}(SEI)$	$CI_{0.95}(SEIR)$	Unit
p_s	[0.6, 32.7]	[0.2, 24.6]	%
c_s	[1, 7]	[1, 6.3]	$[\log_{10}(\frac{CFU}{pig})]$
N_I	[0, 22]	[0, 16]	pigs
N_R	n/a	[0, 20]	pigs

Table 3: Comparison of the model outcome distributions when all initial infected pigs are
 either active shedders (*SEI* model, 202500 scenarios) or non-shedding carriers (*SEIR* model,
 337500 scenarios). The 95% confidence interval ($CI_{0.95}$) is given for the prevalence at slaughter
 (p_s), the average cutaneous contamination at slaughter (c), the number of new infections from
 farm to slaughter (N_I) and the number of infected pigs reverting to excretion from farm to
 slaughter (N_R).

The re-excretion rate (ρ) has a small impact on the model outcomes, except
 for N_R , which could be expected (Figure 9). It just explains around 10% of the
 variability for c_s and less than 1% of the variability for p_s and N_I . Introducing
 285 re-excretion affects the relative impact of the other factors by enhancing the
 effect of the initial conditions. For instance, the initial prevalence at farm p_0
 and the initial contamination of the lairage pen q_A have a greater impact on p_s
 and c_s , respectively.

4. Discussion

290 We presented a stochastic model of *Salmonella* infection dynamics from farm
 to slaughter and we used it to analyse the impact of epidemiological and man-

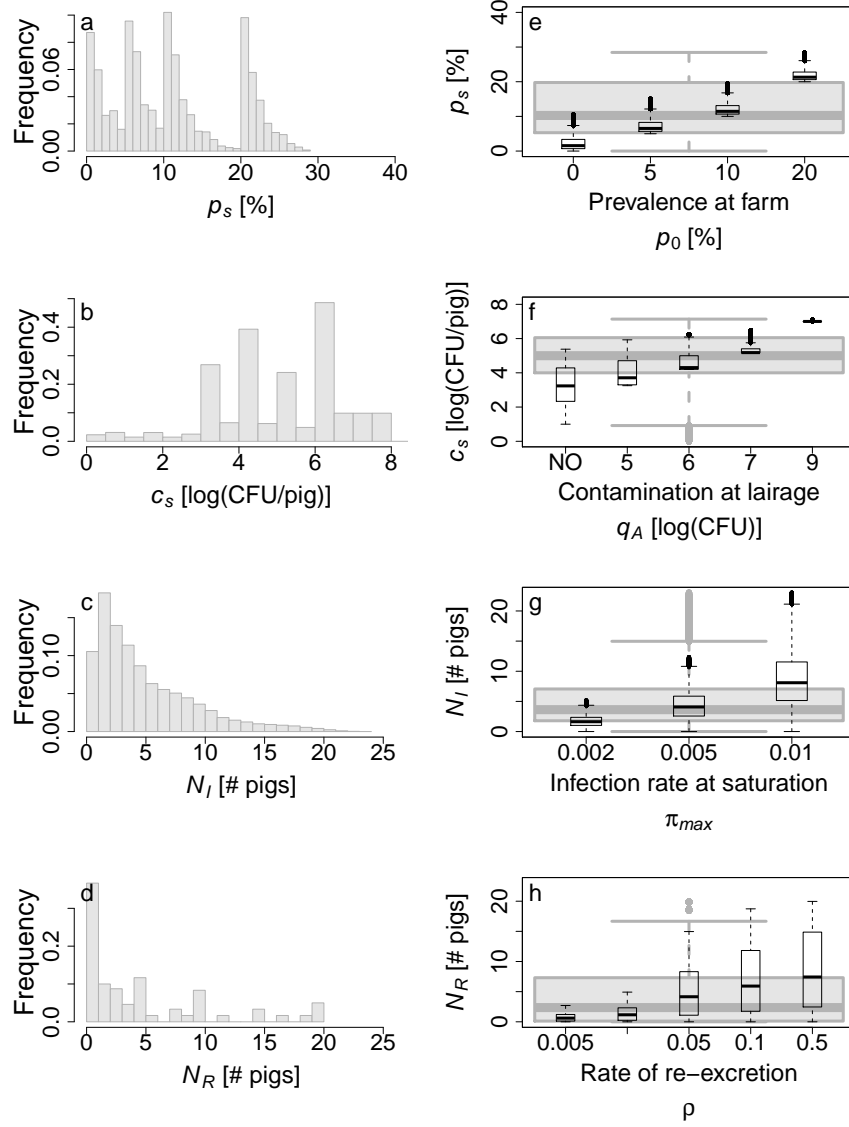


Figure 8: Inter-scenario distributions of the *SEIR* model outcomes: **(a,e)** prevalence at slaughter, **(b,f)** average cutaneous contamination at slaughter, **(c,g)** new infections from farm to slaughter and **(d,h)** number of infected pigs reverting to excretion from farm to slaughter. Histograms **(a–d)** present the model outcomes averaged over 501 repetitions for 337500 scenarios. Scenarios cross 9 dose–response functions with 5 excretion rates, 5 re-excretion rates and 1500 management contexts. Corresponding boxplots **(e–h)** are presented alongside (grey), as well as boxplots split by the values of the factor that most affects the outcome considered (white): **(e)** initial prevalence at farm, **(f)** environmental contamination at lairage, **(g)** maximum infection probability at saturation (parameter of the dose–response function) and **(h)** re-excretion rate.

