Use of Integrated Studies to Elucidate Potential Benefits from Genetic Resistance to *Salmonella* Carrier State in Fowl

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1. Introduction

Salmonella, and especially *Salmonella* enteritidis, are one of the major causes of human toxi-infections (Humphrey, 1990), even if the trend is decreasing (European Food Safety Agency [EFSA], 2011). Many prophylactic means have thus been investigated to control the incidence of *Salmonella* in poultry flocks, including vaccination (Zhang-Barber et al., 1999, Curtiss et al, 2010), competitive exclusion (Nurmi & Rantala, 1973), acidification of feed and genetic resistance. More precisely, animals' ability to clear bacteria was considered. Indeed, the absence of clinical symptoms (which is the most classical definition of resistance) does not preclude human contamination since some animals may remain contaminated for weeks or even months without showing any symptoms. Such silent carriers cannot be identified as dangerous and may therefore enter the food chain. At the opposite spectrum, animals' ability to clear bacteria will reduce the risk of transmission, first to other animals and second to humans. It will thereafter be denoted as resistance to carrier-state.

The feasibility of selection for an improved resistance to asymptomatic carriers has been demonstrated by a divergent selection experiment (§2). Thanks to development of genomics, many results were obtained in the past 10 years: several genome regions controlling different traits were identified and the role of other genes was suggested. It will dramatically increase the efficiency of selection. With traditional selection, it is necessary to perform experimental inoculations and to slaughter animals to register the level of contamination. With marker-assisted selection and furthermore genomic selection, it will be possible to directly choose the future breeders. In addition to improved selection efficiency, it will discard the need for artificial infections.

However, even if numerous data are currently available, strategy for application of these results are still questionable: genetic relations between resistance traits are complex and the choice of selection criteria deserves more investigations. Therefore, the whole process, leading from fowl contamination to transmission to other animals or humans was described in an integrated approach, combining genetics and other means. First results showed possible synergy between resistance and at least one another mode of prevention, vaccination. Other studies are required to compare different combinations of prophylactic means and identify new strategies for eradication of Salmonellosis, in coherence with new regulation implemented by the European Commission. They aim at reducing *Salmonella* prevalence in poultry flocks at less than 2% while a recent study estimated it at 29.7 and 23.7%, respectively, of laying and broiler flocks, respectively, with large differences between countries (EFSA, 2007a, 2007b)

The goal of this chapter is to make a review of data currently available on the possibility of reduction of fowl carrier-state through genetic selection. It will enhance the benefits that may be obtained from an integrated approach of genetics of resistance in hens to improve the control of the incidence of *Salmonella* within a flock.

2. Main results on genetics of resistance to *Salmonella*

First studies were achieved very early (Lambert & Knox, 1928; DeVolt et al., 1941) at a time when many animals died from acute salmonellosis. They thus focussed on the reduction of mortality. No experimental inoculation was needed to observe diseased animals and experiments could be achieved directly on the field. Studies focussed on serotypes pathogenic for birds (*Salmonella* Pullorum, *Salmonella* Gallinarum and *Salmonella* Typhimurium). Later on, the incidence of those diseases dramatically reduced with the reinforcement of preventive measures in poultry breeding. The same held for studies of resistance to *Salmonella*. But, in the end of the 1980's, many outbreaks of human toxi-infections due to *Salmonella* Enteritidis occurred, resulting in new developments in genetics of resistance to *Salmonella*. As in former studies, feasibility of such an approach was considered through selection experiment; genetic parameters were estimated to appreciate the response that could be expected. However, with the beginnings of molecular genetics, the research of genes controlling these traits was also addressed.

2.1 Development of protocols of measures and identification of genetic models

One main feature of studies of genetic resistance to *Salmonella* is the very large number of resistance-related traits that were considered (see Calenge et al., (2010) for a review). They differ in many factors. Inoculation by the oral route is more representative of what occurs in the field but less reproducible than the intravenous or intramuscular route. According to the *Salmonella* serotype, carrier-state (mostly observed after inoculation with *Salmonella* Enteritidis) or acute disease (mostly observed after inoculation with *Salmonella* Typhimurium) will be studied, but to an extent also depending, among others, on the number of inoculated bacteria. The animal's age at infection is a very important parameter. The interval between infection and observation will also be of importance, especially with reference to long-term carrier-state. Animals' conditions of rearing also influence the outcome of the infection. The susceptibility of egg to *Salmonella* multiplication may also be considered as described by Sellier et al. (2007).

