**Vet. Res. (2008) 39:20**
DOI: 10.1051/vetres:2007058

www.vetres.org



**Original article**

# Effect of genetic resistance of the hen to *Salmonella* carrier-state on incidence of bacterial contamination: synergy with vaccination

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(Received 23 February 2007; accepted 18 September 2007)

**Abstract –** *Salmonella* is one of the major sources of toxi-infections in humans. The association between egg consumption and *Salmonella* outbreaks is a serious economic and public health problem. To control the incidence of *Salmonella* in poultry flocks, many prophylactic means have been developed but none allows a total reduction of the risk. In a previous study, we derived mathematical models for *Salmonella* transmission and used them to appreciate the most important factors of variation of egg contamination rate and thus of risk of human contamination. Thanks to recent data of a selection experiment for increased or decreased rate of carrier-state (also called divergent selection), we showed that mixing, in an equal proportion, animals issued from a line selected for a lower (denoted Sal-) or higher propensity to carry *Salmonella* (denoted Sal+) results in a reduction by half of the maximal percentage of contaminated animals but does not accelerate the extinction of the disease. Vaccination and selection should be synergic, since a former contamination reduces the maximal prevalence by 45 and 71%, respectively, in Sal+ or Sal- flocks respectively. These results show the interest in the introduction, even at a rather moderate percentage, of animals selected for a reduced rate of *Salmonella* carrier-state within commercial flocks. This could be achieved by using one or more selected lines in commercial crosses. These results must be confirmed experimentally while the mathematical model could be extended with minor modifications to other animal species or pathogenic species.

***Salmonella* / mathematical model / genetic / resistance / vaccination**

## 1. INTRODUCTION

While food safety is an increasing request of consumers, *Salmonella* remains one of the major sources of toxi-infections in humans, most often as a result of egg consumption[1] [15]. In order to control the incidence of *Salmonella* in poultry flocks, many prophylactic means have been developed: vaccination [16], competitive exclusion [12, 13], acidification of feed, or genetic resistance to infection [3] or carrier-state [2]. But none of them are able to assure zero risk of animal contamination and human transmission, at least when used alone. In a previous study [7, 8], we derived mathematical models for *Salmonella* transmission and used them to identify the most important factors of variation of egg contamination rate and thus of risk of human contamination. More precisely, we built a mathematical model to

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[1] Food and Agriculture Organization of the United Nations (FAO), Risk assessments of *Salmonella* in eggs broiler chickens, [on line] (2002) http://www.fao.org/docrep/005/y4392e/y4392e00.htm [consulted 18 September 2007].

assess *Salmonella* dissemination within the flock. This model takes the main features of hen contamination into account, distinguishing between three steps: digestive contamination, systemic infection (when systemic organs such as the liver or spleen are contaminated after translocation of the bacterium through the digestive barrier), and bacterial clearance leading to recovery. The risk of egg contamination is also considered. Numerical analysis of the model highlighted the influence of the recovery rate (which represents the ability of hens to eliminate *Salmonella*) on both the maximal prevalence and the duration of the epizooty. This rate is partially under genetic control. Such a result could be expected from the large difference in kinetics of bacterial clearance between poultry lines [9]. The L2 egg-type line, in particular, was found to be susceptible to the *Salmonella* carrier-state, whether in the digestive tractus, the systemic organs or the ovaries. The role of genetics was shown by Beaumont et al. [2] who estimated heritability (i.e., the proportion of variability due to genes) of the percentage of hens having cleared *Salmonella* four weeks after an experimental contamination by the oral route at more than 0.35. Recent data of a selection experiment for increased or decreased rate of *Salmonella* carrier-state (also called divergent selection) undertaken within the L2 line confirmed this hypothesis[2]. However, our previous model is based, upon others, on the simplifying assumption that all hens possess the same genetic characteristic. The main objective of this work was thus to take into account the hens' genetic heterogeneity and to investigate the impact of genetic selection on the prevalence of the epizooty as well as its possible synergy with vaccination.

We therefore used observations achieved on poultry lines differing in genetic abilities to clear *Salmonella* to fit model parameters. These values allowed us to investigate the effect of selection and predict what might be expected from various strategies of prophylaxis. This point is of particular importance since the European Commission has recently implemented a new regulation which aims to reduce a *Salmonella* prevalence in poultry flocks to less than 2% while the average value for European countries is about 30%, with large variations from values close to zero to more than 80% in others[3].

## 2. MATERIALS AND METHODS

### 2.1. Mathematical model

The model described in Prévost et al. [7] was implemented with two subpopulations that have different genetic values. We denote by $N$ the total number of hens (thereafter assumed to be constant in time) and $N_1$ and $N_2$ the number of hens in the two subpopulations such that $N = N_1 + N_2$. In the scope of this publication, these two subpopulations will correspond to different lines with a higher or lower rate of *Salmonella* carrier-state. Let $p \in [0, 1]$ be the proportion of animals in the second subpopulation $N_2$, so that we have $N_1 = (1 - p)N$ and $N_2 = pN$. Moreover, for each subpopulation of hens $i (i = 1, 2)$ and at time $t$, let $S_i(t)$ be the number of susceptible hens, $I_i^D(t)$ the number of hens suffering from digestive contamination (i.e., $D$-infectious), $I_i^S(t)$ the number of hens suffering from systemic contamination (i.e., $S$–infectious) and, $R_i(t)$ the number of recovered hens (i.e., having been able to eliminate all bacteria). Thus, we have

