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Resistance to *Salmonella* carrier state: selection may be efficient but response depends on animal's age

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Summary

Increasing resistance to acute salmonellosis (defined as bacteraemia in animals showing symptoms) is not sufficient for food safety, because of the risk of carrier state (when animals excrete bacteria without showing any symptoms). Increased resistance to *Salmonella* carrier state is therefore needed. Two experiments of divergent selection on resistance at a younger and a later age lead to significant differences between lines and allowed estimating genetic parameters on 4262 animals. 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. Genetic correlations between contamination of the different organs ranged from 0·46 to 0·67, while correlations between resistance at both ages were estimated at −0·50, showing that increasing genetic resistance of hens will reduce resistance in chicks. Highest estimated absolute values of genetic correlations between resistance and production traits were, for chicken contamination level, with number of eggs laid between 41 and 60 (0·37) and, for adult contamination, with number of eggs laid between 18 and 24 (0·37) or 25 and 40 (−0·33) weeks of age.

1. Introduction

Improving animals' genetic resistance to disease is a promising way to improve animal health and food safety and reduce the need for antibiotics. However, before any practical application, care must be taken to prevent an increase in prevalence of asymptomatic carriers, i.e. contaminated animals showing no symptoms. Indeed such animals, which cannot easily be identified as a potential source of contamination, are responsible for horizontal or sometimes vertical transmission of the pathogenic agent within the flocks, which constitutes an insidious risk for public health. Genetic control of resistance to acute disease differs from control of asymptomatic carrier state. For example, in mice, the wild allele of the *Slc11a1* gene (formerly called *Nramp1*) codes for higher resistance to disease (measured by the mortality level after an intravenous inoculation) but also for higher susceptibility to a longer term and silent contamination, in a model of inoculation mimicking carrier state (Caron *et al.*, 2002). Moreover, even when carrier state is considered, the genetic control of resistance may differ with animal's age or be dependent on the localization where carrier state is assessed. These are key questions that should be answered but imply a large number of measures. This may explain why, although very important for any practical applications, at least until now, they have been very rarely addressed.

Biological characteristics of fowls, i.e. their small body size and low individual economic value facilitate to some extent such studies. They contribute to explain why extensive work was made on genetic control of fowl resistance to salmonellosis, but the main reason is no doubt the importance of this bacterium

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for food safety and the involvement of poultry products in this risk. *Salmonella* is worldwide one of the major causes of food-borne human poisoning (see de Jong and Ekdahl, 2006 for figures in the European community), with symptoms ranging from mild to severe gastroenteritis and invasive disease, which, can, in some cases, especially for immuno-depressed patients, be fatal. Most of these food-borne outbreaks originate from poultry products and especially egg products: the latter were implicated in 80 % of the 371 known source *Salmonella* Enteritidis outbreaks reported in USA, from 1985 to 1999 (Patrick *et al*., 2004) but were estimated to be even more important by Schroeder *et al*. (2005). This importance of poultry products in human contamination is mostly linked to the difficulty of identifying asymptomatic carriers. That is the reason why, in addition to studies of genetic resistance to disease or mortality due this bacteria, resistance to carrier state was also investigated in fowls (Beaumont *et al*., 2003*a*). Since heritability of resistance to carrier state had been estimated at 0·20 in young birds (Berthelot *et al*., 1998) and higher than 0·35 in laying hens (Beaumont *et al*., 1999), a selection experiment was undertaken for six generations to test the feasibility of such a genetic improvement and obtain genetic models that should be very helpful in understanding the mechanisms of resistance. A large data set of 4262 measures of *Salmonella* contamination in adults and chicks was thus obtained and enriched by measures of laying rate and egg weight and colour. This allowed deciphering the genetic basis of fowl resistance to *Salmonella* by estimating heritabilities and genetic correlations between all these traits. The purpose of this work is thus to present estimated genetic parameters of resistance traits and in particular genetic correlations between resistance at a younger or older age as well as between measures of resistance to *Salmonella* carrier state (that are contaminations of the different organs of a given animal) on the one hand and production traits on the other.