Many studies involve both the development of a protocol of measure of resistance and the identification, with this protocol, of lines differing in resistance. Identifying such genotypes is of great importance: their existence strongly suggests the role of genetics in the control of resistance-related traits that are measured. Genetic models for resistance or susceptibility are needed for research of genome regions controlling those traits. The first study was achieved by Bumstead & Barrow (1988). They considered resistance to acute disease due to *Salmonella* Typhimurium and observed difference between partially inbred lines that are especially interesting for QTL research. Other studies addressed carrier-state and focussed on *Salmonella* Enteritidis. Guillot et al., (1995) observed differences in response to inoculation of chickens with a high bacterial dose. To mimic carrier-state in chicks, Duchet-Suchaux et al., (1995) developed a protocol of experimental inoculation where animals were orally infected with a low dose, showed no symptom but remained infected for several weeks. They could thus identify genetic models for further research, both outbred (Duchet-Suchaux et al., 1997) and partially inbred lines (Tilquin et al., 2005). It is to notice that the former study focussed on the same lines as in Bumstead & Barrow, (1988). Other protocols of inoculation were developed to study persistence for a shorter term (Lamont et al., 2002; Kramer et al., 2003; Hasenstein & Lamont, 2007).

Studies on resistance to carrier-state in adults were less numerous, in spite of importance of laying hens for the risk of human contamination. Protais et al., (1996) orally contaminated hens at the peak of lay and bacteria were searched in caeca, spleen, liver and ovary four weeks later. They observed differences between outbred poultry lines. Later on, Sadeyen et al., (2006) identified differences between inbred lines using the same protocol while Lindell et al., (1994) used a slightly different protocol.

2.2 Response to selection

The first selection experiment for resistance to salmonellosis after an experimental inoculation was undertaken in 1932; it proved to be efficient (Lambert, 1932). A higher resistance was observed for some breeds, among which White Leghorns (Robert and Card, quoted by Hutt & Scholes (1941)). But De Volt et al., (1941) showed that the former were less resistant than selected Rhode Island Red hens. This result and others contributed to the development of selection for a higher resistance (see Beaumont et al., 2003a, for a review).

These results are coherent with the estimations of heritability of resistance to death (0.15 and 0.12) obtained respectively by Beaumont et al., (1999) after intramuscular inoculation of day-old chicks and by Janss & Bolder (2000) after inoculation at two weeks of age. It is to notice that, according to genetic parameters estimated by the latter, more resistant animals would survive longer, contributing to a higher risk for consumers if they were still carriers. This emphasizes the importance of an increase in genetic resistance to carrier-state. An experiment of divergent selection on this trait was achieved by Beaumont et al., (2009; 2010). The base population was issued from a layer-type line. Two series of divergent lines were selected, for increased or decreased resistance at a younger age (as in Duchet-Suchaux et al., 1995) or at the peak of lay (using the protocol described by Protais et al., 1996). In adults, selection was on an all-or-none trait called global contamination, coded "1" if at least one organ (i.e. spleen, liver, caeca or ovary) was found positive and "0" in the other cases. Resistance of chicks was assessed by the logarithm of the number of colonies forming units (c.f.u.) per gram of caeca measured 5 weeks after inoculation (i.e. contamination level). A total of 3817 animals were

thus measured (1408 adults and 2409 chicks). Clear and significant differences were observed in lines selected on adult performance, with difference in prevalence of about 20%. Selection may be efficient in reducing the level of *Salmonella* carrier-state in hens. Differences between the "chicken" lines were, at least until now, smaller. This may be due to lower heritability but also smaller selection pressure and family sizes, due to experimental constraints and to variations in responses to infections. Indeed, in two hatches out of eight, only a small proportion of animals were still contaminated by *Salmonella* at slaughter; others could not be measured for level of contamination, which also slowed down selection.

Values of estimated genetic parameters were the main information from this experiment. Heritability of resistance was estimated at 0.16 in chicks while it varied from 0.14 to 0.23 with analysed organ in adult hens. It was higher in caeca (0.23) while heritability of ovarian contamination was estimated at a lower value (0.11). Heritability of adult global contamination was found at 0.18.

All genetic correlations between contamination rates of individual organs were positive, ranging from 0.46 to 0.67. Genetic correlations between adult global contamination and contamination in individual organs were very high (from 0.75 for liver to 0.85 for spleen and caeca), except for ovary (0.32). These results are related to the central role of intestine in carrier-state of gastro-intestinal bacteria: bacteria pass through it when inoculated or after recontamination; at the opposite, contamination of other organs is dependent on translocation of intestinal barrier. Contamination of ovaries is especially rare: in this experiment only 6% of them were found contaminated versus 49%, 21% and 62%, respectively, for spleens, livers and caeca, respectively (resulting in a percentage of contaminated adults equal to 76%). This low rate of ovarian infection further reduced the expected response to direct selection against ovarian contamination. An indirect selection on another criterion should be more efficient. This result and the positive values of genetic correlations between contamination rates of individual organs reinforce the interest of the overall adult contamination: it is more precisely assessed and combines several traits, all of which being positively correlated.