$$S_1(t) + I_1^D(t) + I_1^S(t) + R_1(t) = N_1, \forall t \geqslant 0$$

and

$$S_2(t) + I_2^D(t) + I_2^S(t) + R_2(t) = N_2, \forall t \geqslant 0.$$

Here the total number of hens is constant and equal to $\overline{N}$, so we have $S(t)+E(t)+I(t)+R(t) = \overline{N}, \forall t \geqslant 0$. Let $C(t)$ be the bacterial environmental contamination (i.e., bacterial load within the hen house) at time $t$. We assume that the transmission rate (the rate at which susceptible hens become exposed) is proportional to bacterium load (i.e., number of bacteria in the environment). This transmission is represented by the term $-\kappa_i C(t) S_i(t), \forall i = 1, 2$. The $D$-infectious status is assumed to be a transient status, so that infected hens will first be $D$−infectious,

[2] Beaumont C., Marly J., Protais J., Protais M., Chapuis H., Trotereau J., Sellier N., Velge P., Salvat G., Selection for increased resistance to *Salmonella* carrier-state, 4th European poultry genetics symposium, Dubrovnik, Croatia, 2005/10/6-8, CDROM, 5 p.

[3] http://www.efsa.europa.eu/en/science/monitoring_zoonoses/reports/1541.html [consulted 18 September 2007].

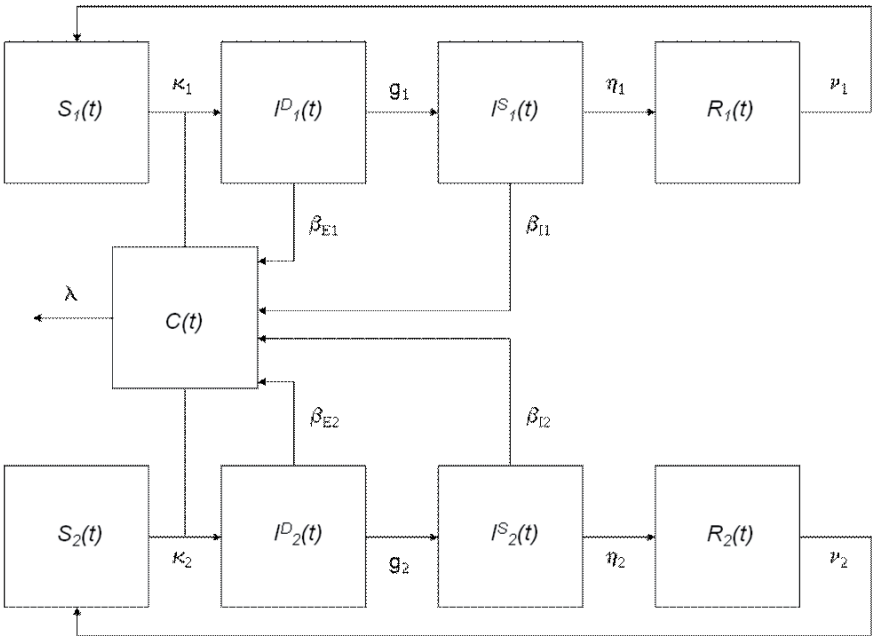


**Figure 1.** Flow diagram of the epidemic population of hens divided into two subpopulations, where $\kappa_i$ is the exposition rate which modulates the transmission of the infection, $g_i$ the rate of translocation of the digestive barrier, $\eta_i$ the recovery rate which depends on innate or acquired immunity, the rate of return to the susceptible state which is characteristic of the immune protection, $\lambda$ the rate of natural mortality of *Salmonella* in the hen house, and $\beta_{Ei}$ (respectively $\beta_{Ii}$) the rates of excretion of bacteria by hens suffering from digestive and systemic contamination respectively.

and after some time, become $S-$ infectious hens. We also suppose that $D$-infectious and $S$–infectious animals shed bacteria in the environment (by an excretion process). This flux of excreted bacteria for each class is hypothesized to be equal to $\beta_{Ei}I_i^D(t) + \beta_{Ii}I_i^S(t), \forall i = 1,2$. After recovery, formerly infected $R$ animals may be infected again once they return back to the susceptible state at a rate dependant on the $\nu$ parameter. The transfer between stages is summarized in Figure 1 and the different steps and the bacterial load are coupled into the following system:

$$\begin{cases} dS_i(t)/dt = -\kappa_i S_i(t)C(t) + \nu_i R_i(t), \\ dI_i^D(t)/dt = \kappa_i S_i(t)C(t) - g_i I_i^D(t), \\ dI_i^S(t)/dt = g_i I_i^D(t) - \eta_i I_i^S(t), \\ dR_i(t)/dt = \eta_i I_i^S(t) - \nu_i R_i(t), \\ dC(t)/dt = \left[\sum_{i=1}^{2} \beta_{Ei} I_i^D(t) + \beta_{Ii} I_i^S(t)\right] - \lambda C(t), \\ \forall i = 1,2. \end{cases}$$

Since the different parameters of the model are described in [7], here we will only give a brief description of them. Within each subpopulation ($i = 1, 2$):

- $\kappa_i$ : is the exposition rate which modulates the transmission of the infection,
- $g_i$ : the rate of translocation of the digestive barrier,
- $\eta_i$ : the recovery rate, which depends on innate and acquired immunity leading to bacterial clearance,
- $\nu_i$ : the rate of return of recovered hens to the susceptible $S$ status,
- $\lambda$ : the rate of natural mortality of *Salmonella* in the hen house,
- $\beta_{Ei}$ : the rate of bacterial excretion by hens suffering from digestive contamination,
- $\beta_{Ii}$ : the rate of bacterial excretion by hens suffering from systemic contamination.