2. Material and methods

The selection experiment was carried out from a base population consisting of 79 animals sampled from a layer-type line. Two series of divergent lines have been selected, for increased or decreased level of carrier state at a younger age or at the peak of lay, respectively.

(i) *Assessment of level of carrier state*

Before challenge, all animals (both candidates to selection and breeders) were reared in protected facilities and shown to be *Salmonella*-free by regular tests. At every hatch, chicken boxes as well as a few chicks were checked, by microbiological tests, for the absence of *Salmonella*. Microbiological tests were thereafter achieved every 2 weeks and serological tests at 20, 26, 42 and 58 weeks of age. Moreover, additional bacteriological tests were performed at the arrival of the hens in the experimental unit to check the absence of *Salmonella*, while eggshells are disinfected at the hatchery. All tests were found to be negative.

All challenges were performed in protected facilities with a class 2 level of protection against pathogens. Moreover, animals were reared according to ethical guidelines. For measures of adult resistance, within each hatch, families were randomly allocated to three cells. When 20–24 weeks old hens were orally contaminated with 10^9 colony forming units (cfu) of a PT4 *Salmonella* Enteritidis strain 5556, a wild strain isolated from a human case of toxi-infection, as in Protais *et al*. (1996). Bacteria were searched in caeca, spleen, liver and ovary 4 weeks later. A total of five traits were thus considered: presence/absence of *Salmonella* in each of these four organs (thereafter called $\text{adult}_{\text{spleen}}$, $\text{adult}_{\text{liver}}$, $\text{adult}_{\text{caeca}}$ and $\text{adult}_{\text{ovary}}$, respectively), as well as the global adult contamination rate ($\text{adult}_{\mathbf{0/1}}$), coded '1' if liver, spleen or caeca was found positive and '0' in the other cases. Another synthetic variable was also studied; the so-called global contamination level (adult_{g}) that could vary from 0, if none of the three organs was contaminated to 3 if all organs were contaminated.

For chicken challenges, within each hatch, animals were randomly attributed to four cages. At 1 week of age, as described in Duchet-Suchaux *et al*. (1995), chicks were orally inoculated with 5×10^4 cfu of *Salmonella* Enteritidis PT4 strain 1009, which is a spontaneous mutant of the strain 5556 resistant to nalidixic acid and streptomycin. Five weeks post inoculation, they were slaughtered and *Salmonella* were numbered in the caeca. Selection criterion was the logarithm of the number of colonies forming units ($\text{young}_{\text{logcfu}}$) per gram of caeca. For two hatches, consisting of 395 and 295 chicks, respectively, *Salmonella* could only be found in a small proportion of animals and at very low levels. In that case, selection criterion was assessed as an all-or-none trait called contamination rate in young ($\text{young}_{\mathbf{0/1}}$). A total of 4262 animals were thus measured, among which 2097 at the adult age and 2165 at the younger age.

(ii) *Data of zootechnical interest*

In parallel, traits of zootechnical interest were recorded on parents of animals measured for resistance; the traits were body weight at 17 weeks of age, numbers of eggs laid, egg weight and eggshell colour assessed at different time intervals (i.e. between 25 and

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Table 1. *Number, per generation and trait, of animals measured for resistance*

Generation	Number of offspring (and hatches) measured	
	For chicken resistance	For adult resistance
1	461 (2)	610 (6)
2	464 (1)	496 (3)
3	784 (2)	–
4	217 (1)	302 (3)
5	483 (1)	318 (3)
6	481 (1)	–

40, 41 and 60, as well as for number of eggs only, 18 and 24 weeks of age).

(iii) *Methods of selection and of estimations of genetic parameters*

As inoculated animals could no more be kept for reproduction, sib-selection had to be achieved. Candidates to selection were produced in one or, from the third generation on, two additional hatches (one for lines selected on resistance at an adult age and one for those measured at a younger age). Numbers of animals per generation and measures are given on Table 1. At each generation, estimated breeding values (EBV) for the $adult_g$ and $young_{logcfu}$ traits were obtained considering the whole pedigree (from the base population), that is, at the last generation, 6433 animals originated from 315 sires, 159 maternal grandsires and 692 dams.