All genetic correlations between carrier-state in chicks and production traits (egg numbers, egg weights and body weights at various ages) were small and positive (ranging from 0 to 0.37) except for the number of eggs laid between 18 and 24 weeks of age, which was slightly but negatively correlated with *Salmonella* load (0.17). These results suggest that selection for increased resistance may be achieved without much detrimental effect on production traits. Unlike what was observed for resistance at a younger age, genetic correlation between adult carrier-state (global contamination) and egg numbers laid at the beginning of lay (between 18 and 24 weeks of age) was positive. At the opposite, those with laying intensity at older ages were negative or very close to 0 (ranging between -0.33 and 0.01). Correlation with body weight at 17 weeks of age was close to 0. These differences in genetic correlations between production traits and resistance at a younger or an older age are consistent with the negative genetic correlation observed between chicken and adult resistance.

The latter was estimated at a quite high and negative value (-0.50) and the probability of the true correlation being positive was estimated at only 5%. This major result holds whether overall contamination is considered or different organs distinguished and whatever the method of estimation. It is probably linked to differences in mechanisms of resistance between chicks, whose immune system is not mature, and hens, who may also benefit from

adaptive immune response. The variation with animals' age in genetic control of adult and chicken resistance was suspected, because of differences in relative resistance of poultry lines to resistance to carrier-state at a younger (Duchet-Suchaux et al., 1997) or an older age (Protais et al., 1996). This result implies that most results obtained at a younger age are expected to be irrelevant in adults, if not of opposite sign. They may not be extrapolated to hens without experimental validation. Indeed, differences in expression of gallinacins observed by Sadeyen et al., (2004) and Sadeyen et al. (2006) were found to be associated, in young chicken, with increased susceptibility but, in adults, with resistance. Similar variations with age should also be the case of a large proportion of genes or genome regions found to be involved in resistance (for a review, see Calenge et al., 2010). This result also implies that increasing genetic resistance of hens should reduce resistance in chicks. This holds for marker-assisted or genomic selection.

2.3 Towards identification of genes or genome regions involved in the control of resistance

Though promising these results may seem, large-scale selection for increased resistance is very difficult to implement since experimental infections, which are both very expensive and time consuming, are required. Identifying the underlying genes of genetic markers could make it possible to alleviate the need of such experiments.

The major drawback of such genomic studies is the numbers of both phenotypes and genotypes which are required. Studying crosses between inbred lines, following the pioneer studies of Bumstead & Barrow, (1988; 1993) is of great relevance, as it makes it possible to identify QTLs with much less animals than with crosses between outbred lines. Backcross (Mariani et al., 2001; Lamont et al., 2002; Fife et al., 2011), F2 cross (Tilquin et al., 2005) or, more recently, advanced intercross lines (AIL) were considered (Hasenstein et al., 2007; Ghebremichael et al., 2008) .

Candidate genes, i.e. genes chosen according to an a priori knowledge of their effect in *Salmonella* resistance were first investigated. That was in particular the case for two genes, SLC11A1 and TLR4, known to be involved in resistance to *Salmonella* in mouse. The first one corresponds to the formerly called Nramp1 (natural resistance-associated macrophage protein) gene. It is responsible for resistance of mice to inoculation with *Salmonella* Typhimurium, Mycobacterium bovis and Leishmania donovani (Vidal et al., 1993). Later, Nramp1 has been described as a member of a solute carrier family and hence renamed Slc11a1. It is involved in the control of the intracellular replication of parasites in phagosomes. An homologue of Nramp1 was mapped on the chicken chromosome 7 (Hu et al., 1995; Girard-Santosuosso, 1997) and subsequently cloned (Hu et al., 1996).

The second candidate gene, TLR4 (Toll-like receptor 4), previously named Lps, belongs to the large family of Toll-like receptors involved, among others, in the recognition of LPS (lipo-polysaccharides), a component specific of Gram negative bacteria (among which *Salmonella*). Its mutation results in a lack of response to LPS and a higher susceptibility to Gram negative bacteria. The positional cloning of Lps led to the identification of TLR4 as a positional candidate. It was mapped to the chicken micro-chromosome 17 and cloned (Leveque et al., 2003).

Hu et al., (1997) observed that both genes explained together up to 33% of the difference in survival of young chicks during the first week after an intra-muscular inoculation at one day

of age with *Salmonella* Typhimurium. This difference was smaller when survival during the whole experiment was considered. The effect of the NRAMP1 gene was shown in many other experiments: in early stages of systemic *Salmonella* infection in meat-type chicks (Kramer et al., 2003) and layer-type hens (Lamont et al., 2002; Liu et al., 2002) or in spleen infection after intravenous inoculation of pullets (Girard-Santossuoso et al. 2002) or hens (Beaumont et al., 2003b). It is interesting to note that Caron et al., (2002) showed that the NRAMP1 allele coding for a better resistance of mice to an early and acute infection was also responsible for a higher excretion rate in later stages. This result has not yet been investigated in fowls. If it held, it could imply that selecting for the NRAMP1 allele coding for higher resistance to disease would result in a more intense excretion of *Salmonella* in the environment and thus quicker and more important transmission between animals. At the opposite, only the study of Beaumont et al (2003b) suspected a role of the TLR4 gene. Other candidate genes involved in the immune response were investigated using either polymorphisms within the gene or genetic markers (for a review, see Calenge et al (2010).