As further detailed in the Appendix, extinction and endemicity depend on the reproductive number $R_{0,p}$ (which can be computed for the original ordinary

differential equation system presented) given by

$$R_{0,p} = (1 - p)\, R_{0,1} + pR_{0,2}, \qquad (1)$$

with

$$R_{0,i} = \frac{N\kappa_i}{\lambda}\left(\frac{\beta_{Ei}}{g_i} + \frac{\beta_{Ii}}{\eta_i}\right), \forall i = 1, 2.$$

$R_{0,p}$ measures the number of secondary *Salmonella* infections generated by (*D*- or *S*-) infected hens in a population divided into two subpopulations with respective proportions of (1-$p$) and $p$. So, when $R_{0,p} > 1$, epizooty will persist, while when $R_{0,p} \leqslant 1$, it will go to extinction. As can be seen in the formula, $R_{0,p}$ is dependant on the heterogeneity of the population. It is a weighed mean of $R_{0,1}$ and $R_{0,2}$, weighed by the relative proportion of hens in each subpopulation.

When $R_{0,p} \leqslant 1$, the only nontrivial steady state is given by $S_i = N_i$, $I_i^D = 0$, $I_i^S = 0$, $R_i = 0$, and $C = 0$, for $i = 1, 2$. In this case, all hens return back to the susceptible status and the bacterial load goes to extinction. When $R_{0,p} > 1$, we have in addition a positive endemic equilibrium (cf. Appendix) meaning that the epizooty is endemic.

Extinction of the epizooty can also be obtained in another case. When assuming that subpopulation 2 consists of hens with a higher bacterial clearance such as $R_{0,2} \leqslant 1$ and $R_{0,1} > 1$, the optimal proportion of $p*$ is given by

$$p^* = \frac{R_{0,1} - 1}{R_{0,1} - R_{0,2}}.$$

Consequently for all $p \geqslant p^*$, the disease goes to extinction.

### 2.2. Estimation of parameters and data used

Model parameters for the base population were fitted using data on the percentage of contaminated animals at various intervals, from 0 to 6 weeks post inoculation) of an egg-type poultry line called L2 [9]. Sixteen hens were inoculated at the peak of lay, per os in the crop with $10^9$ cfu/mL of *Salmonella enteritidis* PT4 (SE) and contamination of the spleen, liver, caeca, oviduct, ovaries, and eggs assessed 1, 2, 4, and 6 weeks post inoculation [9]. The effect of selection was investigated using the results of an experiment for divergent selection achieved in the L2 line that we just modelized. Within this base population, two lines were selected, one for decreased and the other for increased contamination rate four weeks after experimental inoculation. In each line and at each generation, chickens were produced in two hatches issued from the same parents. Since the *Salmonella* carrier-state may only be assessed on necropsied animals, half of the animals were kept to produce the breeders of the next generation while the others were orally contaminated as described in Protais et al. [9] and bacteria were searched in their caeca, spleen, liver, and ovaries four weeks later. Selection was on a trait coded "1" if the caeca, spleen, or liver was found positive and "0" in the other cases, i.e., the presence of the bacteria in at least one of these three organs versus the absence of bacteria in any of them. Breeders of the next generation were chosen, within each line, in the second hatch, among the sibs of the animals using estimated breeding values predicted by BLUP determined with the Pest software [4]. Since divergent selection was achieved, animals with a higher genetic risk of *Salmonella* contamination four weeks after inoculation were selected in the line with a higher rate of *Salmonella* carrier-state (Sal+), and vice versa in the Sal- line, where animals with a better clearance ability were kept. This was achieved for four generations. In the fourth generation, four weeks post inoculation, the percentages of *S*-infectious hens were 40.7% in the Sal- line and 60.25% in the Sal+ line. Parameters for each subpopulation (Sal- and Sal+) were fitted using these data and the model described in Section 2.1. with a null proportion of animals in the second class (i.e., $p = 1$).

### 2.3. Long term selection

Since these populations have only been selected for four generations, longer term response was extrapolated, noting Sal++ and Sal- as the lines resulting from long term selection.