During the first three generations, genetic values for $young_{logcfu}$ were computed using the Pest software (Groeneveld *et al.*, 1990) and BLUP, while those for binary traits were predicted using a threshold model, as described by Gianola and Foulley (1983). Selection was on the mean of breeding values for adult and chick contamination.

Because of increasing evidence of negative correlations between both measures, lines selected on chicken level of contamination were then completely distinguished from those selected on adult level of contamination. The criterion of selection for adult was then the $adult_g$ trait and the lines were named Sal_a+ and Sal_a-, respectively, for increased and decreased adult carrier state, respectively. Selection on chicken resistance was based on the mean of EBVs for both $young_{logcfu}$ and $young_{0/1}$ and the lines named Sal_y+ and Sal_y-.

Breeding values and genetic parameters were estimated, by a Bayesian approach, as the means of marginal posterior densities. For the continuous $young_{logcfu}$ trait, the model took into account the fixed effect of the cage (25 levels), the dam environmental effect (483 levels) and the additive genetic effects of the animals (6433 levels). For each of the discrete traits ($adult_{spleen}$, $adult_{liver}$, $adult_{caeca}$, $adult_{ovary}$ and $adult_{0/1}$), the model assumed an underlying latent variable related to the outward phenotypes (the observed categorical responses) through a link function as classically described (Wright, 1934; Dempster & Lerner, 1950). As detailed in Sorensen & Gianola (2002), the joint posterior distribution of unknown parameters was augmented with the unobserved liabilities to yield fully conditional posterior distributions that have a standard form and are easy to sample from. The model fitted the fixed effect of the cage (21 levels for the whole data set), the dam environmental effect (303 levels) and the additive genetic effect of the animal (6433 levels). It should be noticed that traits measured at different stages were mutually exclusive. A Gibbs sampler was implemented to estimate the marginal posterior distributions of the genetic parameters. Computations were performed using Thrgibbs1f90 (Misztal *et al.*, 2002). Long chains (of more than 5 500 000 iterations) were launched and the first 50 % rounds were discarded. In order to reduce the autocorrelation, only one iteration was sampled every 991 rounds, so that samples of about 3000 observations were used to compute the posterior distributions of the parameters of interest. Convergence was assessed both visually and using the BOA R package (Smith, 2005).

For each parameter of interest, we computed the mean value and the highest probability density intervals (excluding 2·5 % of extreme values in both sides of the distribution). When addressing genetic correlations, we also computed the probability of the correlation to be negative, as the percentage of samples below zero. Similarly, the probability for heritability to be higher than 0·10 was obtained.

Three different sets of analysis were considered. First, genetic parameters for adult contamination were estimated in a four-trait analysis grouping liver, caeca and spleen contamination rate ($adult_{liver}$, $adult_{caeca}$ and $adult_{spleen}$) as well as $adult_g$. Heritability for ovary ($adult_{ovary}$) was also estimated in an independent analysis. In a second analysis, genetic correlations between measures assessed in the younger or the older age were estimated from four distinct three-trait analyses, including the two selection criteria ($young_{logcfu}$) and the adult overall contamination ($adult_g$) and one of the four organ contamination. The last set of three-trait analysis aimed at estimated genetic parameters of the two selection criteria and the productive traits. In that case shorter chains of 900 000 iterations were used and the first 20 % discarded.