Searching for Quantitative Trait Loci (QTLs) is another way to identify genome regions involved in resistance. It is based on a systematic research of effects, on resistance, of anonymous genetic markers, chosen to cover as regularly as possible the whole genome. A first genome scan was achieved by Mariani et al., (2001) on resistance to disease due to *Salmonella* typhimurium. A major QTL controlling spleen bacterial load was identified on chromosome 5 and named SAL1. A 6th generation backcross allowed confirming and refining the QTL (Fife et al., 2009). It also suggested two very promising functional candidate genes, which should lead to the identification of the gene(s) underlying the QTL. Tilquin et al., (2005) identified QTL for resistance to both disease and carrier-state. One genome-wise significant QTL and five chromosome-wise significant QTL were observed on chromosomes 2, 1, 5, 11 and 16, respectively. This genome scan used selective genotyping (i.e. genotyping of only a subset of animals, chosen as particularly informative, because of extreme phenotypic values); two of those QTLs (on chromosomes 2 and 16) were confirmed after targeted genotyping of all animals (Calenge et al., 2009) while the QTLs on chromosomes 1 and 16 were also observed in the lines issued from the experiment of divergent selection on resistance to carrier-state described by Beaumont et al., (2010) (Calenge et al., 2009). More recently, the development of a new generation of genetic markers, Single Nucleotide Polymorphisms (SNP) allowed a denser coverage of the genome and the identification of new QTLs (Calenge et al., 2011; Fife et al., 2011) one of which is close to the zone observed on chromosome 2 by Fife et al. (2011). The results of all publications of QTL research are shown on Table 1.

Functional genomics compares the levels of expression (i.e. of activity) of genes at the genome-wide level. It allows the identification of genes involved in the mechanisms of resistance and may also lead to the identification of the genes controlling resistance, provided that the mutations responsible for differences in resistance also result in variations in the levels of expression. Studies compared levels of expression before and after infection, on animals from the same genotypes, or between animals issued from resistant and susceptible poultry lines (for a review, see Calenge et al., 2010). Focussing on the expression of candidate immune genes allows a better understanding of their role (as in Sadeyen et al., 2004; Sadeyen et al., 2006; Swaggerty et al., 2006) in relation with the type of cells as shown by Chausse et al., (2011), when comparing results obtained on the whole caeca by Sadeyen et al. (2004) to observations obtained on sorted cells from this organ.

Chromosome	Position (in cM or Mb)	Reference
1	85, 207 cM	Tilquin et al., 2005
	509 cM	Calenge et al., 2011
2	87 cM	Tilquin et al., 2005
	20 Mb	Fife et al., 2011
3	123 cM	Calenge et al., 2011
	96 Mb	Fife et al., 2011
4	158, 242 cM	Calenge et al., 2011
5	157 cM	Mariani et al., 2001
	100, 111 cM	Tilquin et al., 2005
	38 cM	Calenge et al., 2011
6	8 cM	Calenge et al., 2011
9	68 cM	Calenge et al., 2011
11	18 cM	Tilquin et al., 2005
	63 cM	Calenge et al., 2011
12	15 Mb	Fife et al., 2011
14	72, 74, 80 cM	Calenge et al., 2011
16	2 cM	Tilquin et al., 2005
18	12, 14 cM	Calenge et al., 2011
22	29 cM	Calenge et al., 2011
24	41 cM	Calenge et al., 2011
25	1 Mb cM	Fife et al., 2011
27	37, 54, 68 cM	Calenge et al., 2011

Table 1. Position of QTLs for resistance to *Salmonella* in chicken already published.

2.4 Potential efficiency of marker assisted or genomic selection

Using results currently available makes it possible to use marker-assisted selection. A first experiment of SNP-assisted selection was achieved by Legarra et al., (2011). A total of 600 animals were genotyped for 831 SNP; these markers explained a large proportion of genetic variance, even if no reduction in residual variance could be observed. This drawback will no doubt be alleviated when a larger number of SNP will be considered, leading to genomic selection, i.e. marker-assisted selection based on genotypes assessed on a very large number of anonymous markers, most often SNP (Meuwissen et al., 2001). Genomic selection investigates the whole genome at least as far as the markers cover it. Even if no application of this procedure to selection of commercial fowls is known yet, that should be the case in the near future. That should especially hold for traits whose measure is expensive and heritability low, as resistance related traits. It should also make it possible to select for several resistance related traits, even if genetic correlations are negative.