Extrapolation was based on the average time spent from the *S*-infectious to the recovered stage. Mathematically, these times are given by $1/\eta$ (resp. $1/\nu$). Moreover, we assumed that increasing the number of generations of selection allowed doubling the response to selection, that is, for the Sal- line, the reduction (resp. the augmentation) in average time spent in *S*-infectious (resp. recovered) stage. If we denote by $\eta_0$, $\eta_4$, $\eta_{long}$ (respectively $\nu_0$, $\nu_4$, $\nu_{long}$), the different parameters in the base line L2 or in the line selected for a lower rate of contamination at the fourth generation of selection (denoted as the Sal- line) or at a longer term (denoted by Sal-), we obtain the estimation of $\eta_{long}$ and $\nu_{long}$ by using

the following formula:

$$\left(\frac{1}{\eta_4} - \frac{1}{\eta_{long}}\right) = \frac{1}{2}\left(\frac{1}{\eta_0} - \frac{1}{\eta_4}\right)$$

and

$$\left(\frac{1}{\nu_{long}} - \frac{1}{\nu_4}\right) = 2\left(\frac{1}{\nu_4} - \frac{1}{\nu_0}\right).$$

### 2.4. Heterogeneous population

To investigate the effect of heterogeneity, the global population $N$ was assumed to be divided into two subpopulations: one composed of animals with a lower rate of *Salmonella* carrier-state (Sal-) and one composed of susceptible animals (Sal+). We tested the effect of heterogeneity using different proportions of Sal- animals (i.e., $p = 0.25$, 0.5, and 0.75). It was compared to a homogenous intermediary population whose parameter values (noted $\eta_{av}$ and $\nu_{av)}$ were equal to the means of those observed in both Sal- and Sal+ lines. For example, we computed the parameters $\eta_{av}$ and $\nu_{av}$ with the formula:

$$\eta_{av} = \frac{2}{\left(\frac{1}{\eta_{Sal+}} + \frac{1}{\eta_{Sal-}}\right)}$$

$$\text{and} \quad \nu_{av} = \frac{2}{\left(\frac{1}{\nu_{Sal+}} + \frac{1}{\nu_{Sal-}}\right)} \qquad (3)$$

### 2.5. Vaccination

Vaccination can be used in addition to genetic selection. Protais et al. [10] investigated the effect of vaccination on two poultry lines (which differed in susceptibility of *Salmonella*) and found that the vaccine efficiency was related to the susceptibility. These authors and general literature on vaccination (see for example the review by Barrow [1]) showed that the vaccination, as other factors is linked to the characteristic of the immune protection. To have a first estimation of the effect that could be expected from vaccination, we used what Barrow [1] calls a gold standard, which is infection with a wild-type strain. We thus assumed that vaccination would result in the same immunity as the one observed in formerly contaminated hens that are in the recovered state. However, we hypothesized that vaccination would be efficient in only 95% of the population. Computations were therefore achieved setting 95% of hens in the recovered state and the other 5% in the susceptible one. We tested the effect of vaccine in the Sal+, Sal-, heterogeneous ($p = 0.50$) and homogeneous average populations using the same parameter values as before.

**Table I.** Values of the model parameters corresponding to the L2 and lines selected for decreased (Sal-) and increased rate of *Salmonella* carrier-state (Sal+) at the fourth generation of selection.

| Parameters | $\kappa_i$ | $g_i$ | $\eta_i$ | | $\lambda$ | $\beta_{Ei}$ | $\beta_{Ii}$ |
|---|---|---|---|---|---|---|---|
| L2 | $10^{-4}$ | 0.5 | 0.022 | 0.005 | 0.1 | 0.03 | 0.1 |
| (Sal-) | $10^{-4}$ | 0.5 | 0.048 | 0.002 | 0.1 | 0.03 | 0.1 |
| (Sal+) | $10^{-4}$ | 0.5 | 0.022 | 0.02 | 0.1 | 0.03 | 0.1 |

## 3. RESULTS

### 3.1. Estimations for the base and selected populations and long-term selection

Estimated parameters for the base population L2 and the Sal- and Sal+ lines are summarized in Table I, and the corresponding evolution with time post inoculation of infection rate in Figure 2. The value of the recovery rate ($\eta$) varied between Sal+ and Sal- lines. It was equal to 0.022 in the Sal+ line (i.e., the same value as in the L2 line, which is the base population). It was larger ($\eta = 0.048$) in the Sal- line. The rate of return to the susceptible state was equal to 0.02 in the Sal+ line and to 0.002 in the Sal- line. We can notice that mixing both populations may lead to extinction of the epizooty provided that the proportion of Sal- hens is greater or equal to 52% of the total population (using the formula of the optimal proportion $p*$ defined in Section 2.1.).

### 3.2. Effect of genetic heterogeneity

We tested the effect of heterogeneity on the rate of infection, comparing a heterogeneous population (that is a mixture in equal proportions of Sal- and Sal+) to a homogeneous population with parameters defined by formula (3). Heterogeneity had an effect on the maximal percentage of $S$-infectious hens. As can be seen in Figure 3, while it was equal to 82% in the homogenous intermediary population, it was only 45% in a heterogeneous population with $p = 0.50$ and thus the same average parameters, and 22% when 75% of the animals were issued from the Sal- line. Figure 3 also shows that heterogeneity plays a

