A spatio-temporal model to describe the spread of *Salmonella* within a laying flock

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**Abstract**

*Salmonella* is one of the major sources of toxii-infection in humans, most often because of consumption of poultry products. The main reason for this association is the presence in hen flocks of silent carriers, i.e. animals harboring *Salmonella* without expressing any visible symptoms. Many prophylactic means have been developed to reduce the prevalence of *Salmonella* carrier-state. While none allows a total reduction of the risk, synergy could result in a drastic reduction of it. Evaluating the risk by modeling would be very useful to estimate such gain in food safety. Here, we propose an individual-based model which describes the spatio-temporal spread of *Salmonella* within a laying flock and takes into account the host response to bacterial infection. The model includes the individual bacterial load and the animals’ ability to reduce it thanks to the immune response, i.e. maximum bacterial dose that the animals may resist without long term carriage and, when carriers, length of bacterial clearance. For model validation, we simulated the *Salmonella* spread under published experimental conditions. There was a good agreement between simulated and observed published data. This model will thus allow studying the effects, on the spatiotemporal distribution of the bacteria, of both mean and variability of different elements of host response.

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**Keywords:**

Individual-based model

Bacterial transmission

Individual heterogeneity

**1. Introduction**

*Salmonella* is one of the major causes of human toxii-infection and poultry products, eggs and egg products, are the most common source of human salmonellosis. *Salmonella enterica* serovar *enteritidis* is the strain most often associated with salmonellosis caused by poultry products (EFSA, 2009; Humphrey, 1990). *Salmonella enteritidis* can colonize the gastrointestinal tract of fowls as well as their systemic organs, such as spleen or liver, for long periods. This colonization does not cause clinical signs. This silent carrier-state will in turn lead to between animals transmission. Horizontal transmission within the flock may occur either directly from one infected animal to another fowl, especially in the same cage, through aerosols (Gast et al., 2002; Lever and Williams, 1996) or indirectly because of environmental contamination, mainly through water and feed, as observed in Nakamura et al. (1994). Vertical transmission though trans-ovarian route may also occur (Humphrey and Lanning, 1988). Moreover, once the animal is infected, there is an individual variation in the duration and level of bacteria excretion (Beaumont et al., 2003; Ishola, 2009).

Because of the importance for food safety of poultry contamination by *Salmonella*, prevention of animal infection is an important research area. Many experiments have been conducted to evaluate control methods to prevent animal colonization: vaccination (Barrow, 2007; Zhang-Barber et al., 1999), competitive exclusion (Rantala and Nurmi, 1973), acidification of food, selection for increased animal genetic resistance (Beaumont et al., 2009).

While none of these control measures results in a zero risk, their relative efficacy and possibility of synergy still remains to be estimated. This may be investigated through modeling of bacteria spread within a hen house including horizontal and vertical transmission and animals heterogeneity once infected (level and duration of excretion).

Models have already been proposed to study *Salmonella* spread within various animal species: hens (Leslie, 1996; Prévost et al., 2006; Thomas et al., 2009), pigs (Hill et al., 2008; Lurette et al., 2008) and dairy herds (Xiao et al., 2005, 2006, 2007; Lanzas et al., 2008). But no model considered, at least until now, both the bacterial transmission and heterogeneity of hens’ response to infection. Individual variability of the immune response may be introduced into stochastic individual-based models. Such model patterns are largely used in ecology (Grimm et al., 2006) and were already used to model the growth and migration of *Salmonella enteritidis* in hens’ eggs (Grijspeerdt et al., 2005).
The objective of this paper is to present a stochastic individual-based model for the spread of *Salmonella* within a hen house. It allows us to consider individual levels of bacterial infection as well as variability of the host response, i.e. maximal dose an animal may eliminate without long term infection as well as duration of bacterial clearance. The paper is organized as follows: in Section 2 we describe our individual-based model; validation and exploration of the model behavior are presented in Section 3; they are followed by a discussion and a conclusion in Sections 4 and 5, respectively.

2. Model

The model description hereafter follows the ODD (overview, design concepts, details) protocol for describing individual and agent-based models (Grimm et al., 2006).

2.1. Purpose

An individual-based model was used to represent the spatial spread of *Salmonella* within a flock of laying hens reared in cages. We assumed that all animals in a cage are infected at the same time (through contaminated food, water, rodents...) so that the individual unit of interest here is a cage. In this model, the risk of long term infection is dependant on both individual bacterial load and a stochastic threshold, corresponding to individuals’ capacities of resistance to infection. When the latter are overwhelmed, persistent infection occurs. Its length and duration of immune protection after recovery are stochastic.

### Table 1

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C(t_n, x)$</td>
<td>Density of bacterial environmental infection</td>
</tr>
<tr>
<td>$B(t_n, x)$</td>
<td>Level of bacterial load within an individual</td>
</tr>
<tr>
<td>$H(t_n, x)$</td>
<td>Health status of the individual</td>
</tr>
<tr>
<td>$D(x)$</td>
<td>Bacterial threshold within the individual</td>
</tr>
</tbody>
</table>

2.2. Entities, state variables, and scales

In this model, individuals are cages harboring hens (all of them harbor the same number of hens). Individuals are aligned in rows and each group of two rows are separated from each other by an interval allowing the farmer to take care of animals. As we are interested in the transmission via the environment, the flock is divided in grid cells, allowing us to describe the contamination in each location of the hen house. Each individual is then identified in the hen house by its position $x=(x_1, x_2)$ (i.e. $x_1$-abscissa and $x_2$-ordinate).

