

Selection for increased resistance to *Salmonella* carrier-state

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Introduction

In France as in other countries, *Salmonella* remain a major cause of human disease related to food consumption. In one third of cases, the serotype responsible for human food poisoning is *Salmonella* Enteritidis (Bouvet *et al.*, 2002). Poultry products are the main source of human *Salmonella* infections, mostly because chickens may be asymptomatic carriers (*i.e.* remain contaminated by *Salmonella* for several weeks without showing any symptom that could help to their detection). Both caecal and ovarian *Salmonella* carrier-states may be involved in human contamination. While the latter may result in vertical transmission of *Salmonella* and in yolk contamination, the former is responsible for a horizontal transmission of the bacteria and for human disease through contamination of the egg shell at the oviposition and of the carcass during evisceration. In both cases, the existence of asymptomatic carriers dramatically complicates the prophylaxis of this disease.

Food safety could potentially benefit from an increase in the genetic resistance of fowls to the *Salmonella* carrier-state (*i.e.* a better ability of animals to clear *Salmonella*), which can be measured by the persistency of the bacterial infection after inoculation. To address this question, experimental models of infection were defined in chicks (Duchet-Suchaux *et al.*, 1995) and adult hens (Protais *et al.*, 1996). Using these models, the heritability of resistance was estimated at 0.20 in young birds (Berthelot *et al.*, 1996) and more than 0.35 in laying hens (Beaumont *et al.*, 1999). These results show that the *Salmonella* carrier-state is partly genetically controlled and strongly suggested that selection for increased resistance to *Salmonella* colonisation could be efficient and reduce the risk of foodborne *Salmonella* infection. A selection experiment has therefore been realized 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. The goal of this work is to present the first results of this experiment, which are both responses to selection and estimated genetic parameters.

Material and methods

The selection experiment was carried out from a base population that consisted of 79 animals sampled from a layer-type line. Two series of divergent lines were selected, for increased or decreased resistance at a younger age or at the peak of lay respectively. The PT4 *Salmonella* Enteritidis (SE) strains 5556 and 1009 used to evaluate resistance at both ages were a wild

strain isolated from a human case of toxi-infection and a mutant resistant to nalidixic acid (Nal) and streptomycin (Sm), respectively.

Assessment of resistance: In adult, hens were orally contaminated at the peak of lay with 10^9 colonies forming units (c.f.u) of SE PT4 5556 and bacteria were searched in caeca, spleen, liver and ovary four weeks later, as described in Protais *et al.* (1996). Within each hatch, families were randomly divided in three cells of the protected area. A total of five traits were thus considered, i.e. presence/absence of *Salmonella* in each of these four organs as well as presence/ absence of the bacteria in either spleen, liver or caeca (thereafter called contamination rate). The latter was coded “1” if at least one organ was found positive and “0” in the other cases. Selection was made on the latter trait.

Resistance of chicks was measured, as described in Duchet-Suchaux *et al.* (1995) five weeks after oral inoculation with 5×10^4 c.f.u. of SE PT4 1009 at one week of age. Within each hatch, animals were dispatched in four cages and care was taken of a homogenous repartition of families among cages. Resistance was assessed by the logarithm of the number of c.f.u. per caeca (i.e. contamination level) except in two hatches (consisting of 395 and 295 chicks respectively) where *Salmonella* could only be found in a small proportion of animals at very low contamination level. In that case, resistance was assessed as an all-or-none trait (presence versus absence of contamination, also called contamination rate).

A total of 3817 animals were thus measured, among which 1408 at the adult age and 2409 at the younger age. In the latter, resistance could be measured as an all-or-non trait in 690 animals and in a quantitative trait in 1719 chicks.

Methods of estimation of genetic parameters: Heritability of chick contamination level was estimated at each generation with the REML software (Neumaier and Groeneveld, 1998). An animal model was used, accounting for the effect of the cages. For all-or-none traits (chicken and adult contamination rates as well as contamination rates of individual adult organs) a software based on the threshold model (Gianola and Foulley, 1983) was used (Chapuis et Beaumont, 2002). In that case, a sire, dam and maternal grandsire model was considered and the fixed effects of cages and cells fitted. In the last generation, Gibbs sampling was used to analyse both types of traits. In every case, the data set grouped all available data from the base population.

Methods of selection: As inoculated animals could no more be kept for reproduction, sib-selection had to be achieved ; at each generation, selection candidates were produced and reared in protected facilities in one or two additional hatches. Lines selected on resistance at a younger age were distinguished from those selected on resistance at the adult age from the second generation on.

For chick contamination level, BLUP estimated breeding values (EBV) were obtained using the Pest software (Groeneveld, 1990) and the model described above. Breeding values of adult resistance were estimated using the software and the model previously described. Until generation 2, selection was based on global resistance, assessed as the mean estimated values for adult and chicken resistance. Because of increasing evidence of a negative correlation between both measures, lines selected on young stage resistance were then completely distinguished from those selected on adult resistance. The selection criterion for adult resistance was contamination rate, while selection for chick resistance was based on the mean of estimated breeding values for both contamination level and rate.