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Table 2. *Means of marginal posterior distribution of genetic parameters (heritability, bold on the diagonal; genetic correlations above the diagonal), with minimal and maximal values of 95% highest posterior densities intervals and probabilities for heritabilities to be higher than 0·10 and for correlations to be positive for chicken caecal load ($young_{logcfu}$ expressed in log(cfu)) as well as the presence/absence of* Salmonella *Enteritidis in spleen, liver and caeca or global contamination ($adult_g$) after oral inoculation of adults with* Salmonella *Enteritidis*

	$Young_{logcfu}$	$Adult_g$	$Adult_{Spleen}$	$Adult_{Liver}$	$Adult_{Caeca}$
$Young_{logcfu}$	0·16 [0·06, 0·29] **88%**	−0·50 [−0·92, 0·21] 95%	−0·41 [−0·98, 0·38] 86%	−0·51 [−0·99, 0·06] 90%	0·30 [−0·62, 0·68] 20%
$Adult_g$		**0·18** **[0·09, 0·30]** **94%**	0·85 [0·64, 0·96] 0%	0·75 [0·44, 0·94] 0%	0·85 [0·67, 0·96] 0%
$Adult_{Spleen}$			**0·19** **[0·09, −0·31]** **94%**	0·46 [−0·11, 0·90] 6%	0·67 [0·18, 0·96] 1%
$Adult_{Liver}$				**0·14** **[0·05, 0·26]** **67%**	0·48 [−0·11, 0·87] 7%
$Adult_{Caeca}$					**0·23** [0·12, 0·36] **99%**

(iv) *Phenotypic responses to selection*

The effect of selection for increased or decreased *Salmonella* contamination was tested for one age at a time (by comparing Sal_a+ to Sal_a- on the one hand, Sal_y+ to Sal_y- on the other). Animals from both lines were reared together and inoculated at the same time and in the same room. The line effect was tested in an analysis of variance using SAS Proc GLM procedure (SAS, 1989).

3. Results

(i) *Response to selection*

Response to selection could hardly be estimated before lines selected on chicken carrier state level were separated from those selected on adult carrier state level. Three generations later, a significant although still moderate between lines difference in chicken contamination level was observed: mean contamination levels differed by 0·4, from 2·19 in the Sal_y- to 2·56 log(cfu) in the Sal_y+ line.

Larger differences were observed between lines selected for decreased or increased carrier state at the adult age. They began to significantly differ at the third generation. At the fifth generation, average contamination levels in the Sal_a- and Sal_a+ lines, respectively, were equal to 30 and 44% in caeca, 5 and 6% in liver and 15 and 30% in spleen, respectively. All of them were significantly different ($P<0·001$). Mean percentages of global contamination ($adult_g$) were equal to 40·7 and 60·3, respectively. No difference could be observed for the ovary contamination level, but, in this case, the number of positive samples was very low.

(ii) *Estimation of genetic parameters*

As shown in Table 2, estimated heritability of the chicken caecal load was estimated at 0·16 and that of adult global contamination ($adult_g$) at 0·18. Genetic correlation between both measures was estimated at a quite high and negative value (−0·50) and the probability of this correlation being positive was only 5%.

With reference to individual organs, heritability was higher in caeca (0·23) and slightly lower in spleen (0·19) and liver (0·14), while heritability of ovarian contamination was estimated at a lower value (0·11). All genetic correlations between contamination rates of spleen, liver and caeca were positive, ranging from 0·46 to 0·67 and probabilities of negative estimates were less than 7%. Genetic correlation between adult global contamination and ovarian contamination rate was estimated at 0·32 with quite a large high posterior density [−0·31; 0·85]. Estimated genetic correlations between adult global contamination and the other three organs were very high: they ranged from 0·75 for liver to 0·85 for spleen and caeca with a probability of 100% of being positive.

(iii) *Data of zootechnical interest* (*Table 3*)

When considering genetic correlations with $young_{logcfu}$, those with egg numbers were positive (ranging from 0·07 to 0·37) except for the number of

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Table 3. *Means of marginal posterior distribution of genetic parameters of some production traits, i.e. heritability and genetic correlations with chicken caecal load (expressed in log(cfu)) and adult global contamination after inoculation with* Salmonella *Enteritidis. Minimal and maximal values of 95% highest posterior densities intervals are also given as well as the percentage of negative estimates*