An individual is characterized by its bacterial load denoted by $B(t_n, x)$ and its health status denoted by $H(t_n, x)$ at time $t_n$ and position $x$. In our model, we assume that there exists an individual bacterial threshold within an individual denoted by $D(x)$ so that the individual bacterial load decreases over the time when the initial bacterial load is lower than $D(x)$, increases when the initial bacterial load is higher than $D(x)$ and remains constant when it is equal to $D(x)$ (see Fig. 2(a)). An individual infection beyond the threshold results in systemic infection (because of overwhelming of individuals capacities to reduce bacterial load).

At the same time $t_n$ and position $x$, the level of contamination in environment is represented by $C(t_n, x)$. Note that, for positions that do not contain individuals, variables $B(t_n, x)$, $H(t_n, x)$ and $D(x)$ are not defined while $C(t_n, x)$ is defined.

![Flowchart showing a time step iteration. For each time step, Rule 1 considers individual infection (ingestion or inhalation in environment) to update individual bacterial load (as described in Section 2.7.1), Rule 2 considers the bacterial load to update health status (as described in Section 2.7.2), and Rule 3 computes the number of excreted bacteria to update the contamination level in environment (as described in Section 2.7.3).](image-url)
The full set of variables for the individual-based model are described in Table 1. Time step for the model is one day.

2.3. Process overview and scheduling

The flowchart in Fig. 1 outlines the events occurring during each time step for one given replication. Details are given by submodels in Section 2.7.

2.4. Design concepts

Interaction: We consider interaction between one individual and those located in the neighborhood through inhalation or ingestion of bacteria excreted in the environment.

Stochasticity: Two components are stochastic: threshold for bacterial load and time interval before change to next health status.

Observation: The model outputs are the health status of each individual and the density of bacteria in environment at each time step for all positions. From these outputs, we compute the percentage of individuals over time in the house and over row for each health status which are determinant for practical issues and for comparison to experimental data.

2.5. Initialization

Initialization consists in defining the size of the house, the number of rows of individuals, the number of individuals by rows and following state variables for each location. Health status (H(tn, x)), bacterial load (B(tn, x)) and density of bacteria in the environment (C(tn, x)) are initialized by the user. The threshold D(x) is initialized stochastically for each replication and each individual and kept constant during the whole replication.

2.6. Input for time varying process

The model does not use input data to represent time-varying process.

2.7. Submodels

Further notations are needed to describe the submodels: the time interval (0, tmax) is partitioned into subintervals (tn, tn+1) with a time step ∆t = tn+1−tn = 1 day.

Let Ω ⊂ R^2 be the area covered by the hen house and denote by x = (x_1, x_2) ∈ Ω a point of hen house. Assume that x_1 ∈ (0, L_1) and x_2 ∈ (0, L_2) where L_1 and L_2 is the length and width of hen house, respectively. We use a uniform Cartesian grid consisting of grid points (x_1, x_2) to partition the x_1-component and x_2-component interval of x where x_1 = iΔx_1 and x_2 = jΔx_2, i = 0, 1, 2,..., N_1, j = 0, 1, 2,..., N_2 and we simplify the writing of the point (x_1, x_2) by x_{ij}.

From now, an individual will be identified by the coordinates of its center x_{ab} where a and b are chosen from {0, 1, 2,..., N_1} and {0, 1, 2,..., N_2}, respectively.

2.7.1. Model for individual bacterial load within each day

An individual must initially be exposed to a sufficient density of bacteria to become infected (Humphrey et al., 1991; Gast, 1993; Lever and Williams, 1996). At very low dose of infection, the bacterial load within an individual will decrease over time due to the ability of the organism to overcome this infection. At the opposite, it will multiply over time when this initial dose of infection exceeds a threshold until saturation value due to the limitation of resources. We denote by M, the carrying capacity of bacterial load within an individual (i.e. maximal number of bacteria that an individual may carry) and D(x_{ab}) (0 < D(x_{ab}) < M), the threshold of bacterial load for which an individual at position x_{ab} is able to overcome an infection and reduce the number of bacteria over time. We use the strong allee effect (Jiang and Shi, 2009; Wang and Kot, 2001) to model the growth of bacteria within an individual in the following way. Let B(t, x_{ab}), f(B(t, x_{ab})) and g(B(t, x_{ab})) be the level of bacterial load, the per capita growth rate and the growth rate of bacteria load within an individual at time t ∈ (t_n, t_{n+1}) and position x_{ab}, respectively. We assume that the functions f and g depend on bacterial load B(t, x_{ab}) so that f is negative when density of bacteria B(t, x_{ab}) is small. Consequently B(t, x_{ab}) decreases when B(t_n, x_{ab}) < D(x_{ab}), increases when the initial condition B(t_n, x_{ab}) > D(x_{ab}) and is constant when B(t_n, x_{ab}) = D(x_{ab}) (see Fig. 2(a)).