However, even with genomic selection, the choice of selection criteria remains a main issue. Whatever the method, it is not possible to consider the numerous resistance-related traits that might be measured using the different protocols of experimental infection described in subsection 2.1. It is necessary to find out the most important ones. Modelling will no doubt be of great interest for such studies. It will consider current knowledge on genetic resistance and other prophylactic means and investigate what may be expected from different methods of selection and prevention as well as from their combination.

3. Mathematical modelling: A way to integrate genetic results for a better understanding of *Salmonella* propagation within a flock of hens

A few models have already been proposed to study *Salmonella* spread within hens (Leslie, 1996, Thomas et al., 2009; Prévost et al., 2006; Zongo et al, 2010a). In two models (Leslie, 1996; Thomas et al., 2009), the transmission of the *Salmonella* is determined by direct contact between animals and the density of bacteria in environment neglected. Prévost et al. (2006, 2007, 2008) and Zongo et al. (2010a) took into account the effect of the bacterial load of the environment. Variability in animal genetic resistance was introduced by Prévost et al, (2008). They distinguished two subpopulations of hens, with a lower or higher resistance to *Salmonella* carrier-state, as the model is a deterministic compartmental model (i.e. the population is divided into categories). In Zongo et al (2010a), individuals are represented, which makes it possible to model the contamination of each hen and to assume them to have varying degrees of resistance. In particular, effects of animal's capacity of defense on the evolution of the individual bacterial load were considered. Since the model is stochastic, the impact of this variability on propagation within the flock can be investigated.

3.1 Model at the population level

Classically, for a large population (as a flock), compartmental deterministic models are defined and the numbers of animals in each category are modelled. Prévost et al., (2006) developed such a model. These authors considered four categories of hens. Naïve (also called susceptible) birds have no protection against *Salmonella*: they are at risk of becoming infected. For hens' contamination, three steps were distinguished. The first is digestive contamination, when the bacteria are located in the digestive tract. Systemic infection occurs after translocation of bacteria through the digestive barrier; it results in contamination of systemic organs, such as liver or spleen, and sometimes also in egg contamination. Afterwards, bacterial clearance leads to recovery (see figure 1).

First contamination depends on the environmental bacterial load. Indeed, propagation of the infection may occur through aerosols from one contaminated animal to another animal, especially when they are reared in the same cage, or indirectly because of environmental contamination, mainly through water and feed. It is to note that this model neglected vertical transmission though transovarian route (Humphrey et al., 1989). Once contaminated at the digestive level, animals will become infected to a systemic level then get immunised. Thus, the rates of transition to the systemic level and, afterwards, the recovered status are constant.

Numerical analyses give hints for developing control measures as they highlight the influence of factors contributing to the variation of egg contamination and thus to the risk of human contamination. The recovery rate, representing the ability of hens to eliminate *Salmonella*, influences both maximal prevalence and duration of the epizooty. The rate of return to the susceptible state, i.e. the loss of protective immunity, is also of major importance. These results could be related to biological mechanisms. The mechanisms explaining the response to selection for a lower or higher contamination level were not the same. Selection on higher resistance had mostly modified the hen's clearance ability while a higher rate of return to the susceptible state was responsible for the higher level of contamination of the other line, which had a shorter immune protection time. This is coherent with results on immune response of lines differing (among other resistance traits) in resistance to carrier state, whether inbred (Sadeyen et al., 2006), or outbred (Proux et al., 2002; Protais et al., 2003).
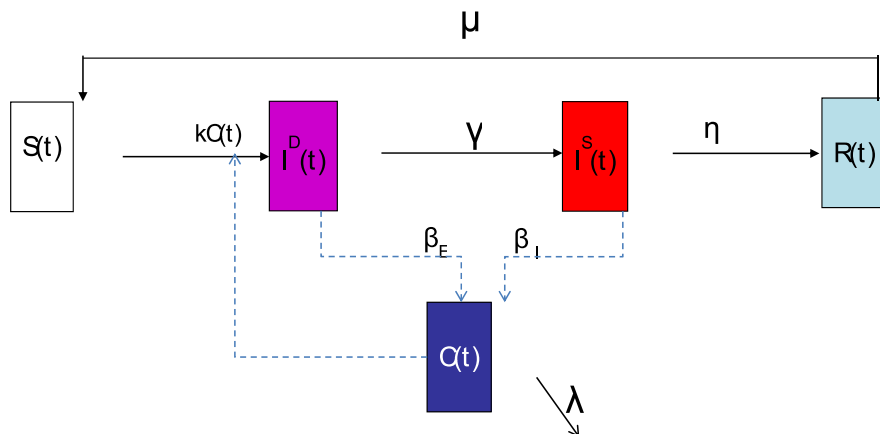
Fig. 1. Diagrammatic representation of the evolution of health status for an individual at a given time t: individual may be susceptible (S), infected at the digestive level (I^D) or at the systemic level (I^S) or recovered (R). β_E and β_I are the rates of excretion of infected animals at the digestive or systemic levels. They contribute to the increase in the number of bacteria in the environment. The rate of transition (k C(t)) from the susceptible (S) to the infected at the digestive level (I^D) status depends on the number of bacteria in the environment (C(t)). Parameters γ, η and μ, respectively, are the rates of transition from one status to another (i.e. from infected at the digestive (I^D) to infected at the systemic level (I^S), from infected at the systemic level I^S to recovered (R) and from recovered to susceptible (S), respectively).