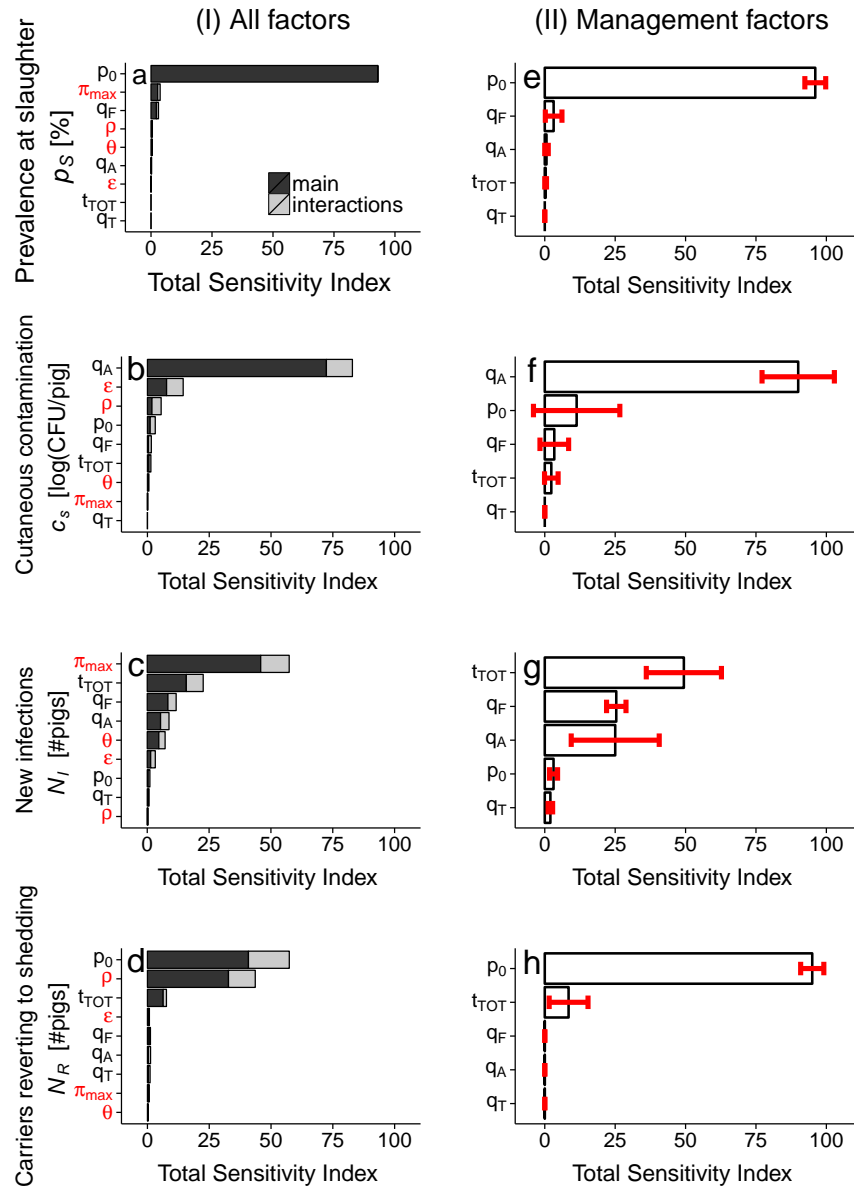


Figure 9: Inter-scenario sensitivity analysis of the of the *SEIR* model outcomes: **(a,e)** prevalence at slaughter, **(b,f)** average cutaneous contamination at slaughter, **(c,g)** new infections from farm to slaughter and **(d,h)** number of infected pigs reverting to excretion from farm to slaughter. Outcomes are averaged over 501 repetitions for 337500 scenarios that cross 9 dose-response functions with 5 excretion rates, 5 re-excretion rates and 1500 management contexts. Column **(I)**: total sensitivity indices for all factors, split into main effect (dark grey) and interactions (light grey). Column **(II)**: total sensitivity indices for the management factors alone, averaged over all scenarios sharing the same management constraints; so the associated errorbars represent the variability arising from the uncertainty over the epidemiological parameters. Factors are described in Table 1.

agement factors on outcomes of interest: prevalence at slaughter (p_s), average cutaneous contamination at slaughter (c_s), number of new infections from farm to slaughter (N_I) and number of pigs reverting from carriers to active shedders from farm to slaughter (N_R). The first two outcomes are important to evaluate the risk of carcass contamination in further steps of the pork processing chain. The last two reflect *Salmonella* circulation. Therefore, they are the effective targets for prevention and control strategies aiming at reducing transmission at this stage of the production chain.