From Jiang and Shi (2009) and Wang and Kot (2001), g is written as function of f (see Fig. 2(b)) as

\[ g(B(t, x_{ab})) = B(t, x_{ab})f(B(t, x_{ab})). \]

**Fig. 2.** (a) Example of evolution of bacterial load, B(t, x) on one day, t ∈ (t_n, t_{n+1}). Curves with three different initial sizes of dose: B(t_n, x) = 4.5 log_{10}D (the bottom solid line), B(t_n, x) = 5 log_{10}D (the dotted line) and B(t_n, x) = 5.5 log_{10}D (the upper solid line). (b) Growth rate of bacterial load g(B). Here, individual threshold, D = D(x) is set at 5 log_{10} carrying capacity, M at 10 log_{10} and the density of bacteria that an individual acquires by ingestion or inhalation at zero.
where
\[ f(B(t_{n+1}, x_b)) = \theta \left( \frac{B(t_{n+1}, x_b)}{\Delta x_b} - 1 \right) \left( 1 - \frac{B(t_{n+1}, x_b)}{M} \right) \]
for all \( \tau \in (t_n, t_{n+1}) \), \( \theta \) is the net growth rate of bacteria within an individual.

We assume the dynamics of individual bacterial load satisfies the following differential equation:
\[ \frac{d(B(t_{n+1}, x_b))}{dt} = g(B(t_{n+1}, x_b)) - I_p(t_n, x_b), \quad \tau \in (t_n, t_{n+1}). \tag{1} \]
where initial condition of Eq. (1) is denoted by \( B(t_n, x_b) \). \( I_p(t_n, x_b) \) is interpreted as the density of bacteria that an individual (i.e., all hens forming one individual) acquires by ingestion or inhalation at time \( t_n \) and position \( x_b \). We model the latter in the form
\[ I_p(t_n, x_b) = k \sum_{y_i \in o(r)} C(t_n, y_i). \tag{2} \]
where \( o(r) \) is the area of contamination around the individual at position \( x_b \), \( k \) is the transmission probability of infection of an individual after ingestion or inhalation of bacteria in environment. We have denoted by \( r \) the radius of contamination around the individual of center \( x \) so that \( o(r) = \{ y \in \Omega \mid ||x - y||_2 < r \} \) and \( || \cdot ||_2 \) is the Euclidean norm.

The immune response is strongly dependent on many individual factors, e.g., genetics (see for example Barrow, 2007; Bumsstead and Barrow, 1988; Beaumont et al., 2003). Therefore, the individual threshold for bacterial load \( D(x_b) \), was assumed to vary from an individual to another resulting in a variability between individuals in the dynamics of Eq. (1) for the same initial condition \( B(t_n, x_b) \). Thus we assume that the thresholds \( D(x) \) are triggered according to a random variable \( Y_0 \) defined by \( Y_0 = Z \times 10^{10 \log_{10} Z} \) where \( Z \) is beta distributed, \( B(p, q) \) (\( p, q > 0 \)), with mean \( p/(p+q) \). \( Y_0 \) takes on values in interval \( (0, 10^{10 \log_{10} Z}) \). For an individual at position \( x_b \) the threshold \( D(x_b) \) was drawn from distribution of \( Y_0 \) at the beginning of each replication and is kept constant during the replication.

2.7.2. Epidemiologic model

An individual located in position \( x_{a,b} \) and at time step \( \delta t = t_{n+1} - t_n \) is in one of the five disease states (see Fig. 3): \( S_0 \): susceptible individual without bacterial load. \( I_0 \): individual suffering from digestive infection at a dose lower than its threshold \( D(x_{a,b}) \) (i.e., with a transient infection), \( I_1 \): individual suffering from digestive infection at a dose higher than its threshold \( D(x_{a,b}) \), \( I_2 \): individual systemically infected after the long term digestive infection, \( R \): recovered individual. We denote by \( \mathcal{H}(t_{n+1}, x_{a,b}) \) the health state of an individual, \( \mathcal{H}(t_{n+1}, x_{a,b}) \in \{ S_0, I_0, I_1, I_2, R \} \).

Transitions from \( S_0 \)-state to \( I_0 \)-state and from \( I_0 \)-state to \( I_0 \)-state are only regulated by the density of bacteria within the individual. For each time-step \( \delta t \), for each individual in \( S_0 \)-state or \( I_0 \)-state, we solve Eq. (1) via Runge Kutta methods (Butcher, 2003) and we obtain a unique solution denoted by \( B(t_{n+1}, x_{a,b}) \).

We assume that for \( B(t_{n+1}, x_{a,b}) = 0 \), individual is in \( S_0 \)-state, when \( B(t_{n+1}, x_{a,b}) \in ]0, D(x_{a,b})] \), individual is in \( I_0 \)-state and can go back to the \( S_0 \)-state. When the bacterial load \( B(t_{n+1}, x_{a,b}) \) becomes higher than the threshold \( D(x_{a,b}) \), then the individual changes its status from \( I_0 \) to \( I_1 \) state. The occurrence of a bacterial load verifying, for an individual at position \( x_{a,b} \), \( B(t_{n+1}, x_{a,b}) > D(x_{a,b}) \) is denoted by \( B_{1x} \).