Mean numbers of candidate sires (brothers of the animals measured for resistance) and of sires used to obtain further generations were, in lines selected on chick resistance, 32.5 and 13.25 respectively, and 18.5 and 11.14 in lines selected on adult resistance.

Results

Response to selection: Response to selection could hardly be estimated before lines selected on chicken resistance were separated from those selected on adult resistance. Two generations later, however, a significant difference in chicken contamination level could be observed: means contamination levels differed by 0.4 log and, when keeping only sires with extreme genetic values, by 1 log.

With reference to adult contamination, clear and significant differences were observed earlier; they increased with generation; in the fifth and last generation, mean contamination rates in the resistant and susceptible line respectively were 55 and 70 % for caeca, 17 and 21% for liver and 42 and 74 % for spleen while no difference in ovary contamination could be observed. Similar or larger differences had been observed in the former generation (i.e. 19, 20, 25 and 4% for caeca, liver, spleen and ovarian contamination).

Estimation of genetic parameters: For all traits, significant heritabilities were estimated and all of them were higher than 0.20. In chicken, estimated heritability was higher for contamination level than for contamination rate. In adult, estimated heritability for global contamination rate was 0.17. With reference to individual organs, heritability was higher in caeca and lower in ovary while genetic correlations between contamination rates of the four organs were positive. When correlation with ovary contamination rate was considered, they were low or very low, while they were moderate in the other cases.

Two major and unexpected results were obtained: two negative genetic correlations were estimated, between adult and chick resistance in one hand and between the ability of chicks to clear *Salmonella* (i.e. presence/absence of *Salmonella*) and the chicken contamination level in the other hand.

Discussion

Indeed, the large and unpredictable variations in *Salmonella* clearance do not facilitate selection at a younger age, especially as level of contamination and presence/absence of *Salmonella* are uncorrelated. One solution could be to slaughter a representative sample of animals at regular intervals, in order to find out the relevant *post inoculation* interval at which resistance should be achieved and resistance be assessed as an all-or-none trait with an optimal contamination rate (of about 50%), as suggested by Duchet-Suchaux (personal communication). But such a strategy would heavily complicate the organization. It was therefore decided to slaughter animals at a given interval and use all available information. This explains why two traits could be observed when measuring chicken resistance. This gave us interesting and expected results on the genetic difference between contamination level and contamination rate.

At the beginning of the experiment, the hypothesis of a partly different genetic control of adult and chicken resistance was assumed but no clear antagonism was expected. This feature probably explains why differences between lines selected on chicken resistance are still moderate. Moreover efficiency of sib-selection was partly reduced by the relatively small family size resulting in low precision and moderate selection pressure. While between-lines differences is expected to increase soon, larger phenotypic differences could be observed when further selecting animals with high or low estimated genetic values. These animals might already be used as genetic models of resistance and susceptibility

Adult selection seems to be, at least until now, more efficient; between lines differences are already important and might be exploited for between lines comparisons. Indeed, estimated genetic correlations between presence/absence of *Salmonella* in different organs appeared to be positive and of average value, which is favourable for selection: for example increasing resistance to spleen contamination should also result in a decreased ovary contamination. This result is also in favour of considering the global animal contamination, since it is more precisely assessed and combines several traits, all of which are positively correlated.

In the whole, genetic control of resistance is very complex: it depends on many factors such as the *Salmonella* strain, the inoculated dose, the organ, the interval *post inoculation*. This emphasizes the importance of a very precise definition of the trait. Because of the large number of selection criteria that could be used, their choice is also a main issue; it should be based on a study of their impact on the whole flock contamination rate and level.

Though promising these results may seem, selection for increased resistance would be very difficult to implement, as it requires experimental infections, which are both very expensive and time consuming. Identifying the underlying genes could make it possible to alleviate the need of such measures. Indeed, the effects of several genes on resistance to *Salmonella* were already demonstrated or suggested, as in Beaumont *et al.* (2002), Girard-Santosuosso *et al.* (2002), Kaiser *et al.* (2002), Lamont *et al.* (2002), Mariani *et al.* (2002) Tilquin *et al.* (2005) or Sadeyen *et al.* (2004). Such an experiment can also be used to test their interest in commercial stocks, as already done in Beaumont *et al.* (2002).

Conclusion

This study confirmed that resistance 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. This selection experiment also emphasized the importance of the definition and choice of the selection criteria, especially because of the complexity of genetic control of genetic resistance. In particular, care must be taken to reduce or avoid detrimental effects that selection for increased resistance at an age could have on resistance at another age. Further investigations will be necessary to choose the relative importance to give to the relatively large number of unrelated selection criteria which could be used. These lines should also be useful to further understanding of genetic control of resistance and of host-pathogen interactions.

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Table 1: Number, per generation and lines, 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)	Not yet done