Trait	Number of observations	Heritability estimate	Genetic correlation with young log(cfu)	Genetic correlation With $adult_g$
Egg number 18–24 weeks	1069	0·36 [0·22; 0·49]	−0·17 [−0·56; 0·21] 80%	0·37 [−0·19; 0·77] 11%
Egg number 25–40 weeks	1039	0·35 [0·22; 0·49]	0·07 [−0·39; 0·57] 46%	−0·33 [−0·72; 0·36] 86%
Egg number 41–60 weeks	1059	0·26 [0·14; 0·40]	0·37 [−0·10; 0·86] 6%	−0·01 [−0·50; 0·46] 55%
Body weight at 17 weeks of age	1073	0·53 [0·41; 0·63]	−0·03 [−0·36; 0·33] 62%	−0·03% [−0·35; 0·31] 62%
Egg weight 25–40 weeks	1058	0·47 [0·35; 0·60]	−0·12 [−0·44; 0·28] 79%	0·05 [−0·30; 0·39] 45%
Egg weight 41–60 weeks	997	0·53 [0·41; 0·66]	0·10 [−0·24; 0·43] 32%	−0·10 [−0·45; 0·26] 75%
Egg colour 25–40 weeks	1042	0·41 [0·27; 0·55]	0·10 [−0·36; 0·49] 35%	−0·09 [−0·52; 0·31] 70%
Egg colour 41–60 weeks	999	0·65 [0·51; 0·77]	−0·05 [−0·42; 0·35] 64%	−0·21 [−0·54; 0·18] 89%

eggs laid between 18 and 24 weeks of age (−0·17), while genetic correlation with body weight was close to zero. Correlations with egg weight were of low absolute value (<0·12).

Unlike what was observed for resistance at a younger age, genetic correlation between $adult_g$ and egg numbers laid at the beginning of lay (between 18 and 24 weeks of age) was positive, while those with laying intensity at older ages were negative or very close to 0 (ranging between −0·33 and 0·07). Correlation with body weight at 17 weeks of age was close to 0.

4. Discussion

These results definitely show that selection may be efficient in reducing *Salmonella* carrier state. First, the probability of being higher than 0·10 was 88 and 94%, respectively, for the heritability coefficients of $young_{logcfu}$ and $adult_g$, respectively. This result confirms that these traits are partly genetically controlled, as first observed on a much smaller data set and, therefore, with a very low precision by Berthelot *et al.* (1998) and Beaumont *et al.* (1999). Moreover, the carried out selection led to significant differences between the Sal+ and Sal− lines in the two sets of line.

The probability of the heritability of $young_{logcfu}$ being higher than the estimation previously obtained by Janss & Bolder (2000) in a meat-type strain (0·09) after intra-muscular inoculation at 2 weeks of age is 90%. This estimate is slightly lower than the heritability estimated by Berthelot *et al.* (1998) in the base population for the presence/absence of caecal contamination ($young_{0/1}$). It should be noted that the traits slightly differ and that we fitted a dam environmental effect, which may explain the discrepancies between these estimations.

Similar reasons probably explain why the means of sampled heritabilities of adult spleen and caecal contamination were much smaller than the estimations formerly obtained with a high standard error by Beaumont *et al.* (1999), i.e. $0{\cdot}32 \pm 0{\cdot}23$ and $0{\cdot}38 \pm 0{\cdot}25$ for spleen and caecal contamination, respectively. Differences in heritability could be observed between the four organs. Caecal contamination appeared to be the most heritable, while spleen and, to a higher extent, liver and ovary contamination rates were less

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heritable. Indeed differences in heritability of spleen and caecal contamination had been suggested by Beaumont *et al*. (1999). Such a difference could be related to the more central role of intestine in persistence of carrier state: it is a major location for gastro-intestinal bacteria such as *Salmonella* and bacteria pass through it when inoculated or after re-contamination following excretion by other animals. At the opposite, bacterial contamination in the other organs is more dependent on translocation of intestinal barrier and contamination of systemic organs, which might result in smaller importance of genetic control and thus a lower value of heritability. This is especially true for ovaries: in this experiment only 6·15% of them were found contaminated versus 48·6% for spleens, 20·9% for liver and 61·8% for caeca (resulting in a percentage of contaminated adults equal to 75·9%). Moreover, this low occurrence of ovarian contamination resulted in a very low quantity of information on ovarian resistance, which further reduces the expected response to direct selection for reduced ovarian contamination. An indirect selection on another criterion should be more efficient, so that this trait was not considered in selection.