The transitions from \( I_0 \)-state to \( I_1 \)-state, from \( I_2 \)-state and from \( R \) to \( S_0 \)-state are stochastic. We denote by \( T(x_{a,b}/I_{0x}) \), \( T(x_{a,b}/I_{1x}) \) and \( T(x_{a,b}/R) \), the residence time of an individual in the \( I_0 \)-, \( I_1 \)- and \( R \)-state, respectively. We assume that at time \( t_0 \), an individual newly reaches the \( I_0 \)-state, \( T(x_{a,b}/I_{0x}) \) is triggered according to an exponential distribution with an average duration equal to \( 1/\lambda_1 \) and the individual will change its status from \( I_0 \) to \( I_1 \) at time \( t_0 - r \), where \( r \) equal to the integer part of \( T(x_{a,b}/I_{0x})/\delta t \). In the same way, successively \( T(x_{a,b}/I_{1x}) \) and \( T(x_{a,b}/R) \) are triggered according to an exponential distribution with an average duration equal to \( 1/\lambda_2 \) and \( 1/\mu \). We denote by \( p_{I_0 \rightarrow I_1} \), \( p_{I_1 \rightarrow R} \) and \( p_{I_2 \rightarrow R} \), respectively, the transition probabilities for \( I_1 \) to become \( I_2 \), \( I_2 \) to become \( R \), \( R \) to become \( S_0 \) per unit of time. Then \( (p_{I_0 \rightarrow I_1}, p_{I_0 \rightarrow R}) = (1 - \exp(-1/\lambda_1), 1 - \exp(-1/\lambda_2), 1 - \exp(-1/\mu)) \).

2.7.3. Model for diffusion of bacteria in the hen house

Bacterial environmental contamination within an industrial hen house is modeled assuming that Salmonella is dispersed in the environment via a diffusion process through dust particles and contaminated aerosols (Gast et al., 2002; Nakamura et al., 1994; Lever and Williams, 1996). Let \( C_{ij} \) be the density of bacteria at time \( t_0 \) and position \( x_{ij} \). \( C_{ij} \) is a approximated solution of a continuous reaction diffusion equation describing the dispersion of bacteria in hen house (Appendix Eq. A.1). It was approximated by a forward finite difference scheme in time and centered finite difference scheme in space, which gives
\[ \frac{C_{ij}^{n+1} - C_{ij}^{n}}{\delta t} + \frac{2}{(\delta x_j)^2} \left( C_{ij}^{n+1} - 2C_{ij}^n + C_{ij}^{n-1} \right) + \lambda C_{ij}^{n+1} = \beta_{I_0} \eta(B_{1x}) + \beta_{I_2} \eta(B_{1x}), \tag{3} \]
with boundary conditions
\[ C_{0j}^{n+1} = C_{0j}^{n+1}, \quad C_{N_1 - 1, j}^{n+1} = C_{N_1, j}^{n+1}, \]
\[ C_{ij}^{n+1} = C_{ij}^{n+1}, \quad C_{j+1}^{n+1} = C_{j+1}^{n+1}, \]
and initial condition \( C_{ij}^0 \geq 0 \). Convergence of numerical scheme in \( \| \cdot \|_2 \) norm is obtained by the following condition \( \delta t \delta x^2/(\delta x_j)^2 < 1/4 \) and \( \delta t \delta x^2/(\delta x_j)^2 < 1/4 \) (Lucquin and Pironneau, 1996, p. 281).

2.8. Calibration

Data for carrying capacity \( M \): We have no data to estimate the value of \( M \). Noticing that in experimental infections, almost all individuals are infected with an inoculum dose ranging between 3log_{10} and 9.5log_{10} colony-forming units (cfu) and that a saturation of bacteria within an individual seems to be observed.
Data for recovery rate, \( \eta \) and rate of transition from \( I_{S} \) to \( I_{R} \), \( \gamma \): Humphrey et al. (1991, Table 1) showed that the duration of fecal excretion is correlated with the size of inoculum dose and can vary from 3.4 to 36.8 days for dose ranging from \( 10^3 \) to \( 10^9 \) while Gast et al. (2005) showed that the duration of fecal excretion can vary from 13.8 to 32.9 for an inoculum dose at \( 10^9 \) of different Salmonella strains. Since individuals excrete at digestive or systemic state, the average duration of the digestive period, \( 1/\gamma \), and of the systemic period, \( 1/\eta(B_{is}) \) is necessarily a function of the inoculum size and of the Salmonella strains. Setting \( 1/\gamma \) at 2 days as in Prévost et al. (2006, 2008), then we generate some residence time in \( I_{S} \), varying in about 95% of the simulation from 0.05 to 3 days. Assuming that \( 1/\gamma(B_{is}) \) depends on the initial value of bacterial load in \( I_{S} \)--status, mean \( 1/\eta(B_{is}) \) was calibrated as described in Table 3. This allowed to generate with exponential distribution residence time in \( I_{S} \) varying in 95% of the simulations from 0.6 to 47 days for doses ranging from \( 10^2 \) to \( 10^9 \).

Data for parameters \( p \) and \( q \) of beta distribution to trigger the individual threshold \( D(x_{ab}) \): We set \( p=35 \) and \( q=45 \) so that 5% of individuals have a bacterial threshold lower than 3.5 log_{10} and higher than 5.8 log_{10}.

Data for net growth \( \theta \): The net growth rate is the key parameter influencing simultaneously the fast-growing (or slow-growing) and the fast-decreasing (or slow-decreasing) of the bacterial load over time when the initial bacterial load is higher and lower, respectively, than the threshold. Many factors such as the bacterial strain, host factors might affect this parameter. In the literature, no experimental data were available for laying hens. We assumed that \( \theta \) is equal to 0.0007 h^{-1}.

