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Faecal shedding and intestinal colonization of *Salmonella enterica* in in-bred chickens: the effect of host-genetic background

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SUMMARY

Considerable and reproducible differences were observed in the amount and duration of faecal excretion when in-bred lines of chickens were infected orally with *S. enterica* serovar Typhimurium at 6 weeks of age after being given a gut flora preparation when newly hatched. Similar but less pronounced results were observed with *S.* Enteritidis or *S.* Infantis. Differences in the viable numbers of the inoculated bacteria in caecal contents were detectable within 24 h of inoculation. No major differences were seen in *Salmonella*-specific serum IgA or IgG titres. Small differences were seen in the numbers of circulating heterophilic cells. Caecal contents taken from the more resistant lines immediately prior to challenge appeared to be no more inhibitory for *Salmonella in vivo* than contents taken from susceptible lines. The more resistant lines showed a slightly higher rate of intestinal flow, as indicated by the rate of production of faecal droppings, although there was no difference in the rate of emptying of the caeca. In an F1 generation resistance was dominant and not sex-linked. There was no MHC linkage or any association with *SAL1*, the gene implicated in resistance to systemic salmonellosis in chickens, or *NRAMP1*.

INTRODUCTION

Salmonella remains one of the major causes of bacterial food poisoning for man and poultry are thought to be the major source. It is thought that introduction of the European Directive on food-borne zoononses [1] and national legislation [2, 3] together with the application of a killed vaccine has led to considerable reductions in the incidence of *S.* Typhimurium- and *S.* Enteritidis-infected flocks and in the frequency of infected birds within flocks [4] although this has again recently started to increase. This, combined with the use of killed vaccines in poultry, has slowly been translated into a reduction in the number of cases of food poisoning attributable to this serotype [4].

Although *S.* Typhimurium, *S.* Enteritidis and the vast majority of the remaining *Salmonella* serovars generally produce little systemic disease in adult chickens they are able to colonize the alimentary tract of poultry. As a result of this they contaminate poultry carcases and enter the human food chain. Control of human food-borne salmonellosis must inevitably involve control of the infectious agent in the host animal. Attempts have been made to do this through increases in the standards of hygiene, housing, feed quality and management [5]. However, the high costs incurred would put the national poultry industry at a financial disadvantage in comparison with countries which do not introduce such measures. Biological methods of control have therefore been sought. These include (i) antibiotic usage, which has the obvious public health risks of selection for, and increased colonization by, resistant bacteria, (ii) use of competitive intestinal flora preparations, the efficacy of which is variable and (iii) the use of live or killed vaccines. There are also financial costs incurred in the extensive application of (ii) and (iii), particularly for broiler use, and their uses may effectively be limited to breeder

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birds until economic incentives for their application are introduced.

The breeding and rearing of chickens which might be inherently more resistant to *Salmonella* infection is thus an attractive option. Considerable work has been carried out on the genetic basis for the variations in resistance/susceptibility observed in out-bred [6–9] and in-bred [10, 11] lines of chickens to the acute systemic *Salmonella* infections produced in newly hatched chickens by *S*. Typhimurium, *S*. Enteritidis and *S*. Pullorum and in older birds infected with *S*. Gallinarum. This resistance appears to be expressed at the level of the reticulo-endothelial system and correlates with increased bacterial killing by blood monocyte-derived macrophages infected *in vitro* with *Salmonella* [12]. The gene concerned, designated *SAL1*, appears to have little effect on most other aspects of systemic infection, including colonization of, or invasion from, the alimentary tract and localization in the reproductive tract [13, 14].

Colonization of the alimentary tract by *Salmonella* does not require association with the intestinal epithelium [15] and in some ways may be expected to be independent of host factors other than immunity. A phenotype such as duration of faecal shedding might be expected to segregate according to MHC type, since this might be expressed as the degree of immunological response to the infection. This might become detectable several weeks after infection as has been found with commercial lines [16–18]. This paper, however, reports differences in the amount of faecal shedding between in-bred lines of chicken soon after oral infection.