All estimated genetic correlations between contamination rates of different organs are positive: decreasing the frequency of contamination of one organ will contribute to decrease the rate of contamination of the others. This reinforces the interest of the overall adult contamination, which is more precisely assessed and combines several traits, all of which being positively correlated.

In coherence with these estimated genetic parameters, differences between the Sal_a+ and Sal_a- lines are significant and quite important. They can already be exploited for between lines comparisons and should increase with further generations. Offspring of extreme breeders (with the most positive or most negative EBVs) might already be used to investigate the mechanisms underlying genetic resistance. They could, for example, be used to confirm the interest, in these lines, of results obtained with different genetic backgrounds, such as F2 crosses between inbred lines (Mariani *et al*., 2001; Tilquin *et al*., 2005) as achieved by Calenge *et al*. (2009).

Selection was, at least until now, less efficient in the 'chicken' lines than in the 'adult' ones. This lower selection efficiency is coherent with the lower heritability estimate. Selection response was also restricted by other factors. Selection pressure and family sizes were smaller than in 'adult' lines. Indeed, although male and female chicks were measured (instead of adult hens only), they were produced in only one hatch to be measured in the same conditions. Moreover, because of the large variations in time needed for *Salmonella* clearance, in two hatches out of eight, only a small proportion of animals could be measured for level of contamination ($young_{logcfu}$). The others could only be recorded as 'infected' or 'non-infected', an all-or-none trait that seems to be very little genetically correlated with log(cfu). This slowed down selection. One solution to avoid such an event could be to slaughter a representative sample of animals at regular intervals in order to find out the relevant *post inoculation* interval at which susceptibility to carrier state could be assessed as an all-or-none trait with an optimal contamination rate (of about 50%), as achieved by Berthelot *et al*. (1998). But such a strategy could hardly be implemented for such a long term selection experiment. It was therefore decided to slaughter animals at a given interval and use all available information. This smaller than expected response to selection is also and probably mostly due to the genetic antagonism that we observed between genetic control of resistance at a younger or older age. Indeed, at the beginning of the experiment, the hypothesis of a partly different genetic control of adult and chicken susceptibility to carrier state was made in relation with 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). But no such large antagonism was expected and the same breeders were used for both sets of lines during the first three generations. Moreover, animals from both susceptible and resistant lines were reared together, which probably reduced between lines differences as suggested by Prevost *et al*. (2008) simulations based on results from the Sal_a+ and Sal_a- lines.

This negative and quite high genetic correlation between adult and chicken contamination is no doubt a major and unexpected result of this experiment, even if the accuracy of the estimates is low. This result holds whether overall contamination is considered or different organs distinguished, except when adult and young caecal contamination are considered. It does not depend on the method of estimation either: using REML with VCE4 (Groeneveld *et al*., 1997) or Bayesian inference led to negative estimations. When pooling all the chains produced for estimating genetic correlations with production traits, an even more negative correlation was observed, with a mean of $-0{\cdot}68$ and a median of $-0{\cdot}81$. This variation with age of genetic control is probably linked to mechanisms of resistance: since the immune system is not mature at hatching, chicks may only be protected by innate immune response, while hens may also benefit from adaptive immune response. This result is concordant to observations made by Sadeyen *et al*. (2004, 2006) when studying two inbred lines: the most susceptible at a younger age was the most resistant at the adult age. It has large consequences. Most results obtained at a younger age are thus expected to be irrelevant in adults, if not of opposite sign. That is for