Data for mortality rate of bacteria \( \lambda \): We assume that the mortality rate of bacteria in the environment is \( \lambda = 0.1 \) day^{-1} as (Prévost et al., 2006, 2008).

Rate of bacterial excretion for \( I_{S} \) and \( I_{R} \) states: As in Prévost et al. (2006, 2008), we assumed that individuals excreted low levels of bacteria and that an individual in \( I_{S} \), excreted less than an individual in \( I_{R} \) state. We set \( \beta_{i0} = 4 \log_{10} \text{cfu Day}^{-1} \), and \( \beta_{i1} = 4.5 \log_{10} \text{cfu Day}^{-1} \).

Data for transmission probability of infection of an individual after inhalation or ingestion of bacteria, \( k \), and radius of contamination around the individual, \( r \). From Eq. (1), variation in parameters \( k \) or \( r \) will result in a similar trend in the the density of bacteria that an individual (i.e. all hens forming one individual) acquires by ingestion or inhalation (\( I_p \)). To calibrate these data, we used the results for the inhalation dose of bacteria calculated by Lever and Williams (1996) in rearing conditions similar to that of flock of laying hens. These results showed that each individual would inhale every 24 h a bacterial load ranging between 0 and 2 log_{10}. We assumed that \( r=2 \text{ m} \) (maximal distance for aerosol transmission) and \( k \) was set at 0.08 so that \( I_p \) remains in the interval \( (0, 2 \log_{10}) \).

Data for diffusion coefficient \( Z \): We assumed that \( Z \) is necessarily a function of the inoculum size and of the Salmonella strains. Setting \( Z \) at a value of \( 10 \) log_{10} for doses ranging from \( 10^3 \) to \( 10^9 \), Gast et al. (2004) showed that the duration of fecal excretion can vary from 13.8 to 32.9 for an inoculum dose at \( 10^9 \) of different Salmonella strains. Since individuals excrete at digestive or systemic state, the average duration of the digestive period, \( 1/\gamma \), and of the systemic period, \( 1/\eta(B_{is}) \) is necessarily a function of the inoculum size and of the Salmonella strains. Setting \( 1/\gamma \) at 2 days as in Prévost et al. (2006, 2008), then we generate some residence time in \( I_{S} \), varying in about 95% of the simulation from 0.05 to 3 days. Assuming that \( 1/\gamma(B_{is}) \) depends on the initial value of bacterial load in \( I_{S} \)--status, mean \( 1/\eta(B_{is}) \) was calibrated as described in Table 3. This allowed to generate with exponential distribution residence time in \( I_{S} \) varying in 95% of the simulations from 0.6 to 47 days for doses ranging from \( 10^2 \) to \( 10^9 \).

### Table 2

Baseline values for the parameters.

<table>
<thead>
<tr>
<th>Description</th>
<th>Dimension</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p, q )</td>
<td>Parameters of beta distribution</td>
<td>Dimensionless</td>
</tr>
<tr>
<td>( M )</td>
<td>Carrying capacity of bacteria within an individual</td>
<td>( \text{cfu} )</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>Rate of transition from ( I_{S} ) to ( I_{R} )</td>
<td>Day^{-1}</td>
</tr>
<tr>
<td>( \eta(B_{is}) )</td>
<td>Recovery rate</td>
<td>Day^{-1}</td>
</tr>
<tr>
<td>( \mu )</td>
<td>Rate of return of individual from ( R ) to ( S_0 ) status</td>
<td>Day^{-1}</td>
</tr>
<tr>
<td>( \beta_{i0} )</td>
<td>Excretion rate of individual at ( I_{S} ) status</td>
<td>Day^{-1}</td>
</tr>
<tr>
<td>( \beta_{i1} )</td>
<td>Excretion rate of individual at ( I_{R} ) status</td>
<td>Day^{-1}</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>Natural death rate of Salmonella in environment</td>
<td>Day^{-1}</td>
</tr>
<tr>
<td>( \sigma^2 )</td>
<td>Diffusion coefficient of Salmonella in environment</td>
<td>m^2 × Day^{-1}</td>
</tr>
<tr>
<td>( k )</td>
<td>Transmission probability of infection</td>
<td>Dimensionless</td>
</tr>
<tr>
<td>( r )</td>
<td>Radius of contamination around the individual</td>
<td>m</td>
</tr>
<tr>
<td>( \theta )</td>
<td>Net growth rate of bacteria within an individual</td>
<td>h^{-1}</td>
</tr>
</tbody>
</table>

0.0007

### Table 3

Calibration of the average duration of the systemic period, \( 1/\eta(B_{is}) \) as a function of \( B_{is} \) (the first value of \( B_{is} \) satisfying \( B_{is} > D(x_{ab}) \)).

<table>
<thead>
<tr>
<th>( B_{is} ) (cfu)</th>
<th>(&lt; 10^4)</th>
<th>([10^4, 5 \times 10^4])</th>
<th>([5 \times 10^4, 10^5])</th>
<th>([10^5, 5 \times 10^6])</th>
<th>([5 \times 10^6, 10^9])</th>
<th>( &gt; 10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 1/\eta(B_{is}) ) in day</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>10</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>( 1/\eta(B_{is}) + 1/\gamma ) in day</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>15</td>
<td>18</td>
</tr>
</tbody>
</table>

### 3. Simulation experiments

#### 3.1. Material and method

Three types of scenarios are considered, the first two for model validation and the third for model exploration. The width, \( L_2 \) and length, \( L_1 \) of hen house were always initialized to 15 and 30 m, respectively, while the number of individuals varies following the scenarios and are summarized in Table 4. To quantify the level of agreement between predicted and simulated data, the Standard error of simulated and observed data was evaluated for the scenarios 1 and 2.