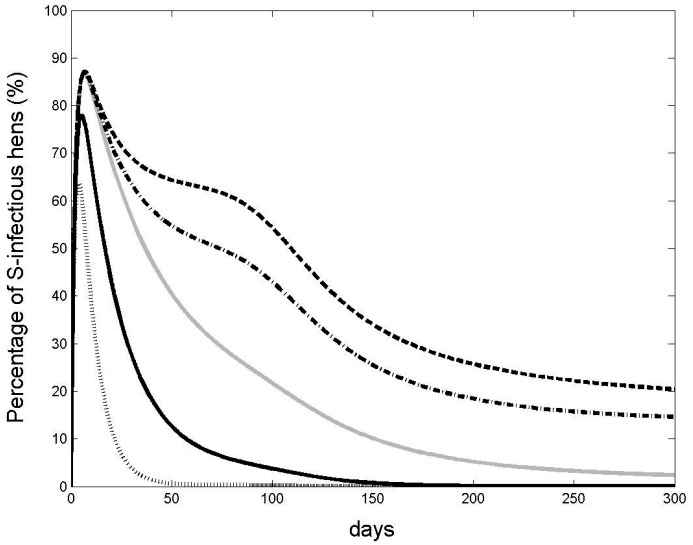


**Figure 2.** Comparison between base population L2 (grey line), lines selected for increased (black solid line) and decreased (dash-dot black line) rate of *Salmonella* carrier-state at the fourth generation of selection (Sal+ and Sal-) and extrapolated longer term selection denoted Sal++ (dashed black line) and Sal- (dotted black line).

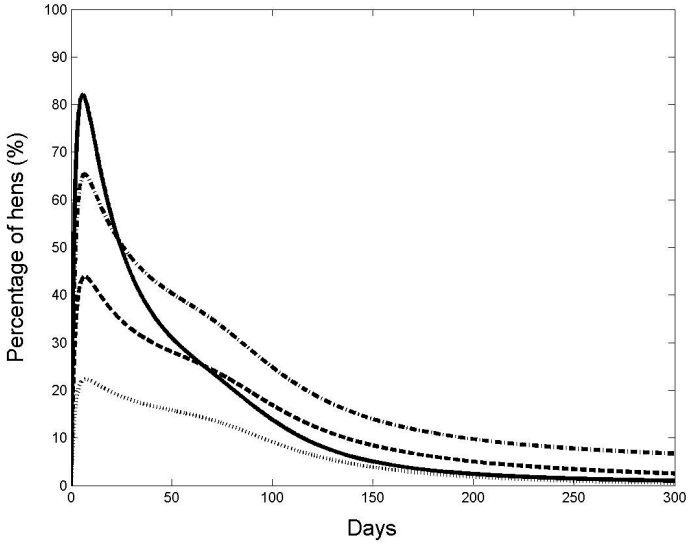


**Figure 3.** Evolution of the percentage of *S*-infectious hens in heterogeneous populations; dash-dot line: 25%, dashed line: 50%, dotted line: 75% hens from a line selected for a decreased rate of Salmonella carrier-state (Sal-), the complementary percentage of susceptible hens from the Sal+ line and, black solid line: an homogeneous intermediary population corresponding to a population whose parameters are equal to the means of those from the Sal- and Sal+ lines.

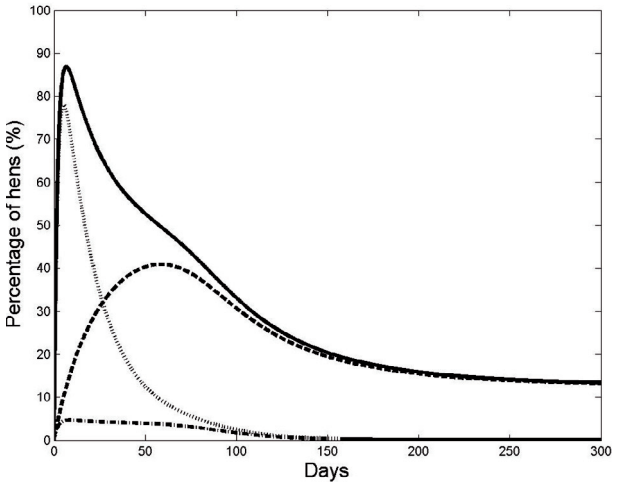


**Figure 4.** Comparison of evolution, with time after the first contamination, of the percentage of infected hens in unvaccinated (respectively vaccinated) hens issued from the line selected for decreased rate of *Salmonella* carrier-state (Sal-, dotted and dash-dot line, respectively) and increased rate of *Salmonella* carrier-state (Sal+, solid and dashed line respectively).

role on the extinction of the epizooty. Heterogeneity is not favorable in this case, with 5% (resp. 2%) $S$-infectious hens 300 days after inoculation whereas, in the average population, there are less than 3% infectious hens 250 days post inoculation. This simulation shows that, for a given value of model parameters, a heterogeneous population allows a reduction of the peak of infection but delays the extinction of epizooty.

### 3.3. Vaccination

First, we made a first estimate of the effect of a former contamination on the Sal- and Sal+ populations. On Figure 4, we can see that vaccination has a much more important effect in the Sal- line with a peak of infection of 4% in comparison to 40% in the Sal+. But the vaccine does not seem to play any significant role on the extinction of the epizooty: in the Sal+ line, about 12% $S$-infectious hens may still be observed 300 days post infection. In the Sal- line, extinction happens at a similar interval post contamination, whether the population is vaccinated or not.

When testing the interaction between homogeneity and vaccination, we can see (Fig. 5) that the vaccine reduces the maximal peak of infection in both cases (23% at 50 days). But it does not accelerate extinction. In fact, we see that the vaccine gives similar results on the evolution of infection rate in both homogeneous and heterogeneous populations.