The effect of the introduction of a proportion of more resistant animals among the population was investigated. It reduces the peak of infection (i.e. the maximal percentage of infected animals), because the more resistant animals excrete less bacteria in the environment, reducing cross contamination. But it also delays the extinction of the epizooty as a higher proportion of animals are still naïve and infected later. Increasing genetic resistance to a greater extent but in a proportion of the population or increasing it to a lesser extent but in all animals will lead to different results. Inversely, results of genotype comparisons will not be the same when animals with different degrees of bacterial clearance are reared together or not. This point should be further studied in practice.

The effect of vaccination on flocks with different levels of genetic ability to clear bacteria was also investigated. The vaccine was more efficient in more resistant animals, in relation with the differences in the persistence of immunity. These differences are coherent with observations made by Protais et al. (2003). It is important to note that the combination of vaccination and genetic selection resulted in a percentage of contamination similar to what the European community is asking for.

3.2 Taking into account the variability at the animal level

A stochastic individual-based model was developed for a finer modelling of the variability of hen's response to contamination (Zongo et al., 2010a). Such models 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 model extends the model previously derived by Prévost et al. (2006).

The variation over time (that is the derivative) of the individual bacterial load B(τ,x) was modelled as resulting from bacterial multiplication within the individual (i.e. growth rate), and from contamination by environmental bacteria. The latter was dependant on the number of bacteria present in the neighbourhood of the individual, that is the density of bacteria. It is to note that such a more precise modelling could also be derived using a deterministic approach as follows:

$$\underbrace{\partial B(\tau, x) / \partial \tau}_{\text{Variation within individual}} = \underbrace{B(\tau, x))}_{\text{Growth rate}} + \underbrace{I_P(t, x)}_{\substack{\text{Inhaled or ingested} \\ \text{within environment}}}$$

Equation 1: Derivative, according to time, of individual bacterial load (B(t,x) at time t and position x is equal to the sum of growth rate, g, of B(t,x) and of contamination through ingestion or inhalation of bacteria present in in the neighbourhood, that is $I_P(C(t,x))$. This derivative is computed at each time step and position.

Contrary to Prévost et al. (2008), two steps in digestive contamination were distinguished (Figure 2): a transient contamination and a long term one. When in the transient status, animals may overcome the contamination and get rid of the bacteria, provided that their bacterial load (thereafter denoted B) remains lower than a threshold D. Once the hen is contaminated at a bacteria load higher than D, its capacities of defence are overwhelmed and it will become systematically infected.
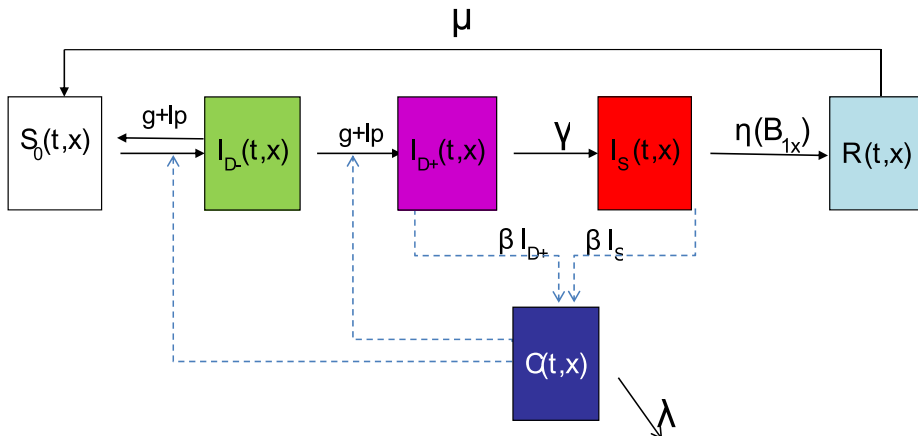

Fig. 2. Diagrammatic representation of the evolution of health status for an individual at time t and position x and its interaction with the level of environmental contamination at this position, C(t,x). The individual may be susceptible (S_0), infected at the digestive level with a low dose of contamination (I_{D-}), suffering from a long term digestive contamination (I_{D+}), contaminated at the systemic level (I_S) or recovered (R). The number of bacteria carried by an individual in the I_{D-} status depends on the growth rate g of its bacterial load and of the quantity of ingested bacteria; the latter depends on the level of environmental contamination in the neighbourhood ($I_{P(t,x)}$). The individual bacterial load regulates transitions from S_0 to I_{D-} (reciprocally from I_{D-} to S_0) and from I_{D-} to I_{D+}. Parameters γ, η and μ, respectively, regulates durations of status I_{D+}, I_S and R, respectively. $β_{D+}$ and $β_I$ are the rates of excretion of infected animals at respectively the transient digestive and systemic level.