#### 3.1.1. Scenario 1: validation of kinetics of infection

Experiments in which all individuals were initially inoculated with the same dose of bacteria were considered. In that case,
reinfection between individuals was reduced as much as possible (by rearing with individual food and water). That was the case of the first experiment described by Gast (1993) where 40 individuals were considered (Table 4(a) and (b)) and an another experiment described by Gast et al. (1997) where 36 individuals were considered (Table 4(c)). In both cases, all of them were inoculated at Day 0. Three bacterial doses, $10^4$, $10^6$ and $7.5 \times 10^5$ cfu were studied (one per replicate) and infections were regularly investigated on feces samples. As individuals excrete Salmonella in feces in $I_S$, and $I_L$-state, the observed prevalence were compared to the simulated percentages $I_{L+} + I_S$. The percentages of $I_S$ were also considered to investigate the repartition between the two status. A total of 300 simulations were achieved with the same dose and the same number of individuals.

Since transmission via the environment was very low if any and can be neglected, the value of the parameter $k$ in Eq. (2) was set equal to zero.

### 3.1.2. Scenario 2: validation of the Salmonella spread from individuals to individuals via the environment

We selected for model validation two experiments where experimentally infected individuals were reared with healthy ones. Gast (1993) studied a total of 72 individuals where he inoculated one-third of them (i.e. every 3rd individual) with a dose of bacteria at $10^6$ cfu. Nakamura et al. (1994) infected eight individuals out of 16 (i.e. every 2nd individual) with a dose of bacteria at $10^5$ cfu. The latter precisely described the distribution of individuals considering one pair of adjacent rows (i.e. eight individuals in one row and last four individuals in the second row at the beginning of experiment. At the opposite, the former gave no information on the spatial distribution of individuals but only the route of cross contamination was known (uninoculated individuals shared drinkers and feeders with inoculated ones). We therefore assumed that the individuals were housed in one pair of rows as in Nakamura et al. (1994) (Table 4(d)). Since in both experiments, Salmonella were searched in feces and our model assumes that excretion occurs in the $I_S$ or $I_{L+} + I_L$ state, results must be compared to the sum of $I_{L+}$ and $I_L$ individuals. However, since the rate of excretion is five-fold higher in the systemic state, comparison with number of hens in the systemic state was also considered. As in scenario 1, observed and simulated percentages of $I_{L+} + I_L$ and $I_S$ were compared. For simulations, a total of 300 simulations were achieved.

### 3.1.3. Standard error of simulated and observed data

In scenarios 1 and 2, we evaluate the root mean squared error (RMSE) known as the standard error of simulated and observed data:

$$
RMSE = \sqrt{\frac{\sum_{t=1}^{N}(obs(t) - sim(t))^2}{N}},
$$

where $N$ is the number of observed data, $obs(t)$ and $sim(t)$ are the observed and simulated data at time $t$, respectively. RMSE has the same units that the simulated and observed data which are in percentage.

### 3.1.4. Scenario 3: influence of the position of the first infection and the distance between pair of rows in the hen house

To investigate the influence of the position of the first infection, initialization of hen house was achieved as described in the Table 4(f). Two cases were considered according to this infection occurred either in one corner (i.e. pair of row 5) or on the middle (i.e. pair of row 3) of the hen house. In both cases, only one individual was infected on Day 0 with a high dose ($10^6$ cfu).

The effect of the spatial distribution of individuals in hen house was also considered by comparing results with data from scenario in Table 4(f) or (g). In the former case, the distance between pair of row is 2 m versus 1 in the latter.

A total of 300 simulations were achieved and the simulated percentages of $I_S$ or $I_{L+} + I_L$ was represented.

### 3.2. Results

#### 3.2.1. Scenario 1: validation of kinetics of infection

Results obtained when simulating data and those observed after experimental inoculation are shown on Fig. 4a–c for doses of $10^4$, $10^6$, and $7.5 \times 10^5$ cfu, respectively. Simulated percentage of $I_{L+} + I_L$ and observed results are very close for the three doses (Table 5(a)–(c) for $I_{L+} + I_L$ and lay between the 5th and 95th percentiles. Simulated percentages of $I_S$ are also very close to observation made at the first two doses (Table 5(a) and (b) for $I_S$). For the highest dose, however, the observation obtained one week post inoculation is close but higher than the 95th percentile (Table 5(c) for $I_S$).

#### 3.2.2. Scenario 2: validation of the Salmonella spread from individuals to individuals via the environment

Simulated percentages of $I_S$ or $I_{L+} + I_L$ and observed prevalence from experiments are shown in Fig. 5a for the experiment of Gast (1993) and Fig. 5b for the experiment of Nakamura et al. (1994).

In the former the greatest disagreement between any of the data sets and simulated percentage of infected individuals was found with regards to $I_{L+} + I_L$ (Table 5(d)). This is especially true for data obtained one week post inoculation (p.i.) while later on both sets of results were very close.