## 4. DISCUSSION

The kinetics of the within flock propagation of *Salmonella* is partly dependant on the genetic ability of the population to clear the bacteria. Indeed, Protais et al. [9] observed large differences when comparing poultry lines inoculated at the same time and with the same dose of inoculation. The model derived by Prévost et al. [7] may be used for such data: since each line was reared separately, it was possible to process one subpopulation at a time. However, that is not the most general case, especially since genetics is partly involved in the control of the hens' rate of infection [2]. The model developed by Prévost et al. [7] was therefore extended to fit the heterogeneity of the degree of resistance within a population. Theoretical results showed that, as it was the case in a homogenous population [7], the existence of an equilibrium is dependant on the reproductive number $R_{0,p}$, that is the number of secondary *Salmonella*

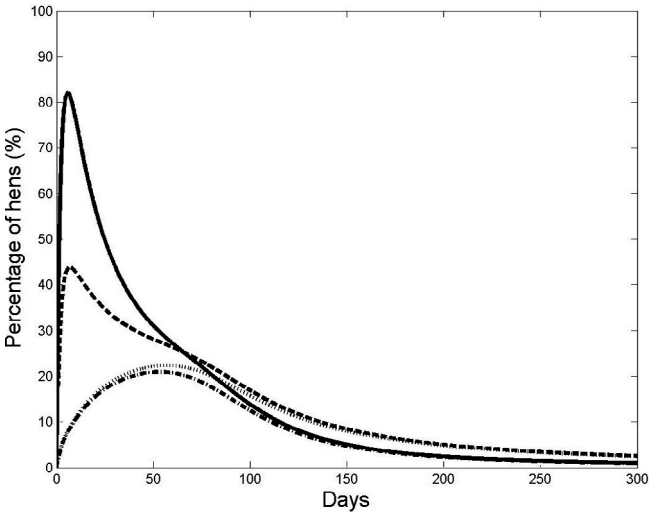


**Figure 5.** Comparison of evolution, with time after the first contamination, of the percentage of infected hens between a heterogeneous population composed with 50% hens issued from the line selected for decreased rate of *Salmonella* carrier-state (Sal-) and 50% hens issued from the line selected for an increased rate (Sal+), vaccinated (dotted line) or not (dashed line), and an intermediary homogeneous population vaccinated (dash-dot line) or not (solid line) whose parameters are equal to the means of those from the Sal- and Sal+ lines.

infections generated by an infected animal. It is equal to the weighed mean of the reproductive numbers in each subpopulations $R_{0,1}$ and $R_{0,2}$, with weighings equal to their relative proportion, (1-$p$) and $p$. Its value is thus the weighed mean of the contribution of the bacteria to the poultry contamination (represented by $N\kappa_i/\lambda$) and of the contribution of $D$–infectious and $S$–infectious hens to the bacterial contamination in the environment. This result might be of great help to choose the optimal proportion of more resistant animals. However, for any practical implications, care must be taken of the characteristics of such an equilibrium which is dependant on all parameters.

This model was first used to fit data measured in a selection experiment on two lines differing in their level of the rate of *Salmonella* carrier-state but reared together. Similar values were obtained for all parameters except for recovery and recontamination rates. This result first confirms the major importance of both parameters in the evolution with time of infection, as already shown by Prévost et al. [7] for homogenous populations. Consistently, we showed that genetic selection for decreased rate of *Salmonella* carrier-state four weeks after an oral inoculation also modified both parameters. Indeed selection was on the rate of *Salmonella* carrier-state four weeks after inoculation, when most animals are either in the $S$-infectious or recovered states. It was aimed at modifying the ability of the animals to clear the bacteria and to accelerate the transition between the $S$-infectious and Recovered states. The $D$-infectious stage should also be accelerated as a prerequisite to the $S$-infectious state and recovery. Moreover, the criterion for selection took into account both systemic and digestive contamination. The extent of the modification of parameters depended on the direction of selection: the difference between the Sal+ line (that was selected for increased contamination) and the base line was smaller than the difference between the latter and the Sal- line. Such asymmetrical responses to selection are rather often observed [6]. They could be expected in this case since the base population was very susceptible [9]. In the

Sal+ line, selection resulted in a 4-fold increase of the rate of return to the susceptible state. This difference is related to the longer persistency of the epizooty as can be seen in Figure 2 and thus higher contamination levels and percentage four weeks after inoculation, when selection measures are made. On the contrary, selection for decreased rate of *Salmonella* carrier-state seems to have modified the recovery rate to a higher extent than the rate of return to the susceptible state: hens issued from the Sal- line eliminate bacteria faster and are less susceptible to recontamination than those from the base population. Globally, the selection has more modified the clearance ability of the Sal- line and the immune protection of the Sal+ hens. Indeed, comparisons of lines differing in their ability to clear *Salmonella* show differences in both types of traits. Sadeyen et al. [14] compared adult hens issued from poultry lines with different rates of *Salmonella* carrier-state; they observed differences in expression levels of several genes involved in primary immune responses while Proux et al. [11] showed the existence of differences in immune humoral response (appreciated by antibody response) between two lines (among which the L2 line). Protais et al. [10] obtained similar results on different inbred lines among which those studied by Sadeyen et al. [14]; moreover these authors showed that these differences were associated with different vaccine efficiencies, which is in favor of a lower rate or return to the susceptible state in the lines showing higher antibody response. Our parameter estimates make it possible to compare kinetics of *Salmonella* propagation in the two lines: differences were more marked for the percentage of contamination at the peak than for the duration of contamination.

