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Host Specificity of *Salmonella* Infection in Chickens and Mice Is Expressed In Vivo Primarily at the Level of the Reticuloendothelial System

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By experimental infection, host-specific *Salmonella* serotypes were shown to demonstrate specificities for chickens, mice, and other laboratory animals. Following oral inoculation, four strains of *Salmonella gallinarum* and two *S. pullorum* strains, isolated from diseased poultry, were more virulent for chickens than for mice. By contrast, four strains each of *S. choleraesuis* and *S. dublin*, isolated from diseased pigs and cattle, respectively, were more virulent for mice than for chickens. These results were also reflected in the degree of virulence expressed after parenteral inoculation. In addition, *S. choleraesuis*, but not other serotypes, killed rats, guinea pigs, and rabbits. *S. typhimurium* strains varied widely in their virulence, and some strains were virulent for both mice and chickens. Four other serotypes isolated from poultry or human food poisoning cases and a nonpathogenic *Escherichia coli* strain were much less virulent for both experimental host species. Most of the host-specific *Salmonella* serotypes studied were able to colonize the distal alimentary tract and invade the tissues in both mice and chickens to various degrees. There was, however, a greater difference in the ability to survive and multiply in the visceral organs, particularly the spleen and the liver, once invasion had occurred which correlated with the virulence for the host species involved.

Salmonella is a parasitic genus whose members infect homeo- and poikilothermic animals by the fecal-oral route, and most, if not all, *Salmonella* species and serotypes are able to produce gastroenteritis and/or systemic disease. A small number of serotypes of subspecies I, *S. enterica*, typically produce a severe systemic disease, initially involving primarily the reticuloendothelial system, in a restricted number of host species. In hosts other than the main target species, the clinical picture tends to be atypical, although little precise information is available (34, 35, 57).

Some of these host-specific serotypes are more frequently associated with mammalian than avian host species. *S. choleraesuis* is a pig pathogen of worldwide economic significance, although inexplicably, it has virtually disappeared from western Europe (26). Although it rarely infects cattle in the field, disease can be produced under experimental conditions (46). Human infection is infrequent but severe and may consist of an enteric fever with pyemia and localized suppuration and is accompanied by a high mortality (57). *S. dublin* typically produces systemic infection and gastroenteritis in cattle of all ages, with abortion in pregnant females (19, 23). Other mammals such as sheep, goats, and pigs may occasionally become infected with this serotype (9), and localized or generalized human infections arising from contact with animals or via the food chain may occur (42, 53). Both of these serotypes are isolated very rarely from avian species (13, 33, 39). Other serotypes, such as *S. typhi* and *S. paratyphi* A and C, *S. abortus ovis*, and *S. abortus equi*, also generally infect only a narrow range of host species, in these cases humans, sheep, and horses, respectively.

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In avian hosts, the serotype which produces the most widespread disease and is of the greatest economic significance is *S. gallinarum* and its biotype *S. pullorum*. *S. gallinarum* produces fowl typhoid which may be accompanied by high mortality in poultry of all ages, whereas the *S. pullorum* biotype usually produces significant mortality only in young chickens (36, 51). Both biotypes infect other avian genera (9). Isolations from mammalian species are rare, although the duisburg variety of *S. gallinarum* (25, 30) and the *S. pullorum* biotype (29) have been found in the past to occasionally enter the food chain and cause human food poisoning.

In contrast, the few other serotypes which routinely produce systemic disease, perhaps most notably *S. typhimurium* and *S. enteritidis*, do so in a wide range of animal species including cattle, sheep, pigs, poultry, humans, and rodents (34). The most severe manifestations, sometimes involving mortality, generally occur in young animals such as calves and newly hatched chicks, which are microbiologically and immunologically immature, or in elderly institutionalized human patients or those who may be immunologically compromised in some other way (35, 54). These two serotypes also currently account for three-quarters of reports of human *Salmonella*-associated food poisoning in the United Kingdom (37).

Individual strains of a number of other serotypes occasionally produce high mortality in young poultry (31) and abortion in sheep (56). However, with such exceptions, the vast majority of remaining serotypes produce little systemic disease, with infections in humans and animals usually being restricted to the alimentary tract.