MATERIALS AND METHODS

Bacterial strains

S. Typhimurium F98 [15, 19] and *S*. Enteritidis P125109 [20, 21] are virulent for newly hatched chickens and colonize the chicken alimentary tract well [15, 20]. They are very invasive [15, 20] and generate strong immune responses in birds [20, 22, 23] *S*. Infantis 1326/28 is an avian strain which colonizes the chicken intestine and is avirulent for chickens [24]. To facilitate enumeration and re-isolation from faeces and caecal contents, spontaneous mutants, resistant to nalidixic acid, were used for all these strains. This mutation has no effect on intestinal colonization ability or virulence [25].

Bacteria were cultured in 10 ml volumes of LB broth (Difco), incubating for 24 h in a shaking incubator (100 r.p.m.) at 37 °C. This resulted in counts of between 1×10^9 and 3×10^9 c.f.u./ml. Chickens were inoculated orally with either 0·1 ml (1- or 2-day-old chickens) or 0·3 ml (older birds) undiluted cultures.

Chickens

All birds were from specified-pathogen-free flocks reared at the Institute for Animal Health, Compton, UK. The in-bred lines which have been described previously and their MHC haplotypes are known [26]. Their susceptibility to acute systemic salmonellosis, attributable largely to the *SAL1* gene, has also been characterized [10, 11]. Lines 6_1, W1 and N are resistant and lines 7_2, 15I and C are susceptible. They were reared in metal cages and on animal protein-free feed as described previously [27].

Experimental plan

Chickens of different lines were inoculated orally when 1 day old with 0·1 ml of an overnight LB broth culture, incubated statically, of caecal contents taken from an adult SPF out-bred chicken from the IAH flock. This was done in order to avoid the development of different flora in the different lines, as may have occurred with earlier work [16, 18, 28]. Chickens from different lines were tagged with two wing-bands and were then reared together for 6 weeks, by when they were immunologically mature [29, 30]. At this time they were divided into separate cages according to genetic line and were immediately inoculated orally. In the first experiment (Expt 1) three rooms were used, each containing two lines. In Expt 2 all three lines were housed in separate cages in the same room.

Experiment 1

Thirty chickens, each of the six different lines, were reared and infected as indicated above. In this experiment three rooms were used, each containing two lines. Each chicken was infected orally with *S*. Typhimurium F98 Nal^r. Cloacal swabs were taken from each bird and plated as described below.

Experiment 2

In Expt 2 all six lines, each of 30 birds, were housed in separate cages in the same room. The birds were infected and sampled as described in Expt 1. The rate of production of faecal and caecal droppings was

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measured over a 2-h period for several mornings between the ages of 5 and 7 weeks for lines N and 6_1.

Experiment 3

Two groups of birds each from lines 6_1 and N were reared as described in Expt 1. Groups of 20 birds from both lines were infected with the *S.* Enteritidis or *S.* Infantis strains as described above and cloacal swabs were taken and processed as described below.

Experiment 4

Three groups each of 20 birds from lines 6_1 and N were caged separately as newly hatched chickens. One group from each line was infected with a gut flora preparation as described above but the birds were challenged 24 h later with *S.* Typhimurium F98. The remaining four groups did not get a gut flora preparation. Two of the remaining N and 6_1 lines were infected with the same *S.* Typhimurium strain when 4 days old and the remaining two infected with *S.* Enteritidis, also at this time. The age of 4 days was chosen since in the absence of a gut flora extensive colonization would occur in the absence of clinical disease. Birds mature considerably immunologically between 2 and 4 days of age [31] and become resistant to systemic disease caused by these two serotypes [32]. Cloacal swabbing was carried out weekly on all birds as described above.

Experiment 5

F1 birds produced by crossing birds from lines N and 6_1 reciprocally were reared together with the pure parent lines. Thirty progeny of each of the crosses together with 15 of the pure N and 6_1 lines, were inoculated with a gut flora preparation when newly hatched. They were reared and challenged when 6 weeks old with *S.* Typhimurium as described in Expt 1. Cloacal swabbing was carried out weekly and seven birds from both of the pure lines were bled for specific serum antibody titration.

