Comparison of resistance of various poultry lines to infection by Salmonella enteritidis (genetic resistance)

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Original article

Comparison of resistance of various poultry lines to infection by *Salmonella enteritidis*

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Summary — A study was undertaken to determine the susceptibility or resistance of 9 outbred experimental or commercial poultry lines to *Salmonella enteritidis* PT4. Young chicks were inoculated either intramuscularly or orally just after hatching. After intramuscular challenge the lines could be divided into susceptible lines (LD 50% ≤ 10^2 *Salmonella* per animal), intermediate lines (LD 50% about 10^4 *Salmonella*) and resistant lines (LD 50% > 10^5 *Salmonella*). The results obtained after oral challenge confirmed these 3 groups for both mortality rates and the probability of the presence of salmonellae in the spleen and liver. There was no difference between lines concerning caecal carriage.

poultry / genetic resistance / *Salmonella* / infection

Résumé — Comparaison de la résistance de différentes lignées aviaires à l'infection par *Salmonella enteritidis*. *La sensibilité ou la résistance de 9 lignées aviaires sélectionnées, expérimentales ou commerciales, vis-à-vis de* Salmonella enteritidis *lysotype 4 a été testée. Les poussins ont été infectés dès la naissance, par voie intramusculaire pour le premier essai, et par voie orale pour le second. Après inoculation intramusculaire, 3 classes de sensibilité sont apparues : des lignées sensibles (DL 50% ≤ 10^2 salmonelles/animal), des lignées intermédiaires (DL 50% de l'ordre de 10^4 salmonelles) et des lignées résistantes (DL 50% > 10^5 salmonelles). L'inoculation orale a confirmé ces classes en ce qui concerne le taux de mortalité et la fréquence de contamination des organes internes : rate et foie. Le portage cæcal différait peu selon les lignées.*

poule / résistance génétique / salmonelles / infection

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INTRODUCTION

Eggs infected with *Salmonella enteritidis* have been identified as a major source of human food poisoning in several countries (Coyle *et al*, 1988; Hopper and Mawer, 1988; Hubert, 1988; Peroles and Audicana, 1989; St Louis *et al*, 1988).

This serotype is pathogenic for both humans and poultry; in the latter it may be transmitted both horizontally and vertically (Protais *et al*, 1989; Gast and Beard, 1990). The vertical route of transmission is amplified by the pyramidal structure of the poultry industry (O'Brien, 1990) which may be partly responsible for the increased incidence of food poisoning due to *S enteritidis*. Producing eggs from genetically resistant hens could be a way to circumvent this problem. Bumstead and Barrow (1988) have compared inbred and partially inbred chicken lines. They found differences in resistance to *S typhimurium* in both intramusculary and orally inoculated newly hatched chickens. The same lines ranked in a similar way after an intramuscular challenge with *S enteritidis* (Bumstead *et al*, 1991).

The aim of this study was to determine the susceptibility or resistance to *Salmonella* of several outbred poultry lines (which are more similar to those used commercially). Resistance of chickens after intramuscular or oral infection at 1 d of age were compared, in addition to the level of infection on the spleen, liver and caecal contents, 4 weeks after oral inoculation.

MATERIALS AND METHODS

Birds

Nine outbred poultry lines were tested for resistance to intramuscular inoculation.Y11 is a meat-type control strain selected at the Station de Recherches Avicoles (INRA, Nouzilly, France). PA12 is a White Leghorn strain, B13 a histocompatible inbred White Leghorn (line GB1, originated from Dr Schierman), both developed by the Station de Pathologie Aviaire et Parasitologie (INRA). C1, C2 and C3 are 3 commercial egg-type strains. H-SRBC, L-SRBC and C-SRBC are the lines selected for high or low antibody levels against a multi-determinant and non-pathogenic antigen, sheep red blood cells (Van der Zijpp, 1983; Pinard *et al*, 1992) as well as the parental line (control) from which they originated.

Bacteria

A virulent strain of *S enteritidis* PT4 isolated from an outbreak of food poisoning was used (M Popoff, Institut Pasteur, Paris, France).

Challenge protocol

One day-old chickens were inoculated either intramuscularly or orally. All lines were challenged using intramuscular inoculation. Four lines (1 resistant: Y11; 1 intermediate: PA12; and 2 susceptible: B13 and C2) were challenged by oral inoculation. All chickens were wingbanded for both routes of inoculation. In order to avoid any confusion between dose and family effects, the families were uniformly distributed across the doses.

For intramuscular inoculation, 0.1 ml portions of dilutions of an overnight culture of the strain incubated at 37°C in brain heart infusion broth (Difco) were inoculated by the same person in the breast muscle of at least 5 chickens per dose per line. The calculated doses were 10^2, 10^3, 10^4 and 10^5 *Salmonella* per animal. The real doses inoculated, enumerated after inoculation, varied from 1–1.2 x 10^2 to 1–1.2 x 10^5 per animal.