The host and microbial characteristics which effect the ability of *Salmonella* serotypes to survive, multiply, and produce systemic disease in particular hosts are now beginning to be elucidated. Host factors include the increased resistance to salmonellosis seen in adults compared with young animals, attributed in part to greater immunological maturity of the reticuloendothelial system (24) but also in part to differential resistance to the bactericidal effects of sera of adult and very

young animals (10). In some cases, variation in susceptibility between genetic lines within individual host species can be attributed to a single gene, *Ity* in mice (41) and a similar gene in fowl (8). However, the interactions between *Salmonella* organisms and hosts that account for the host specificity, restriction, or adaptation observed with some major serotypes have not been studied.

Considerable information on the microbial characteristics responsible for infection and disease is being accumulated. Although the ability to colonize the alimentary tract in numbers sufficient to interact with the intestinal mucosa has not been studied, the basis of adhesion to and invasion of the mucosa by *Salmonella* spp. is now being investigated (1, 14, 16, 52). From a knowledge of the diseases involved and from earlier work with *S. dublin*, *S. choleraesuis*, and *S. gallinarum* (43, 48, 50), invasiveness is obviously of significance. In the pathogenesis of *S. typhimurium* infection in newly hatched chicks, it is the virulence determinant of greatest significance (3). Survival in the tissues implies resistance to the antibacterial effects of normal serum, and this has been found with a number of serotypes (3, 4, 20, 21). Virulent *Salmonella* strains survive and multiply in the reticuloendothelial system, the genetic basis of which is also being studied in detail (21). The cause of death of the host and whether toxins are involved remain to be determined. Survival of *Salmonella* organisms in the environment after release from the host is also important but has been little studied.

Infection experiments were carried out to study the extent to which host specificity might be reflected in infection being restricted in mice, chickens, and other laboratory animals and to attempt to define the stage of infection at which host restriction in *Salmonella* spp. is expressed.

Throughout the text, the terms host specific, host adapted, and host restricted are all used without implying any understanding of the nature of host specificity with respect to the host-pathogen relationship.

MATERIALS AND METHODS

Bacterial strains. Four strains each of *S. gallinarum* O:1,9,12, *S. choleraesuis* O:6,7, and *S. dublin* O:1,9,12 and two strains of *S. pullorum* were originally isolated from diseased adult chickens, pigs, calves, and chicks, respectively. The 15 *S. typhimurium* O:1,4,(5),12 strains were also isolated from diseased calves, chickens, or humans. *S. agona* O:1,4,12 and *S. heidelberg* O:1,4,12 were isolated from cases of human food poisoning. *S. infantis* O:6,7 and *S. montevideo* O:6,7 were isolated from healthy chicken feces. A nonpathogenic *Escherichia coli* strain, P4 (O9:K36:H19), was isolated from the intestine of a healthy pig. Strains were stored as freeze-dried cultures or were maintained on Dorset egg slopes at 4°C. All strains were considered to be smooth because they agglutinated with O-specific antisera and did not agglutinate either spontaneously in phosphate-buffered saline (PBS) or in the presence of acriflavine (44). They were all serum resistant in normal adult chicken and rabbit sera when tested by a standard method (4).

For the assessment of intestinal invasiveness, strains were used as nalidixic acid-resistant (Nal^r) mutants. Smith and Tucker (50) showed that Nal^r *Salmonella* mutants were as virulent for chickens as was the parent strain. In one experiment, chickens and mice were infected with a mixture of *Salmonella* strains resistant to different antibiotics. These were *S. choleraesuis* 195 Nal^r, *S. infantis* 1326/28 Rif^r (rifampin resistant), and *S. typhimurium* F98 Spc^r (spectinomycin resistant). *S. gallinarum* 9 in which the 85-kb virulence-associated plasmid had been labelled with Tn*3* (ampicillin resistance [Ap^r]) was used. This strain was fully virulent for chickens (7).

Broth cultures of all strains were made in 10-ml volumes of nutrient broth (CM67; Oxoid, United Kingdom) incubated at 37°C for 24 h in a shaking water bath (100 strokes min^{-1}). They contained approximately 10^9 CFU ml^{-1}.