Experiment 6

A standard infection experiment as described in Expt 1 was set up in 35 6-week-old birds of lines N and 6_1 that had been given a gut flora preparation when newly hatched. The chickens were infected with *S.* Typhimurium. Birds were killed at 1, 4, 7, 14 and 21 days post-infection (p.i.) for enumeration of the inoculated *Salmonella* in the caecal contents and histological examination of blood.

Experiment 7

Groups of 10 birds of lines N and 6_1 were inoculated with a gut flora preparation when newly hatched and reared together for 6 weeks. They were then separated and inoculated 3 days later with *S.* Typhimurium and cloacal swabs were taken for 3 weeks. A few days prior to challenge with the *Salmonella*, fresh caecal droppings were collected from beneath the cages in which the two lines were being kept. This was done as follows. A sheet of clean polythene was placed beneath the cage at the beginning of the day. Fresh caecal droppings were then collected over a period of 2 h and the capacity of the flora in these droppings to inhibit the colonization by *S.* Typhimurium was tested in three ways.

(*a*) Several caecal droppings, collected by cotton wool swab, were pooled and emulsified in 5 ml aliquots of sterile phosphate buffered saline (PBS). The preparations from different lines, together with an emulsion of caecal contents obtained from an adult SPF out-bred chicken from the IAH flock, were then used to inoculate three groups of seven newly hatched out-bred (Light Sussex) chickens which were reared in small cages. An additional group of seven chickens did not receive any gut flora preparation. Each bird received 0·1 ml of the preparation immediately prior to being given access to food. Twenty-four hours later each bird was challenged with 0·1 ml of a dilution of an overnight culture of *S.* Typhimurium F98 Nal^r. The birds were killed 3 days later and enumeration of the inoculated strain was carried out on the caecal contents. This experiment was done twice, once with lines N and 6_1 and once with lines 6_1, W1, N and 7_2.

(*b*) Caecal contents were collected by separate swabs and were gently emulsified immediately and separately in 2 ml aliquots of PBS. Preparations from five droppings from each of the two lines were used to inoculate ten groups of five newly hatched Light Sussex chickens. These were inoculated, challenged and examined as described in (*a*) above.

(*c*) Pooled caecal contents emulsified in PBS from lines N and 6_1 were used to inoculate two groups of 30 newly hatched Light Sussex chickens housed in standard wire cages. These were inoculated with *S.* Typhimurium 24 h later. Cloacal swabs were taken weekly and faecal excretion of the inoculated strain was assessed as described below.

Table 1. *Faecal excretion of* S. *Typhimurium F98 following oral inoculation of the Nal^r mutant by in-bred lines of chickens which had been administered a gut flora preparation* (*Expt 1*)

Time (weeks) after infection	Percentage chickens excreting *Salmonella*					
	Resistant line (with MHC haplotype)			Susceptible line (with MHC haplotype)		
	6_1 (Ea B^2)	W (Ea B^{14})	N (Ea B^{21})	7_2 (Ea B^2)	15I (Ea B^{15})	C (Ea B^{12} and Ea B^4)
1/7	88	77	100	100	97	83
1	74	91	100	97	100	100
2	0	17	88	37	47	80
3	0	9	49	29	21	24
4	0	9	55	31	3	6
Caeca	0	0	42	23	3	6

Comparisons were made by χ^2 between rates of excretion between the following pairs of data: 6_1 and N, 6_1 and 7_2, W1 and N, W1 and 7_2. In all cases $P < 0{\cdot}01$.

Bacterial enumeration

Swabs from the cloaca were plated, as described previously [19] on Brilliant Green agar containing sodium nalidixate (20 μg/ml) and novobiocin (1 μg/ml), to enable semi-quantitative enumeration. They were also further incubated in selenite broth for enrichment. The numbers of bacteria excreted were expressed as a percentage of chickens whose faecal excretion resulted in 50 or more colonies of the inoculated strain per plate (>50), 1 colony or more per plate (D = direct) and those positive whether by direct plating or enrichment (T = total). For quantitative enumeration caecal contents were diluted and homogenized in PBS. The viable count of *Salmonella* in the samples was estimated by plating aliquots of decimal dilutions on to the selective Brilliant Green agar which contained sodium nalidixate and novobiocin, as indicated above.