example the case of results obtained when comparing, between inbred lines differing in their susceptibility to salmonellosis (Bumstead & Barrow, 1988, 1993) and to carrier-state, expression of different genes involved in the innate immunity. Indeed Sadeyen *et al.* (2004, 2006) found differences in expression of gallinacins but they were found to be associated, in young chicken, with increased susceptibility but in adults with resistance. This also holds for selection and for marker-assisted selection. Indeed, when investigating the interests, at the young and adult age, of the quantitative trait loci (QTLs) identified (Tilquin *et al.*, 2005) for their effect on chicken, results largely differed with age with the only exception of the region carrying the *SLC11A1* gene (Calenge *et al.*, 2009). This gene was shown to be involved in resistance to acute salmonellosis in chicken (assessed through the mortality level during the week following an intravenous inoculation with a high dose of bacteria) (Hu *et al.*, 1997) and to carrier state in adult hens (Beaumont *et al.*, 2003*b*) or pullets (Girard-Santosuosso *et al.*, 2002). Similar variations with age should also be the case of a large proportion of genes found to be involved in chicken antibody response to vaccination (Kaiser *et al.*, 2002) or caecal contamination a few days after inoculation (Lamont *et al.*, 2002).

When considering production traits, it must first be observed that estimated values of heritability of production traits were within the range of literature estimates (see Szwaczkowski, 2003, for a review). Since genetic correlations were estimated with low accuracy, results should be taken with caution. Most estimated genetic correlations were of moderate value, showing loose biological relations between resistance, laying rate and egg or body weight and suggesting that selection for an increased resistance should not have much effect on selection on those traits. The same holds for eggshell quality (i.e. resistance to deformation and breaking, data not shown). Interestingly, correlations between $young_{logcfu}$ and number of eggs laid after 25 weeks of age were positive and the probability of being negative was only 6% for correlation with the number of eggs laid between 41 and 60 weeks of age, suggesting that selection for increased laying rate would increase susceptibility to *Salmonella* carrier state at a younger age. This result is coherent with the higher susceptibility of the commercial egg-type line that was compared, in the same conditions, with different experimental lines by Duchet-Suchaux *et al.* (1997), among which the meat-type Y11 line was the most resistant. The negative value of the estimated correlation between $young_{logcfu}$ and early laying rate (with a probability of being negative of 80%) could correspond to a positive effect for resistance of a quicker maturity with regard to both reproductive and immune tissues but this hypothesis must be confirmed.

The signs of most genetic correlations between production traits and adult contamination differed to that of correlations with chicken resistance. This is consistent with the negative genetic correlation between chicken and adult resistance. That was especially the case for genetic correlations with number of eggs laid at different time periods, especially at the beginning of lay. On the whole, the signs of correlations with laying rate varied with age, indicating no clear putative effect of selection for increased laying rate on resistance to adult contamination. If confirmed, the negative correlations between egg colour and $adult_g$ should also be further investigated.

Genetic control of resistance appears to be very complex, which emphasizes the importance of a very precise definition of the trait, including *Salmonella* strain, route and dose of inoculation, organ where resistance is assessed, interval post inoculation, etc. Because of the large number of selection criteria that could be used, their choice is a main issue, which should be based on estimated genetic parameters and thus expected selection response and on a study of the impact of such a selection on the whole flock contamination rate and level. Though promising these results may seem, selection for an increased resistance to carrier state would be very difficult to implement since experimental infections, which are both very expensive and time consuming, are required. Identifying the underlying genes could make it possible to alleviate the need of such measures. This step should take advantage of data and samples collected in this experiment to validate the interest, in animals close to commercial ones and at both young and adult ages of those genes whose effect are already demonstrated or suggested.

5. Conclusion

This study confirmed that resistance or susceptibility to carrier state at both ages and in all organs exhibited a genetic background. Selection for reduced carrier state is possible and might profitably be used as an additional mean of prevention of human food poisoning. It also emphasized the importance of the definition and choice of the selection criterion, especially because of the complexity of genetic control of carrier state. Since genetic correlations between results obtained at a younger or older age are low and negative, results obtained in chicks should not be extrapolated to adult hens without any validation. These lines could be useful to such investigations but also for further understanding of genetic control of carrier state and of interaction between host and pathogen. This selection experiment should therefore be followed until larger differences are observed.

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