Transitions from susceptible to digestive contamination status (both I_{D-} and I_{D+}) are regulated by the individual bacterial load, which is computed at each time step and for each animal. When it is greater than zero and lower that the individual threshold, $D(x)$, individual status changes from S_0 to I_{D-}. The individual may go back in S_0-state unless $B(t_{n+1},x)$ becomes greater than $D(x)$ and individual status changes from I_{D-} to I_{D+}. Other transitions are stochastic with average durations equal to $1/\gamma$, $1/\eta(B_{1x})$ and $1/\mu$, respectively, for the transitions from I_{D+}-state to I_S-state, I_S-state to R-state and from R state to S_0 respectively.

The threshold $D(x)$ is assumed to vary from an animal to another as it depends on many factors: the bacterial strain (as can be seen for example from Bumstead and Barrow, 1993), gut flora (Nurmi & Rantala, 1973), animals' genetic resistance (§ 1). This variability and the differences in initial contamination result in a variability in bacterial load as can be seen in Figure 3.
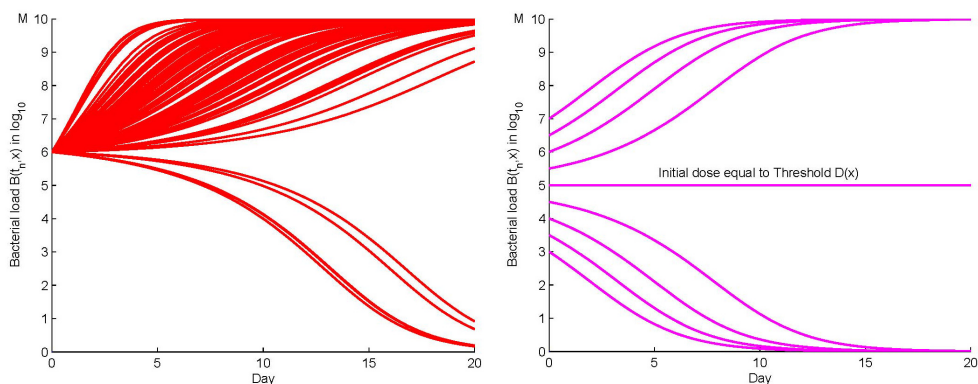

Fig. 3. Evolution of individual bacterial load (B(t,x)) within an individual at the position x and time t according to the number of bacteria ingested by the animal and the threshold for animals' ability to overcome the contamination (D(x)). M is the maximal bacterial load that an animal may carry (set here at 10 log10 c.f.u.). In (a), D(x) is set to the same value for each animal (5 log10 c.f.u.). Four initial doses are above the threshold, one equal to it and four doses below it. In (b), the thresholds D are random, leading to different thresholds for each animal so that, for the same initial dose (set here at 6log10 c.f.u.), there is individual variability in evolution of the bacteria load.

The density of bacteria (C(t,x)) in the environment at time t and position x depends on the rate of diffusion of bacteria, on the natural rate of mortality of bacteria, λ, as well as on the density of bacteria excreted by infectious individuals close to position x.

As the model is spatial, Zongo et al (2010a) show that the position of the first contamination influences both the kinetics of infection and the maximal percentages of infected animals (see figure 4).
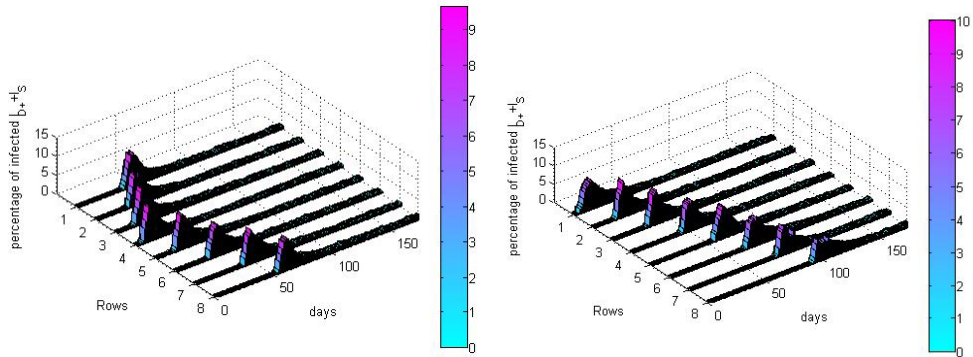
Fig. 4. Influence of the position of the first infection on the evolution over time of the percentage of infected animals (at the digestive level, in a persistent way (I_{D+}), or systematically (I_S)), when (a) infection starts in the middle (row 4 out of 8) or (b) in the corner of the hen house (row 1 out of 8). Only median values of the sum of percentages of infected animal are represented. The color bars indicate the relation between color and percentage of infected animals.