ELISA

Aliquots of 20 μl of blood were taken from the wing vein and diluted in 980 μl PBS containing 20 μl/ml Tween 20 (PBST). These were frozen at −20 °C until needed. The titres of specific IgG and IgA were estimated for each sample by a standard indirect ELISA in Dynatech (Dynex Technologies Inc., Chantilly, VA, USA) microtitre plates [22] using lipopolysaccharide (LPS) from the *S*. Typhimurium as antigen. LPS was used at a concentration of 60 μg per well and alkaline phosphatase-linked rabbit anti-chicken IgG (Sigma) and IgA (Serotec, Kidlington, Oxfordshire, UK) at a dilution of 1:1000.

Haematological examination

Blood films were prepared and stained by haematoxylin and eosin.

RESULTS

Experiment 1

The faecal excretion rates, expressed semi-quantitatively, of *S*. Typhimurium F98 Nal^r in six lines of chickens which had been given the same gut flora when newly hatched, reared together for 6 weeks before being caged according to line and infected, are shown in Table 1. The lines have been grouped according to their resistance to systemic salmonellosis which is mediated largely by the *SAL1* gene. Statistically significant differences in excretion were observed between lines (see Table 1). Line 6_1 chickens eliminated the infection very rapidly and a similar but slightly less marked rapidity of elimination was seen in line W1 birds. At the end of the experiment the inoculated *Salmonella* was not found in any of the birds from either of these lines. In contrast birds from lines N and 7_2 showed much higher rates of excretion and isolation from the caeca at the end of the experiment. Lines 15I and C showed an intermediate pattern of excretion.

The MHC haplotypes indicated no apparent association between faecal excretion and MHC type.

Experiment 2

Since, in Expt 1, lines 6_1 and W1 were housed in one room, lines N and 7_2 were in another and lines 15I and

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Table 2. *Faecal excretion of* S. *Typhimurium F98 following oral inoculation of the Nal^r mutant by in-bred lines of chickens which had been administered a gut flora preparation* (*Expt 2*)

Time (weeks) after infection	Percentage chickens excreting *Salmonella*					
	Resistant line			Susceptible line		
	6	W	N	7	15	C
1	92	100	100	100	100	89
2	100	92	100	97	100	89
3	15	56	76	27	50	70
4	6	0	42	13	11	33
5	9	9	24	10	15	26
6	9	—	26	17	4	15
7	3	—	6	17	4	4
Caeca	0	21	24	20	0	11

Comparisons were made by χ^2 between rates of excretion between the following pairs of data: 6_1 and N, W1 and N, W1 and 7_2. In these cases $P<0{\cdot}01$. For the comparison of lines 6_1 and 7_2, $\chi^2=8{\cdot}67$ and $P=0{\cdot}20$. —, not done.

C were in a third, the experiment was repeated such that birds of each line were housed in the same room. The duration of the experiment was also extended. A similar, if slightly less marked, difference in the patterns of excretion was observed between the different lines, with lines 6_1 and W1 again showing the lowest excretion rates, lines N and 7_2 showing highest excretion rates and lines 15I and C being intermediate but having lower rates of excretion than in Expt 1 (Table 2). Line 6 had the lowest isolation rate and line N the highest rate from the caecal contents at the end of the experiment. The differences in excretion rates between lines 6_1 and W1, and N and 7_2 were again statistically significant (Table 2).

The production of caecal and faecal droppings for the high and low excreting lines N, and 6 were measured several times between the ages of 5 and 7 weeks. The higher number of faecal droppings (612) produced by line 6_1 was of marginal statistical significance ($P=0{\cdot}05$–$0{\cdot}10$ by Wilcoxon's signed rank sum test) in comparison with line N (522) whereas no differences were observed in the number of caecal droppings produced by both lines (22 and 23 from lines 6_1 and N, respectively).