Moreover, our results show that *Salmonella* dissemination will be partly dependant on population homogeneity. Indeed large differences in kinetics of *Salmonella* propagation were observed between heterogeneous and homogenous populations, even if the average values of the parameters were the same. These discrepancies show the relevance of our model. The presence, even in a rather low proportion, of animals of higher clearance ability (for example issued from the Sal- line) reduces, to an important extent, the peak of infection. The latter decreases from 64% to 22% when the percentage increases from 25% to 75%, since animals from the Sal- line will remain for a shorter duration in the infected state, thus excreting less bacteria and recovering more quickly. However, in that case, time to extinction will be longer since the more susceptible animals will be responsible for persistence, at a low percentage, of contamination. For example 150 days after inoculation, when $p = 0.75$, 7% of the animals are still contaminated but 90% of them are issued from the Sal+ line. At the whole, for a given average value, the maximal percentage of contaminated animals will be lower with a heterogeneous population. Such flocks might escape detection, even when systematic detection is achieved, as it is the case in European countries. However, in the whole, heterogeneous populations should reduce the risk of human contamination. This could be rather easily achieved by introducing resistant lines in the four-way crosses currently used in poultry selection schemes. Even if more data are needed to compute the optimal proportion of animals with a higher bacterial clearance, these results show that it could be possible to reduce and slow down bacterial dissemination within a population by incorporating the appropriate proportion of animals with a reduced rate of the *Salmonella* carrier-state. This point is of importance to choose the strategy of genetic improvement: it will not be the same to increase this genetic ability to a greater extent in only a proportion of the population or to increase it to a lesser extent in all animals.

Conversely, the results of genotype comparisons will differ when animals with a different degree of bacterial clearance are either reared together or not. In the former case, the difference between lines could be underestimated. For example, our simulations show (Fig. 3) that, four weeks post infection, the percentage of infected animals is 33% in the Sal+ versus 9% in the Sal- line when both lines are reared together, while they are equal to 60% and 30% respectively in the other case. We

therefore expect to observe larger differences when rearing the Sal+ and Sal- lines separately instead of together as was done until now. This point should be further studied in practice.

Average values of duration of each step and not of parameters were considered because the focus was on the kinetics. However, similar results were obtained when comparing populations with the same average parameter values.

We investigated the effect of vaccination in interaction with the genetic ability to clear bacteria. A former infection had a very large effect: the maximal percentage of infection decreased from 70 to 40% in the susceptible Sal+. It was more marked in the more resistant Sal- line where it went from 80% to less than 5%. This large interaction with genotypes is linked to differences in the $\nu$ parameter, which corresponds to the persistence of protective immunity. The differences found in our simulated data are coherent with observations made by Protais et al. [10], when comparing the effect of vaccination in inbred lines with different rates of the *Salmonella* carrier-state, with the exception of the large interval between the beginning and the peak of infection in the vaccinated Sal- hens. Using the same model of contamination, these authors observed, in two replicates, that the contamination rates of vaccinated animals was about 45% higher in the line with a lower bacterial clearance. It is noteworthy that combining vaccination and genetic selection results in very low percentage of contamination similar to what the European Community is asking for.

Once vaccination is used, genetic heterogeneity has a very small effect on *Salmonella* propagation. Since all but 5% animals are supposed to be immunized against *Salmonella*, there is no more use of animals with higher clearance ability to reduce bacterial dissemination. The differences in duration of contamination that were observed in the absence of vaccination also disappeared since there were no more reservoirs of bacteria.

All these results were obtained with reference to *Salmonella,* taking advantage of the literature available on this subject. But this model could also be relevant to investigate propagation of other bacteria in the same animal species or in others, provided that the mode of contamination is similar to what is described here. This should be the case for other gastro-intestinal bacteria, among them *Campylobacter* whose incidence in chicken meat seems to be high and which could be responsible for some dramatic syndromes in humans [5].

*Acknowledgements.* The authors thank all those who made this work possible. They are grateful to the two anonymous referees for their useful comments and suggestions. Kevin Prévost has a scholarship from the INRA departments of Animal Genetics and Animal Health and from the region Upper Normandy. This study benefited from a grant from the EADGENE Network of Excellence.

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## APPENDIX

The analysis of extinction or persistence of the epizooty was achieved using the same method as in Prévost et al. [8] so that only the computation of the equilibrium will be given here. In this part, we assume that all parameters of the model are strictly positive. The goal of this section is to investigate equilibrium and extinction of the epizooty in the case of heterogeneity of the population. We use the system of ordinary differential equations defined in Section 2.1. It is clear that $S_i = N_i$, $I_i^D = 0$, $I_i^S = 0$, $R_i = 0$, and $C = 0$, for $i = 1,2$, is a trivial equilibrium of this system. Moreover, since

$$S_1(t) + I_1^D(t) + I_1^S(t) + R_1(t) = N_1, \forall t \geqslant 0,$$

and

$$S_2(t) + I_2^D(t) + I_2^S(t) + R_2(t) = N_2, \forall t \geqslant 0,$$

we can reduce this system to the following

$$\begin{cases} dI_i^D(t)/dt = \kappa_i \left[N_i - \left(I_i^D(t) + I_i^S(t) + R_i(t)\right)\right] \\ C(t) - g_i I_i^D(t), \forall i = 1,2, \\ dI_i^S(t)/dt = g_i I_i^D(t) - \eta_i I_i^S(t), \forall i = 1,2, \\ dR_i(t)/dt = \eta_i I_i^S(t) - \nu_i R_i(t), \forall i = 1,2, \\ dC(t)/dt = \left[\sum_{i=1}^{2} \beta_{Ei} I_i^D(t) + \beta_{Ii} I_i^S(t)\right] - \lambda C(t). \end{cases} \tag{1}$$