For oral inoculation, 15–30 chickens per line per dose were inoculated, directly into the crops. Two doses were inoculated: 0.5 ml of an overnight culture of the strain (4–6 x 10^8 *S enteritidis*/animal) and a 10-fold dilution (4–6 x 10^7 *S enteritidis*/animal).

All chicks were reared in isolators and observed twice a day for a period of 15 d for those inoculated intramuscularly, and for a period

of 28 d after oral inoculation. The LD_{50} (lethal dose 50%) were computed using the probit analysis of the SAS statistical package (Mather, 1965).

Six weeks after oral inoculation, the surviving chickens were killed and about 0.1 g of liver and spleen and the whole caecal contents were cultured for *Salmonella* directly on *S shigella agar* (Sanofi Diagnostics Pasteur, France) and following enrichment in Mueller-Kauffmann broth (Sanofi Diagnostics Pasteur, France).

RESULTS

Intramuscular challenge LD_{50}

The estimated LD_{50} are shown in table I. The meat type strain Y11 was the most resistant. B13 and the 3 commercial lines were at the same level; PA12, H-SRBC, L-SRBC and C-SRBC were all intermediate. The mortality rates are shown in figures 1–4 for lines Y11, C2, B13 and PA12, respectively. The mortality rates of lines C1 and C3 were similar to that of line C2, and the evolution of mortality of L-SRBC and H-SRBC was similar to that of line PA12. The mortality rate depended on both the lines and the inoculated dose. Chickens of the more resistant lines (Y11 and, to a lesser extent, PA12) survived longer. Among the susceptible lines, B13 seemed to survive longer than the commercial ones. It is therefore possible to classify these lines as resistant (Y11), intermediate (PA12, H-SRBC, L-SRBC and C-SRBC) or susceptible (with a 'long' survival time: B13; or a 'short' one: C1-C3). The same results were found after a principal component analysis of both LD_{50} and mean survival time.

Oral challenge

The results of the oral challenge are shown in table II. The strains differed significantly and were ranked in the same way for mortality after either intramuscular or oral challenge: Y11 was the most resistant, PA12 intermediate and B13 and C2 the most susceptible. *S enteritidis* was isolated significantly less frequently from the spleen and liver of resistant animals and a similar trend was observed for the livers (but was not significant). *Salmonella* were less frequently isolated from the caecal contents of the Y11 line.

Table I. Susceptibility of chicken lines following intramuscular challenge of 1-day-old chickens.

Chicken line	*Logarithm of estimated LD_{50}*	*Confidence interval*	*Rank*
B13	< 2.0	(No lethality rate < 0.57)	1
PA12	4.35	3.26 – 15.15	8
Y11	> 5	(No lethality rate > 0.28)	9
Commercial 1	2.34	0.25 – 2.95	4
Commercial 2	2.12	1.08 – 2.7	3
Commercial 3	< 2.08	(No lethality rate < 0.11)	2
H-SRBC	3.9	3.0 – 5.1	5
L-SRBC	3.8	3.2 – 4.5	6
C-SRBC	4.1	–	7

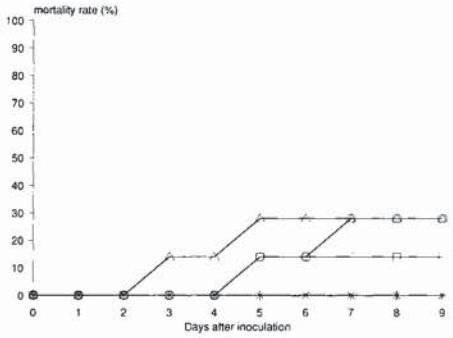

Fig 1. Evolution of the cumulative mortality rate with the number of days following inoculation and the theoretical dose (10^2 (—□—), 10^3 (—✳—), 10^4 (—O—), or 10^5 (—Δ—) *Salmonellae* per animal) in the Y11 line.

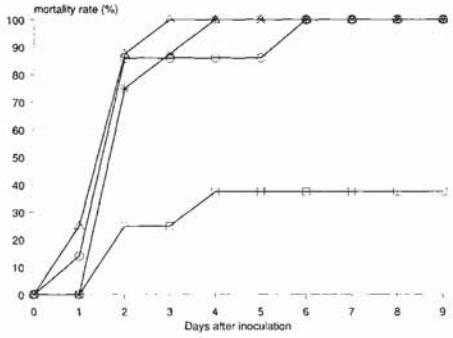

Fig 2. Evolution of the cumulative mortality rate with the number of days following inoculation and the theoretical dose (10^2 (—□—), 10^3 (—✳—), 10^4 (—O—), or 10^5 (—Δ—) *Salmonellae* per animal) in the C2 line.