Experiment 3

The faecal excretion patterns for *S.* Enteritidis, as a second invasive serotype, and *S.* Infantis, as a very poorly invasive serotype, in lines 6_1 and N are shown in Table 3. The differences in excretion in this experiment are less clear cut (see results of statistical analysis in Table 3) as with *S.* Typhimurium but showed a similar pattern. The birds in the groups became overcrowded and were thinned out after week 3 since this may have caused the rises in faecal excretion in week 4. However, the rates of excretion of both serovars in line N were higher than they were in line 6, although the degree of significance was greater for *S.* Infantis ($P=<0{\cdot}01$) than for *S.* Enteritidis ($P=0{\cdot}03$).

Experiment 4

The patterns of faecal excretion of *S.* Typhimurium by four lines infected when they were 4 days old after the birds were given a gut flora preparation within 24 h of hatching, are shown in Table 3. Differences similar to those seen in the 6-week-old chickens were not seen until the birds were several weeks old when rates of excretion were lower in the line W1 birds. The differences in excretion rates were not different between lines N and 6_1 ($\chi^2=6{\cdot}93$, 5 D.F., $P=0{\cdot}20$) and became of marginal significance only by week 6 ($\chi^2=4{\cdot}52$, 1 D.F., $P=0{\cdot}035$). The increased significance at 6 weeks was again seen with the difference between lines W1 and 7 ($\chi^2=4{\cdot}89$, $P=0{\cdot}03$). Chickens not given a gut flora but infected with *S.* Typhimurium when 4 days old were expected to have high excretion rates. This was the case with very high rates and no effective difference between birds of lines N (between 18 and 22/22 birds excreting) and 6_1 (20–22/22 birds excreting) between 1 and 7 weeks after inoculation.

Experiment 5

The patterns of faecal excretion of *S.* Typhimurium in lines N and 6_1 and F1 birds produced by reciprocal crosses between the parent lines are shown in Table 4 and Figure 1. The pure lines showed the expected differences in faecal excretion with line 6_1 eliminating infection within 2 weeks and line N remaining infected at 6 weeks p.i. The two F1 groups produced from reciprocal crosses, showed early rates of excretion which more closely resembled those of line 6, with a small number of birds showing more persistent infection or re-infection.

Seven birds of each of the two pure line groups were bled at 2 and at 5 weeks post-*Salmonella* infection.

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Table 3. *Faecal excretion of* S. *Enteritidis P125109 and* S. *Infantis following oral inoculation of the Nal^r mutant by in-bred lines of chickens which had been administered a gut flora preparation* (*Expt 3*)

	Percentage chickens excreting											
	S. Enteritidis in lines						*S.* Typhimurium in lines					
	N			6_1			N			6_1		
Weeks	>50	D	T	>50	D	T	>50	D	T	>50	D	T
1	94	100	100	55	85	100	0	76	100	52	100	100
2	59	88	100	50	90	100	18	41	71	11	32	53
3	41	76	94	15	40	75	24	24	71	5	21	31
Over-crowding												
4	53	88	94	30	65	100	82	100	100	0	11	26

>50, Fifty or more colonies of the inoculated strain per plate; D, one or more colonies per plate; T, samples positive by swab or by enrichment.
For comparison between the two lines with *S.* Enteritidis: $\chi^2 = 5{\cdot}54$, $P = 0{\cdot}03$ for heavy excretion; $\chi^2 = 7{\cdot}59$, $P < 0{\cdot}01$ for direct plating; $\chi^2 = 1{\cdot}49$, $P = 0{\cdot}25$ for total excretion. For *S.* Infantis: $\chi^2 = 30{\cdot}24$, $P < 0{\cdot}01$ for heavy excretion; $\chi^2 = 21{\cdot}35$, $P < 0{\cdot}01$ for direct plating; $\chi^2 = 11{\cdot}03$, $P < 0{\cdot}01$ for total excretion.