Our model reflects management conditions consistent with the pork production in Brittany, while it considers a great degree of uncertainty over the epidemiological parameters. We evaluated how management and epidemiological factors affect the outcomes at a single batch level, thus focusing on within-batch transmission. Interactions with other batches are represented by the initial environmental contaminations at the farm, in the truck and in the lairage pens. Explicit contamination between batches, together with the analysis of the impact of batch management and cleaning protocols will be addressed in future communications.

The stochastic nature of transmission implies that, under the same scenario (*i.e.* same management and epidemiological parameters), the model outcomes may vary among repetitions. However, we found that these variations are significantly smaller than the variations observed when changing the scenario. This means that the intra-scenario variability, due to the stochastic nature of transmission, is significantly smaller than the inter-scenario variability, due to the variability in management protocols and the uncertainty in epidemiological parameters. For that reason, we carried out an inter-scenario sensitivity analysis on the simulation outcomes averaged over the intra-scenario repetitions ($N_{\text{reps}} = 501$). As pigs are not individualised in the model, analysing the variability at the individual level is beyond the scope of this study.

We found that transmission during transport and lairage significantly increases the prevalence and the average cutaneous contamination of the processed batches with 50% of the scenarios resulting in at least 4 newly-infected pigs and

5% of the scenarios resulting in more than 50 newly-infected pigs (estimates for batches of 100 pigs). It produces new infections and activate non-shedding carriers. This suggests that prevention and control strategies at this stage may prove worthwhile to decrease internal carriage and cutaneous contamination from farm to slaughter.

Such strategies should tackle several management conditions rather than focus on a single aspect of *Salmonella* spread, because different factors are important for each outcome. Prevalence at slaughter is most affected by the prevalence at farm, which strongly depends on the herd management and biosecurity (Lurette et al., 2011). Average cutaneous contamination is most affected by the environmental contamination at lairage. Depending on the slaughtering process, pigs may be showered, which should reduce the cutaneous contamination. Cleaning the lairage pens is also recommended, even if it could prove difficult in certain slaughterhouses, where lairage pens are rarely emptied. New infections are most affected by the total time from farm to slaughter. Reducing the transport and lairage time is recommended to reduce *Salmonella* circulation, but it should be balanced by its limited applicability and by the increased risk of carcass contamination associated with full stomachs.

Our model showed that stress, represented by the re-excretion of non-shedding carriers, has a moderate impact on its own, but it modulates the relative impact of other factors. Indeed, the effect of initial conditions is reduced as stress increases.

Finally, our model suggests that the effectiveness of different control strategies depends on the transmissibility of the *Salmonella* strain: for highly transmissible strains, reducing the prevalence at the farm of origin is expected to have the greatest impact on limiting the number of new infections; whereas for less transmissible strains, control measures at lairage should be more effective. In practice, the transmissibility of *Salmonella* strains in farms is seldom assessed and it would be costly to carry it out comprehensively. Moreover, pigs at lairage originate from various farms, so different strains are likely to be present during transport and lairage. Hence, our findings reinforce the idea that interventions

should occur at each step of the production chain, to robustly reduce *Salmonella*
355 contamination whatever the strains that are circulating.

Our results are in line with the conclusions of the “Quantitative Microbio-
logical Risk Assessment on *Salmonella* in slaughter and breeder pigs” module
of the EFSA report (VLA, DTU, RIVM, 2010) and the subsequent publication
(Simons et al., 2015). Their approach differs from ours: they developed a model
360 considering multiple batches and explicit between-batch transmission through
the accumulation of environmental contamination; they also performed a risk
analysis where intra-scenario and inter-scenario variabilities are evaluated to-
gether. Meanwhile, we focused on an inter-scenario sensitivity analysis, which
allowed us to single out the effects of specific management conditions and to
365 determine their relative impact under different regimes of *Salmonella* transmis-
sibility.

The main discrepancies between the results from both approaches are twofold.
Firstly, our model shows that each outcome is mainly influenced by a single man-
agement factor: prevalence at slaughter mainly depends on the prevalence at
370 farm, cutaneous contamination on the contamination of lairage pens and new
infections on the total duration of transport and lairage. In contrast, in the
EFSA report model, cutaneous contamination at slaughter is estimated from
lymph-node-positive prevalence, so they are both understandably driven by the
same factors. Secondly, we found a notably smaller relative impact of stress-
375 related parameters on the model outcomes, specifically on the number of new
infections from farm to slaughter.

Such discrepancies can be explained by differences in the structure, details
and scope of application of both models. Furthermore, they do not interfere with
the main message of both approaches: transmission and lairage may constitute
380 an effective source of infection for pigs from farm to slaughterhouse and the
contamination risk may be significantly reduced by control strategies targeting
management from farm to slaughter.

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