In the latter case, observed results lay between the 5th and 95th percentiles of simulated percentage of $I_{L+} + I_L$ except for the observation obtained three days post inoculation which is close to the 95th percentile. When simulated percentage of $I_L$ is considered, differences are larger. Observed data at 3 and 6 days p.i. were much higher than simulated percentage of $I_L$; the former ranged from 68.7 to 62.5% while median percentage of $I_L$ varied from 19 to 40%. Comparing results on Table 5(e), $I_{L+} + I_L$ fits observed data better than $I_S$.

#### 3.2.3. Scenario 3: influence of the position of the first infection and of the distance between pair of rows in the hen house

Influence of the position of the first infection is illustrated in Figs. 6 and 7, respectively, for a first occurrence in the middle and in the corner of the hen house, respectively. The position of the first infection influences both the kinetics of infection and the maximal percentages of infected individuals. As late as 210 days post inoculation, the first pair of rows is still not colonized for an infection starting in the opposite corner (Fig. 7a) while when the infection starts in the middle, all five pair of rows are infected about 210 days post inoculation (Fig. 6a). The infection starting in the middle of the hen house results in a higher maximal of percentage of $I_S + I_{L+}$ than an infection starting at a corner: 25% versus 13% (Figs. 6b and 7b, 70 days post inoculation).

Influence of the distance between pair of rows in the hen house (1 m versus 2 m) can be seen by comparing the Figs. 8 and 7. When the pair of rows are close, the colonization is rapid with a lower maximal of percentage of $I_S + I_{L+}$: for example all eight pair of rows are infected about 110 days post inoculation (Fig. 8a) with maximal percentage of $I_S + I_{L+}$ of 17.5% at 100
days post inoculation (see Fig. 8b). At the opposite, the colonization is slow with a close maximal percentage of $I_+I_+$ but a lower minimal percentage.

### 4. Discussion

This model extends the previous model derived by Prévost et al. (2006) where individuals infected at the digestive level were

![Fig. 4.](image-url)  

**Fig. 4.** Evolution over time of the percentages of $I_+I_+$, $I_+$ and $I_+I_+$ when all individuals were inoculated with the same bacterial dose: (a) $10^4$ cfu; (b) $10^6$ cfu as in Gast (1993); and (c) $7.5 \times 10^7$ cfu as in Gast et al. (1997). Observed data (corresponding to percentage of individuals shedding Salmonella in feces) are shown by crosses. Median value of simulations are shown by solid lines. The 5th and 95th percentiles observed on the 300 replicates are shown by dotted curves.

![Fig. 5.](image-url)  

**Fig. 5.** Evolution over time of the percentages of $I_+I_+$, $I_+$ and $I_+I_+$ after rearing infected individuals in the vicinity of healthy ones: (a) i.e. inoculating $(10^6$ cfu) one single individual out of three as in Gast (1993) and (b) letting eight infected $(10^5$ cfu) as in Nakamura et al. (1994). In both cases, individuals are in two adjacent rows and inoculated individuals share water with not uninoculated ones. Observed data (corresponding to percentage of individuals shedding Salmonella in feces) are shown by crosses. Median value of simulations are shown by solid lines. The 5th and 95th percentiles observed on the 300 replicates are shown by dotted curves.

<table>
<thead>
<tr>
<th>Scenario 1</th>
<th>Scenario 2</th>
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<tbody>
<tr>
<td>(a)</td>
<td>(b)</td>
</tr>
<tr>
<td>Standard error for $I_+$</td>
<td>0.19</td>
</tr>
<tr>
<td>Standard error for $I_+I_+$</td>
<td>0.19</td>
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### Table 5
Standard error in percentage using $I_+$ versus $I_+I_+$ as simulated data.
undergo a longer term infection.

D higher that threshold. At the opposite, when the individual bacterial dose is threshold will be determinant in the propagation of practice, the balance between bacterial doses and individuals Beaumont et al., 2009). The threshold was thus chosen as random. In and Nurmi, 1973), individuals' genetic resistance (see for example Bumstead and Barrow, 1988), gut flora (Rantala depends on many factors such as the bacterial strain (as can be seen the individual may clear without persistent and systemic infection, as long as the bacterial load remains lower that the D threshold.

The threshold, \( D(x_a,b) \), which is the maximal bacterial load that the individual may clear without persistent and systemic infection, depends on many factors such as the bacterial strain (as can be seen from Bumstead and Barrow, 1988), gut flora (Rantala and Nurmi, 1973), individuals' genetic resistance (see for example Beaumont et al., 2009). The threshold was thus chosen as random. In practice, the balance between bacterial doses and individuals threshold will be determinant in the propagation of Salmonella, since in the field bacterial doses are most often rather small. Introducing this threshold thus allows investigating the effects of both average values and variability of these factors.

In the model immune response was considered only through its impact on the bacterial load, without refining the description of biological processes which are far too complex (see for example Host, 2000) to be modeled at the same time as the whole flock is considered. Moreover, parametrization of the immunity response would have been hardly feasible because of large number of biological steps that may be considered. This would have lead to the introduction of a higher uncertainty on the process that would decrease the confidence on the model results. Using the strong allel effect, only three parameters were needed: net growth rate of bacterial within the individual, \( \theta \), the individual threshold of bacterial load corresponding to the balance between bacterial multiplication within the host and host response leading to bacterial clearance by immune response, \( D(x_a,b) \) and the carrying capacity \( M \). All three of them have a biological meaning which facilitated their estimation and allowed to base it on experimental data: the threshold \( D(x_a,b) \) and carrying capacity were chosen from numerous results of experimental infections were individuals are infected with inoculum doses ranging between \( 3 \log_{10} \) and \( 9.5 \log_{10} \) colony-forming units (cfu) (Humphrey et al., 1991; Gast, 1993; Lever and Williams, 1996; Gast et al., 2004). Large differences in prevalence even in the first days after inoculation were observed when the dose were higher or lower than \( 5 \log_{10} \) leading us to choose this value as the average value for the threshold.

