ESTIMATED HERITABILITIES OF DIFFERENT MEASURES OF RESISTANCE TO *SALMONELLA ENTERITIDIS* IN FOWLS

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SUMMARY

When resistance to *Salmonella enteritidis* (SE) was assessed through survival of day-old chicks intra-muscularly inoculated with the lethal dose 50 %, h^2 was at 0.34 ± 0.13. When 13-week-old chicks were intravenously inoculated with 10^6 SE, estimated heritabilities of SE levels in spleen, liver and genital organ three days after inoculation ranged from 0.06 ±0.04 to 0.13 ± 0.07. Heritabilities of resistance to SE contamination of spleens and caeca four weeks after oral contamination of laying hens with 10^9 SE were at 0.32 ± 0.23 and 0.38 ± 0.25 respectively. Selection for reduced contamination should be feasible but further investigations are needed to estimate the genetic correlations between these four measures as well as with laying intensity.
Keywords: Genetic resistance, fowl, Salmonella, heritability, carriage.

INTRODUCTION

The mortality due to *Salmonella* varies much between poultry lines as shown by Hutt and Scholes in 1941. In 1988, Bumstead and Barrow inoculated day-old chickens of various inbred lines and recorded mortality of animals. Their results strongly suggested that the differences between lines were due to the existence of a major gene. It could be the chicken equivalent of the mouse *Ity* gene of resistance to Infection by *Salmonella typhimurium* (Plant and Glynn, 1976). NRAMP1, the candidate gene for the latter gene has been cloned in chicken (Hu et al., 1996). But nothing was known about resistance to contamination and carriage. This problem is of great importance because of the high number of human toxi-infections due to *Salmonella*, in peculiar *Salmonella enteritidis* (SE) (Desenclos *et al.*, 1996). The role of genetics could be suspected, in this case too, as large variations of resistance had been observed between lines. Three different protocols of infection and measure of resistance have been considered and performed on the same four poultry lines. Guillot et al. (1995) studied mortality after intramuscular inoculation of day-old chickens as in Bumstead and Barrow (1988). Girard-Santosuosso (1997) developped a model of infection similar to those used to study the effects of the *Ity* gene in mice; she observed significant differences in SE levels in livers and caeca three days after an intravenous inoculation. Protais et

al. (1996) observed that the frequencies of SE contamination of spleens, genital organs and caeca four weeks after oral inoculation with 1.5×10^8 c.f.u. also significantly varied.
These results strongly suggest the role of genetics but do not prove it. The goal of this paper was therefore to estimate the heritabilities of these three measures of resistance. But all these inoculations require protected areas. After recording of their resistance, even the surviving animals may no longer be used as breeders so that selection should be on collaterals. Mimicing an infection by vaccinating with an attenuated strain and measuring antibody response would facilitate selection much, provided that the heritability of this criterion as well as the genetic correlation between the latter and the resistance to infection with virulent strain are high enough. The heritability of antibody response after vaccination was therefore also estimated. The analysis were performed on an egg-type line which had been classified as susceptible in all the former analysis.

MATERIAL AND METHODS

Resistance to mortality of chickens. A total of 419 chicks issued from 53 sires and 120 dams, produced in three hatches, were intramuscularly inoculated at one day of age with a dose close to the lethal dose 50 % of the poultry line and reared in isolated units. Chicks that were alive 10 days after inoculation (*i.e.* 32.4 %) were coded as resistant and the others as susceptible. The heritability of this "all-or-none" trait was estimated using Gianola and Foulley's (1983) threshold model and fitting the fixed effects of the three hatches and the random effects of the sire and dam of the animals.

Resistance to contamination after intravenous inoculation at 13 weeks of age. A total of 307 13-weeks-old animals were produced in one hatch. All of them were intravenously inoculated with 10^6 SE. All the animals were slaughtered three days after inoculation and the livers, spleens and genital organs of all animals cultured for SE. The heritabilities of the number of colonies per each of the 3 organs were estimated using Groeneveld's (1996) VCE 3.2 package. An animal model which took into account the fixed effects of the hatch and of the sex was fitted. As a PCR polymorphism had been detected by Girard-Santuosso et al. (1996) for VIL1, which is very close to NRAMP1 (Hu et al. (1995), the within sire effect of the VIL1 allel on the SE levels was tested.