To identify a positive equilibrium of system (1), we set the differentials to zero and solve

$$\begin{cases} 0 = \kappa_i \left[N_i - \left(I_i^D + I_i^S + R_i\right)\right] C - g_i I_i^D, \forall i = 1,2, \\ 0 = g_i I_i^D - \eta_i I_i^S, \forall i = 1,2, \\ 0 = \eta_i I_i^S - \nu_i R_i, \forall i = 1,2, \\ 0 = \left[\sum_{i=1}^{2} \beta_{Ei} I_i^D + \beta_{Ii} I_i^S\right] - \lambda C. \end{cases} \tag{2}$$

(2) is equivalent to

$$\begin{cases} 0 = \kappa_i \left[N_i - \left(I_i^D + I_i^S + R_i\right)\right] C - g_i I_i^D, \forall i = 1,2, \\ I_i^S = \frac{g_i}{\eta_i} I_i^D, \forall i = 1,2, \\ R_i = \frac{\eta_i}{\nu_i} I_i^S = \frac{g_i}{\nu_i} I_i^D, \forall i = 1,2, \\ 0 = \left[\sum_{i=1}^{2} \beta_{Ei} I_i^D + \beta_{Ii} I_i^S\right] - \lambda C. \end{cases}$$

Hence, we obtain

$$I_i^D = \frac{\kappa_i N_i C}{g_i \left(1 + \kappa_i C \left(\frac{1}{g_i} + \frac{1}{\eta_i} + \frac{1}{\nu_i}\right)\right)},$$

$$I_i^S = \frac{\kappa_i N_i C}{\eta_i \left(1 + \kappa_i C \left(\frac{1}{g_i} + \frac{1}{\eta_i} + \frac{1}{\nu_i}\right)\right)}, \forall i = 1,2.$$

Thus, we obtain

$$\lambda C = \sum_{i=1}^{2} \beta_{Ei} \frac{\kappa_i N_i C}{g_i \left(1 + \kappa_i C \left(\frac{1}{g_i} + \frac{1}{\eta_i} + \frac{1}{\nu_i}\right)\right)} + \beta_{Ii} \frac{\kappa_i N_i C}{\eta_i \left(1 + \kappa_i C \left(\frac{1}{g_i} + \frac{1}{\eta_i} + \frac{1}{\nu_i}\right)\right)}. \tag{3}$$

Here, since we only search positive equilibrium, we assume that $C > 0$ and may thus divide the formula (3) by $\lambda C$ and obtain:

$$1 = \sum_{i=1}^{2} \frac{\frac{N_i \kappa_i}{\lambda} \left(\frac{\beta_{Ei}}{g_i} + \frac{\beta_{Ii}}{\eta_i}\right)}{\left(1 + \kappa_i C \left(\frac{1}{g_i} + \frac{1}{\eta_i} + \frac{1}{\nu_i}\right)\right)}. \tag{4}$$

Let $p \in [0, 1]$ so that we have $N_1 = (1 - p)N$ and $N_2 = pN$. When denoting by

$$R_{0,i} = \frac{N\kappa_i}{\lambda}\left(\frac{\beta_{Ei}}{g_i} + \frac{\beta_{Ii}}{\eta_i}\right), \forall i = 1, 2$$

we can note that the right hand side of equation (4) is proportional to this $R_{0,p}$ parameter. Since all parameters all hypothesized to be positive, equation (4) involves that the numerator $R_{o,p}$ must be higher than 1 so that the existence of endemic equilibrium may only be assured if

$$R_{0,p} = (1 - p)\, R_{0,1} + pR_{0,2} \quad > \quad 1.$$

And the endemic equilibrium is given by

$$S_i = N_i\left(1 - \frac{\kappa_i C\left(\frac{1}{g_i} + \frac{1}{\eta_i} + \frac{1}{\nu_i}\right)}{\left(1 + \kappa_i C\left(\frac{1}{g_i} + \frac{1}{\eta_i} + \frac{1}{\nu_i}\right)\right)}\right),$$

$$I_i^D = \frac{\kappa_i N_i C}{g_i\left(1 + \kappa_i C\left(\frac{1}{g_i} + \frac{1}{\eta_i} + \frac{1}{\nu_i}\right)\right)},$$

$$I_i^S = \frac{\kappa_i N_i C}{\eta_i\left(1 + \kappa_i C\left(\frac{1}{g_i} + \frac{1}{\eta_i} + \frac{1}{\nu_i}\right)\right)},$$

$$R_i = \frac{\kappa_i N_i C}{\nu_i\left(1 + \kappa_i C\left(\frac{1}{g_i} + \frac{1}{\eta_i} + \frac{1}{\nu_i}\right)\right)},$$

where $C$ is the value of contamination at endemic equilibrium.