Variations in durations of bacterial clearance were also considered, taking advantage of experimental data obtained in
Humphrey et al. (1991) and Gast et al. (2005). Indeed, this duration was shown by Prévost et al. (2008) to be partly under a genetic control. Preliminary investigations showed that this duration had a large impact on kinetics of colonization, especially at longer intervals post inoculation.

This model allows reproducing experimental results. Indeed, the simulated percentages and observed data are very coherent when experimental inoculations are considered. They are also consistent with observations resulting from infection between individuals via environment, although simulations overestimated the outcome compared to the second experiment by Gast (1993) where the spatial distribution of individuals in the hen house was unknown. As the spatial distribution of individuals such as the distance between pair of rows influences both the kinetics of infection and the maximal percentages of infected individuals (see scenario 3), it might explain the disagreement between experimental and simulated data. Moreover, no experimental data were available for the first six days post-inoculation for model validation as in Nakamura et al. (1994).

Distinguishing individuals $I_x$ and $I_x^+$ allows to understand what happens during the first days post inoculation. At the opposite, the sum $I_x^++I_x$ gives the real prevalence of infection since fecal samples may found be negative even in the case of cecal infection. The differences between both sets of data disappear at longer post inoculation intervals. Then only systematically infected individuals may be observed so that both simulations and observations refer to the same category of individuals.

Moreover, this model makes it possible to study the spatial diffusion of the bacteria and disease, while until now, to our knowledge at least, no data or model were available to study spatial diffusion of Salmonella or any other pathogenic agents in a hens’ flock.

Most studies showed that flock size has an effect on the prevalence of Salmonella within a laying flock (EFSA, 2009; Huneau-Salaun et al., 2009; Carrique-Mas et al., 2009) but the influence of the position of the first infection or the distance between pair of rows in hen house may have an important effect in the prevalence (see scenario 3). Our results show that these assumptions are exact and that the speed of colonization may depend on the position of the first infection.

However, the model considers all hens in a cage as an epidemiological unit. Indeed, most often all of them will be infected at the same time (by contaminated feed, water, rodent…) and/or cross infected through aerosols contamination via excreted bacteria of the environment (among which food and drinkers).

This model will make it possible to investigate new strategies of reduction of Salmonella prevalence. That will be the case for the spatial effect of introducing more resistant individuals. Prévost et al. (2008) showed that introducing a proportion of more resistant fowls, assuming a homogenous mixing of the two subpopulations of resistant and of susceptible individuals resulted in a reduction of the overall proportion of infected fowls. With our model, we will be able to study possible effects of the relative spatial positions of susceptible and resistant individuals on the spatial spread of the Salmonella in hen house. Considering individual variations in bacterial threshold and duration of clearance will also make it possible to study possible effects of differences in excretion rates which may have a major impact on environmental contamination.

5. Conclusion

In this article, we formulated an individual-based model to describe the spread of Salmonella within a laying flock. This is the first stochastic model describing Salmonella spatio-temporal spread within a poultry flock. It is able to reproduce experimental data; the conceptual understanding of environmental mediated Salmonella spread appears complete. It will thus allow studying the interest of various prophylactic means against this disease as well as the effect of changes or variability of various factors. The model could also be adapted to study the propagation of other pathogens within laying hens.

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Appendix A. Continuous formulation of sub-model 2.7.3

Let $C(t,x)$ be the density of bacteria in the environment at time $t$ and position $x=(x_1,x_2)\in\Omega$, $t \geq 0$. $C(t,x)$ depends on the initial
where $D$ at $\text{ID}^{-}$, $\text{contamination}$, $C(x,t)+2$, and $\text{mortality}$, $\ldots$, as well as on excretion rate, $\beta_{\text{ID}}^{-}$, and, $\beta_{\text{ID}}^{+}$, by individuals at $\text{ID}^{-}$, and $\text{ID}^{+}$-state, respectively. The density dynamics satisfies the following equation:

$$\frac{\partial C(t,x)}{\partial t} = \alpha \frac{\partial}{\partial x} C(t,x) + \beta_{\text{ID}}^{-}(x) + \beta_{\text{ID}}^{+}(x),$$  \hspace{1cm} (A.1)

where $\alpha = \frac{\partial^{2}}{\partial x^{2}} + \frac{\partial^{2}}{\partial x^{2}}$, with no flux boundary condition $\partial C(t,x)/\partial x = 0$ on $\partial \Omega$, $x$ is the outward normal. $\beta_{\text{ID}}^{-}(x) = 0$ if at position $x$ there is no individual at $\text{ID}^{-}$-state; $\beta_{\text{ID}}^{+}(x) = 0$ if at position $x$ there is no individual at $\text{ID}^{+}$-state. We assume that $\beta_{\text{ID}}^{-}$ and $\beta_{\text{ID}}^{+}$ are constant. Therefore, their values are uniformly distributed in the area covered by the individual during excretion.

References