Antibody response to contamination. Antibody response 4 weeks after vaccination at 20 and 24 weeks of age with 1.01×10^8 and 1.45×10^8 c.f.u. respectively with an *aroA* strain of *Salmonella* enteritidis were measured on 228 hens, hatched on the same day and reared in the same building. IgG response was measured using the ELISA test described in Kles *et al.* (1993). The positive antigen was composed of SE lipopolysaccharids. The heritability was estimated using Groeneweld's (1996) VCE software and an animal model.

Resistance to contamination of hens at peak of laying. 304 hens issued from 57 sires and 162 dams were produced in two hatches. They were inoculated at 24 and 27 weeks of age in the two hatches respectively with 1.5×10^8 c.f.u. as described in Protais *et al.* (1996). The presence of SE in spleens and caeca was investigated and animals coded as resistant if no SE could be detected

(*i.e.* on 35.5 % of animals for both organs). The model of analysis described for mortality was used.

RESULTS AND DISCUSSION

Resistance to inoculation. Most estimated heritabilities were high: 0.34 ± 0.10 for resistance to mortality of day-old chicks, 0.32 ± 0.23 and 0.38 ± 0.25 respectively for spleen and caeca contamination of laying hens 4 weeks of inoculation. Heritability estimates were lower when the intravenous route was used: 0.10 ± 0.05; 0.06 ± 0.04 and 0.13 ± 0.07 for SE levels in spleen, liver and ovaries respectively. All these estimates are significantly higher than zero (when making the assumption that the asymptotic normal distribution is reached). This was also the case when considering resistance to caecal carrier-state of chickens (Berthelot *et al.*, 1997) using the protocol described by Duchet-Suchaux *et al.* (1995). This suggests that selection for increased resistance to inoculation should be efficient and should lead to reduced mortality rate or decreased percentage of contaminated animals.

Moreover the resistance could be partly due to a major gene of resistance as suggested by Bumstead and Barrow (1988) when considering resistance to mortality. The effect of VIL1 was significant when considering the SE level in spleen and liver three days after intravenous inoculation ($P < 0.01$). If this result was confirmed it would provide a marker gene of resistance. This result is in good agreement with what is known about the role of the Nramp1 gene in mice. However in chickens its effect appears to be slightly different (Girard-Santosuosso, 1997). Other genes could be implicated as it is the case in mice.

Nothing is known yet about the genetic correlations between these measures which differed by the age, the interval post inoculation, the route of inoculation and the criteria of resistance. The age could play a role as the ranking of the 4 lines mainly varied with the age (Guillot *et al.*, 1995; Protais *et al.* (1996) and Girard-Santosuosso (1997). The intervals post inoculation were chosen according to kinetics of contamination of the four poultry lines previously studied in order to maximize the differences between poultry-lines, (Beaumont et al. (1995) and Girard-Santosuosso (1997)). As the measures were made at different ages it is impossible to appreciate the influence of the criteria of resistance (mortality, SE level or SE contamination) on heritability estimation. At the opposite the difference between estimated heritabilities of contamination after oral inoculation of laying hens or after intravenous inoculation of 13 week-old animals was unexpected. As the latter protocol of infection bypasses the intestinal barrier, mean SE levels obtained on different hatches are more reproductive (Girard-Santosuosso, 1997). This had lead to the hypothesis of a lower effect of environment in the latter protocol of infection and thus of a higher heritability. This result makes us reject this hypothesis. If it was confirmed, it would suggest that, at least for older animals, the intestinal barrier is under a genetic control.

Antibody response to vaccination. The estimated heritability was not significantly different from zero (0.03 ± 0.08 at 24 weeks and 0.10 ± 0.08 at 28 weeks). Selecting for this response would thus be very long and most probably unsuccessful. Further investigations on heritability of antibody response measured at a smaller interval post inoculation or on IgM response are needed before definitely giving up such an indirect criterion.

CONCLUSION

All these results show that selection for increased resistance could be envisaged. But further investigations are needed to estimate the genetic correlations between all these measure as well as with traits of economic importance. The first result will allow to choose the most appropriate criterion of selection, the latter to estimate the expected indirect response to selection for increased resistance. Showing the role of major or marker genes would also be of great help as it could make inoculation with pathogen bacteria useless, thus avoiding the need of protected areas as well as of selection on collaterals.

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