Table 4. *Faecal excretion of* S. *Typhimurium in 6-week-old chickens with gut flora-chickens of lines N, 6 and F1 crosses* (*Expt 5*)

	Percentage of chickens from the following lines excreting *Salmonella*							
	6		N		N/6		6/N	
Weeks	D	T	D	T	D	T	D	T
1	20	73	93	100	37	43	37	60
2	0	0	67	87	0	20	0	15
3	0	0	7	60	3	6	0	10
4	0	7	8	58	6	6	3	6
5	0	0	0	33	0	3	0	0
6	0	0	17	33	0	3	0	0
Cc	0	0	0	25	0	0	0	4

Data are percentage of chickens in each group excreting the inoculated *Salmonella* strain in the faeces. D, inoculated *Salmonella* isolated by direct plating; T, inoculated *Salmonella* isolated by direct plating or after enrichment culture.

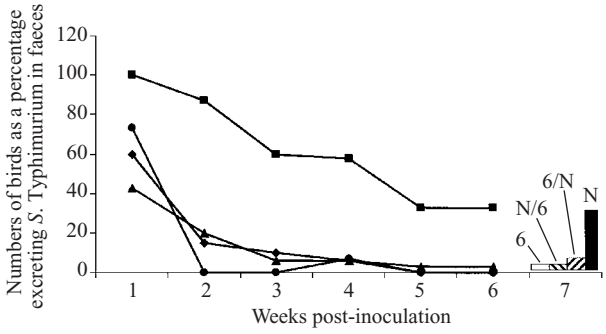

Fig. 1. Faecal excretion of *S.* typhimurium F98Nal^r by chickens from lines N (■) and 6_1 (●) and from F1 crosses $N \times 6_1$ (◆) and $6_1 \times N$ (▲). The columns represent the number of birds from each group, as a percentage, from which the inoculated strain was isolated from the caeca.

The specific anti-LPS titres were standardized against a negative control serum. The geometric mean of the Log_2 IgG titres at 2 and 5 weeks were 3·35 and 6·05 for line N and 3·25 and 4·95 in line 6_1. The mean Log_2 IgA titres at 3 weeks were 4·08 and 3·23 for lines N and 6_1.

Experiment 6

The viable numbers of the inoculated strain in the caecal contents of birds of lines N, 6_1, W1 and 7_2 are shown in Table 5. At 1 day p.i. differences in the bacterial numbers could be detected in the caecal contents, with the counts from lines 6_1 and W1 being lower than those from line 7_2 and N. Similar differences were seen at 4 and 7 days after inoculation. The differences showed greater statistical significance at day 1 than later in the experiment. The counts fell away with time until the bacteria could not be reliably quantified using these methods. However, the numbers that were positive reflected the counts and the results from the first part of this experiment and Expt 2.

At 2 and 7 days p.i. increased numbers of heterophils were observed in the blood of line 6_1 birds compared to similar samples from line N birds (Table 6).

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Table 5. *Bacterial numbers* (*mean* ± *S.E. of* Log_{10} *viable numbers per gram*) *in caecal contents of in-bred chicken lines at times after inoculation with* S. *Typhimurium* (*Expt 6*)

	Low excretor		High excretor	
Days	6*	W†	N*	7†
1	4·82 ± 0·45	4·58 ± 0·19	6·00 ± 1·02	5·38 ± 1·01
4	5·77 ± 1·56	5·72 ± 1·25	6·40 ± 1·62	7·02 ± 0·49
7	4·44 ± 1·12	4·6 ± 0·79	5·29 ± 1·38	5·18 ± 0·52
14, 21	2/18 +	3/10 +	12/16 +	8/10 +

* Eighteen animals per time-point per line.
† Ten animals per time-point per line.

There were less clear differences in the numbers of lymphocytes and monocytes.