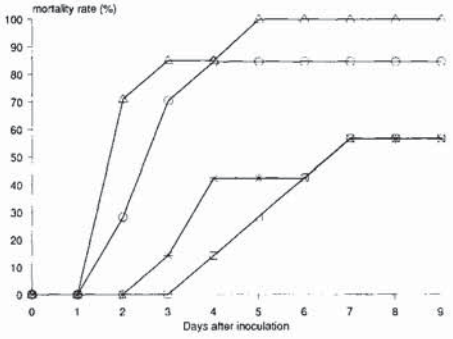

Fig 3. Evolution of the cumulative mortality rate with the number of days following inoculation and the theoretical dose (10^2 (—□—), 10^3 (—✳—), 10^4 (—O—), or 10^5 (—Δ—) *Salmonellae* per animal) in the B13 line.

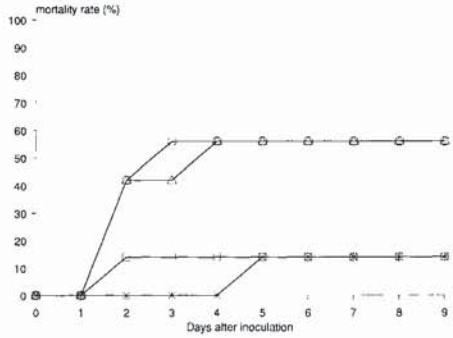

Fig 4. Evolution of the cumulative mortality rate with the number of days following inoculation and the theoretical dose (10^2 (—□—), 10^3 (—✳—), 10^4 (—O—), or 10^5 (—Δ—) *Salmonellae* per animal) in the PA12 line.

DISCUSSION

The LD_{50} of the different lines following intramuscular challenge varied by 100-fold. These differences are similar to those reported by Bumstead *et al* (1991) or Bumstead and Barrow (1993) with inbred poultry lines and 2 different *Salmonella* strains. It is worthwhile noticing that the most resistant strain was a meat-type strain, which could suggest a coselection of growth rate and resistance to salmonellosis. However this strain had been previously eradicated by a stamping out procedure for several diseases including salmonellosis which could result in improved resistance, but this was also the case for the other strains. Alternatively, the higher susceptibility of laying-type lines could result from selection for laying intensity.

Table II. Mortality and presence of *Salmonella* in the organs following oral challenge of chicken lines.

Chicken line	*Dose (cfu/animal)*	*Dead/inoculated*	*Presence of* Salmonella *(No positive organs/No surviving animals)*		
			Caecum	*Spleen*	*Liver*
Y11	5×10^8	2/29	$14/15^a$	$9/15^a$	$2/15^a$
Y11	5×10^7	2/36	$13/15^a$	$9/15^a$	$3/15^a$
B13	5×10^8	21/21			
B13	5×10^7	15/21	$6/6^a$	$6/6^b$	$2/6^{bc}$
PA12	6×10^8	2/30	$15/15^a$	$11/15^{ab}$	$3/15^a$
PA12	6×10^7	4/30	$15/15^a$	$8/15^a$	$0/15^{ab}$
Comm 2	4×10^8	9/19	$10/10^a$	$10/10^b$	$6/10^c$
Comm 2	4×10^7	7/19	$12/12^a$	$9/12^{ab}$	$4/12^{ac}$

* Two rates followed by different superscripts in the same column are significantly different ($P < 0.05$).

Time to death also varied widely and depended on both the challenge doses and the poultry line. This is compatible with the existence of an '*Ity*-like' gene, a major gene conferring resistance to infection by *S typhimurium* in mice (Plant and Glynn, 1974, 1976). This hypothesis was also suggested by the crosses of susceptible and resistant lines of chickens by Bumstead and Barrow (1988). We have also observed that the susceptible lines vary in survival time, which suggests that different genes and mechanisms of resistance could be involved. This point needs further analytical investigations, which will be complicated by the fact that the putative resistance genes are very probably segregating in outbred lines.

Results of the oral challenge showed the same ranking of the lines for both mortality rate and probability of presence of *Salmonella* in the internal organs as those of intramuscular challenge. This result is significant since it suggests that the lethality test after intramuscular inoculation, which is cheaper and easier to perform, leads to similar results as the oral challenge.

The most resistant lines carried *Salmonella* less often and hence the potential risk for consumers of eating infected meat derived from them is reduced. According to Bumstead *et al* (1991), these lines are also presumably more resistant to other *Salmonella* serotypes pathogenic for chicken. Further investigations are needed to determine whether adults of resistant lines also carry *Salmonella* less often in the ovaries, this organ being one of the most likely to be infected by invasive serotypes (Hopper and Hawer, 1988). It is probable that other host genes will be involved since in mice the *Ity* gene controls only the initial exponential growth rate of the bacteria (Hormaeche, 1979).

Resistance of poultry lines to mortality following oral or intramuscular inoculation of a virulent *Salmonella* strain varies in large proportions. This suggests the possibility of increasing the resistance of the most susceptible commercial flocks. A further important step is to determine whether adult resistant hens also lay fewer (or no) infected eggs. A second important step is to reduce the healthy intestinal carriage.

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