One investigation considered two levels of excretion, since it may vary between hens (Ishola, 2009). As can be seen on figure 5, both the propagation speed and the maximum level of infection are strongly influenced (Zongo et al, 2010b). But more studies are still needed to investigate this question.
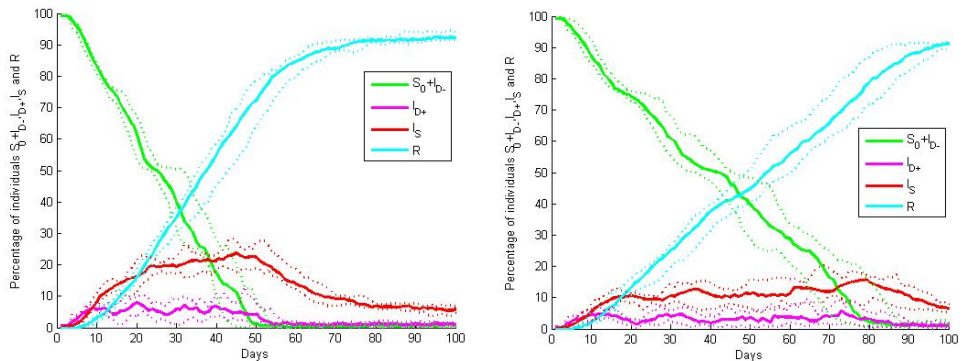

Fig. 5. Evolution of status of individuals (susceptible (S_0), infected at the digestive level with a low dose of contamination (I_{D-}), suffering from a long term digestive contamination (I_{D+}), contaminated at the systemic level (I_S) or recovered (R)), according to days after the first contamination, when (a) all individuals in the I_{D+} or I-state excrete (b) half of individuals at I_{D+}-state or I_S-state excrete. Median values obtained on 100 simulations are shown by solid lines curves, while the 5th and 95th percentiles are shown by dotted lines.

3.3 Perspectives

First, the relative effects of animals' capacity of bacterial clearance and level of excretion should be studied. Both have important effects and most probably interact with each other and with the animals' capacities to overcome transient digestive contamination (threshold D). Since, in hen houses, bacterial doses are most often rather small, it will be important to study the effects of both average values and variability of these factors.

Second, interactions between animals may be more precisely modelled. The model derived by Zongo et al. (2010a) assumes that all hens within a cage are contaminated at the same time and carry the same bacterial load. When the number of hens within cage becomes large, this major hypothesis does not totally hold. Moreover, new systems of rearing should be considered, such as aviaries, extensive rearing or enriched cages. They should be more and more frequent with the European commission banning of traditional cages after 2012. They no doubt interact with sanitary risks. *Salmonella* propagation should also be considered in flocks of younger animals.

Rearing together animals with different profiles of resistance should also be considered. It is worth comparing whether it is more efficient to select one type of resistance (as for example capacity of bacterial clearance or low level of excretion) or several resistance-related traits. In particular, flocks composed of several lines selected on different resistance related traits or performance should be considered. At a longer term, such studies should integrate the links between immune capacities and performance, as investigated by Van der Most et al. (2010). Complementary studies should also consider egg yolk's genetic ability to resist bacterial infection as evidenced by Sellier et al., (2007).

4. Conclusion

Selection for higher resistance to carrier-state may be an efficient way to control *Salmonella* propagation within a flock. It might profitably be used as an additional mean of prevention of human food poisoning. The choice of the selection criteria must be considered carefully as it will have a strong influence on the results of selection. Other means of prevention must also be considered to choose the best strategy of prevention according to its impact on the level of animal contamination and then on the *Salmonella* propagation. Modelling will contribute to integrate genetic and experimental data at individual level to evaluate the propagation at the population level. It will allow the comparison of the impact of different scenarios on the propagation within a flock with, for example, different profiles of resistance or different prophylactic measures. Reversely, these studies will ask new questions and necessitate new experiments to confirm results or give values of some parameters. In particular, if the resistance and the propagation are different according to *Salmonella* strains, different models can be developed, providing there are sufficient data to define them. For other pathogenic agents, such as Campylobacter, integrated studies should also be envisaged.

5. Acknowledgment

6. References

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