Experiment 7

The caecal counts of the challenge *S.* Typhimurium strain in young chickens which have received suspensions of pooled caecal contents from different lines of birds are shown in Table 7. Because a number of the viable counts were less than the limit of detection, the results are presented as the median count with range. The counts in birds which had not been given a gut flora preparation were very high whereas those given the caecal contents from out-bred birds were very low. Considerably variability was observed between the individual birds given the contents from in-bred lines with no particular trend discernible. Because of this, five groups of birds were given suspensions of caecal contents from individual birds of two different lines and were then challenged. The geometric mean counts for the five groups from each line were 4·24, 6·12, 6·19, 7·03 and 7·29 (line 6_1) and 4·71, 6·57, 6·66, 7·92 and 8·10 (line N). The small difference in the counts of the challenge strain from the two sets of birds was of marginal statistical significance ($t = 1{\cdot}58$, $P = 0{\cdot}07$). Pooled caecal contents suspensions were administered orally to newly hatched chickens and the faecal excretion patterns monitored after challenge with *S.* Typhimurium. The percentage excretion rates over 4 weeks, followed by the percentage isolation from the caecal contents at slaughter from the birds given caecal contents from line 6_1 were 47, 32, 42, 42 and (caeca) 79 and from line N birds were 42, 26, 53, 37 and (caeca) 32, respectively. The differences in excretion were not statistically significantly different ($\chi^2 = 2{\cdot}35$, $P = 0{\cdot}3$).

DISCUSSION

We have demonstrated consistent differences in the amount and duration of faecal excretion by in-bred lines of chickens of *S.* Typhimurium and other *Salmonella* serotypes associated with food poisoning. In developing the model we took considerable care to avoid the accidental introduction of sources of variation. We decided to use chickens that were 6 weeks old since they would possess considerable immunological maturity at this age [29, 30]. At this age no interference through sexual hormones would be involved. The intestinal gut flora normally develops over a period of up to 6 weeks [33, 34]. We ensured that all birds were given a standard gut flora preparation and that all birds from different lines were reared together until 6 weeks old. By this means we hoped to have chickens which differed primarily in their genetic background or in factors which resulted from a combination of host phenotype/genotype and the environment, including the gut flora. Ignoring this potential source of variation imposed by differences in the gut flora of different batches of birds can result in difficulties in interpretation [16, 18]. Differences in faecal excretion patterns between different batches of birds as a result of different gut flora have been observed previously [35].

The caeca are the main sites of colonization [15, 36–38]. It seemed likely, therefore, that the differences in caecal colonization led to the differences in faecal excretion observed. The differences were observed over a period of more than 1 year during which the experiments were carried out.

Differences between the lines were also observed with the highly invasive *S.* Enteritidis and also with the poorly invasive *S.* Infantis strain, infection with which results in much lower titres of circulating specific IgG than occurs after infection with *S.* Typhimurium and *S.* Enteritidis (P. A. Barrow & M. A. Lovell, unpublished observations). This result, together with the different LPS and flagellar antigenic structures of these three serovars, suggests that differences in adaptive immunity are not likely to be the basis of the differences seen between lines 6_1 and N since this would probably be stronger in response to an invasive, as opposed to a poorly invasive strain. The similar IgA and IgG titres observed in the birds infected with *S.* Typhimurium soon after infection (Expt 5), together with the rapidity of the appearance of the difference in the caecal bacterial counts (24 h p.i.) also precludes an adaptive immune response. The

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Table 6. *Differential cell counts in blood from in-bred chicken lines*

Days post-infection	Line	Proportion of each cell type expressed as mean (±S.D.) percentage of total white blood cell count in blood of five chickens					
		Lymphocytes		Monocytes		Heterophils	
2 d	6_1	48·2±3·8	$P=0·04$	13·8±2·6	$P=0·35$	38·0±4·3	$P=0·086$
	N	57·4±3·2		11·0±1·4		30·6±1·5	
7 d	6_1	45·2±4·4	$P=0·15$	22·4±3·6	$P=0·22$	32·4±2·5	$P=0·056$
	N	51·2±5·3		26·0±1·9		22·8±4·0	

Table 7. *Viable numbers in caecal contents of individual out-bred chickens after they had been inoculated orally with suspensions of caecal contents obtained from groups of 6-week-old in-bred lines*

Birds challenged with caecal contents from lines (high or low excretor)	$\log_{10}$ individual counts of challenge organism	Median (range)
Line 6 (low)	<2, 6·34, 5·15, 7·2, 6·85, 6·46, 6·83	6·46 (<2–7·20)
Line N (high)	<2, <2, <2, 4·71, 3·70, 3·60, 4·95	3·60 (<2–4·95)
Line 6 (low)	6·15, 5·52, <2, 2·7, 6·46, 2·0, 7·41	5·52 (<2–7·41)
Line N (high)	6·92, 5·90, 8·65, 4·71, 8·38, 2·90, <2	5·90 (<2–8·65)
RiR (out-bred)	3·85, <2, <2, <2, <2, <2, <2	<2 (<2–3·85)
Nothing	8·48, 8·38, 8·38, 8·08, 7·92, 7·65, 8·11	8·11 (7·65–8·48)

greater heterophil numbers in the blood observed in line 6_1 (more resistant) birds was probably the result of differences in bacterial invasion rather than being a determining factor in limiting colonization. However, although there is no evidence that a direct physical association between *Salmonella* organisms and the intestinal/caecal mucosa is involved in colonization [15], intestinal mucopolysaccharides may be important carbon sources for *Salmonella* in the gut and there is a school of thought that this is the main site of *Salmonella* colonization [39]. This site is at least accessible to heterophils infiltrating into the lumen as a result of limited bacterial invasion which would induce IL-6, a powerful chemo-attractant for heterophils produced by cells as a result of invasion [40, 41]. A recent study has also found increased phagocytic activity in chicken lines showing increased colonization [42].

The differences in excretion rates were observed in the presence of a mature gut flora and we thought it unlikely that we would see such differences in chickens without a gut flora since such birds are highly susceptible to oral infection and relatively small differences in excretion rates might not be seen. This was indeed the case and differences were not seen at all over 7 weeks (Expt 4). It also seemed reasonable to suppose that differences might be seen if newly hatched chickens were given a gut flora and infected with *Salmonella* a day later. However, this was not the case and differences between the resistant and susceptible lines were not seen until after several weeks. These results suggested that the presence of a mature gut flora from an out-bred line of birds was not sufficient in itself and that either this must be modified by the host in different ways during the 6-week period prior to challenge or that additional factors, present in 6-week-old but not in newly hatched chickens, was involved. That the gut flora did not differ greatly in inhibitory activity in the different lines at the time of challenge was suggested by the exclusion studies in which newly hatched chickens were infected with flora preparations from the older birds.

Salmonella is likely to compete for nutrients with obligate anaerobic members of the bacterial gut flora [43], which may outnumber it by factors of between 10^5–10^8 and which are inhibitory to its colonization [35]. It seems likely that after caecal emptying and refilling, *Salmonella* may multiply for a short period before the numerically dominant anaerobes induce starvation conditions. A high rate of flow of chyle within the intestine may physically remove *Salmonella* before they are able to multiply. Although this might seem to be a relatively minor effect a small physiological change may have considerable effects on its ability to colonize.

Other host factors more closely connected to the innate immune system have not been examined but may be considered for further work. Recent work with oral infections in mice with a non-pathogenic *Escherichia coli* KBC-236 showed that the *E. coli* count in the mid and distal ileum of matrilysin-deficient ($MAT^{-/-}$) mice, lacking mature cryptidins, was (Log_{10}) 8 and 9, respectively compared to 6 and 8, respectively in $MAT^{+/+}$ mice [44]. The contribution of α- [45] and β-defensins [46–48], produced by Paneth and possibly other cells in poultry in response to intestinal bacteria, including pathogens, should be investigated.

There appeared to be no association with resistance to visceral infection and since lines 6_1 and 7_2 show a common MHC type it seems likely that, in these lines at least, there is no association with MHC. Comparison with sequence information on the coding region of *NRAMP1* [49] suggests no association with the lines involved. The absence of association with *NRAMP1*, which is macrophage associated and *SAL1*, which is also likely to be so, is not surprising. The result of the initial cross indicated that there was no sex linkage and that the rapid clearance (designated resistance) was a dominant effect. The figure suggested that two traits may be present, (*a*) the rapid clearance over the first few weeks and (*b*) the ability to eliminate infection in the later stages of the infection. Selection for the ability to clear *Salmonella* rapidly from the alimentary tract would be a desirable trait in domestic poultry. Further work on practical aspects of application, including the combination of genetic background and use of killed vaccines and extension to other zoonotic pathogens such as *C. jejuni* would be of practical value.

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