Experimental animals. Light Sussex chickens were obtained from a specific-pathogen-free flock. Their sex was not determined. Their rearing conditions have been described previously (49). Mice were BALB/c females weighing 20 g (approximately 6 weeks old). Rabbits were New Zealand White males, 8 to 10 weeks old. Adult male guinea pigs and Sprague-Dawley rats were used.

Inoculation of animals and experimental design. Bacterial virulence in mice and chickens was assessed by oral and parenteral inoculation. Rabbits, guinea pigs, and rats were inoculated orally only. The animals were inoculated orally with undiluted broth cultures in the following volumes (in milliliters): 0.1 (newly hatched chicks), 0.3 (3-week-old chickens), 0.25 (rats), 1 (rabbits), and 0.5 (guinea pigs). Unless otherwise indicated, mice were inoculated with 20 μl of a broth culture that had been concentrated 10-fold by centrifugation. Intramuscular (gastrocnemius) and intravenous inoculations were all of 0.1 ml of decimal dilutions of cultures. Intracranial inoculations of 50-μl volumes were done under light anesthesia as described by Smith and Huggins (47). Mortality was recorded and, where appropriate, 50% lethal doses (LD_{50}s) were calculated (38). LD_{50}s were not calculated for orally inoculated newly hatched chicks since the rapid bacterial multiplication possible in the alimentary tract at this time would render such a value meaningless.

Intestinal invasiveness of strains was assessed by counting viable bacteria in the spleen and liver at intervals after oral inoculation. These intervals were 1 and 2 days (newly hatched chicks) and 1 and 4 days (mice and 3-week-old chickens).

Survival and multiplication in the organs following parenteral inoculation were assessed by counting viable bacteria in organ samples at 1 h and at intervals of 2 to 7 days post-intravenous inoculation with 10^5 (for chickens) or 10^4 (for mice) organisms.

Organ samples were removed aseptically in the following order: breast muscle (for chickens), heart blood, kidney, spleen, liver, heart muscle, leg muscle (for mice), and cecal contents. Samples were homogenized in PBS and the numbers of viable organisms were estimated by the method of Miles et al. (28). Bacteria were counted on MacConkey agar (CM7; Oxoid) containing no antibiotics, a combination of nalidixic acid (20 μg ml^{-1}) and novobiocin (1 μg ml^{-1}), or ampicillin (100 μg ml^{-1}), spectinomycin (30 μg ml^{-1}), or rifampin (100 μg ml^{-1}) as required.

Initial studies of invasiveness. A group of newly hatched chicks were inoculated orally with cultures of a Nal^r mutant of *S. typhimurium* F98 and an Spc^r mutant of *S. infantis* 1326/28. At various times after inoculation, chicks were culled and the liver, spleen, and alimentary tract were removed. For each section of the gut studied, the contents were removed and the segment of intestine was opened longitudinally and then subjected to gentle washing in water and cursory drying. The mucosal layers were scraped off with a scalpel blade. All samples were homogenized, and viable counts were estimated as described above on MacConkey agar and brilliant green agar containing spectinomycin (30 μg ml^{-1}) or the combination of nalidixic acid (20 μg ml^{-1}) and novobiocin (1 μg ml^{-1}).

In a second experiment, the cecal wall after removal was incubated for 90 min in tissue culture medium 199 (Flow Laboratories) containing 250 μg of kanamycin sulfate ml^{-1}

TABLE 1. Virulence of *Salmonella* strains for mice and chickens by oral and parenteral inoculation

Strain	Source[a]	Virulence when inoculated by the indicated route[b]							
		Oral			Intravenous, mice	Intramuscular, chickens at day:		Intracranial, chickens at day:	
		Mice	Chickens at day:						
			21	0		21	0	21	0
S. gallinarum 9	C	>7.3	4.2	100	6.0	1.2	0.6	0.8	1.0
S. pullorum 3	C	>7.3	7.4	45	7.1	7.7	4.9	1.1	1.2
S. choleraesuis 195	P	5.8	>7.5	0	1.2	>7.5	6.0	1.1	0.7
S. dublin 188	B	4.9	>7.7	0	4.5	>7.7	5.4	0.3	1.0
S. typhimurium Bangor	C	5.0	>7.1	40	<1.0	>7.1	1.2	0.8	1.3
S. infantis 1326/28	C	>7.3	>7.4	0	>8.0	>7.4	2.3	0.8	0.6
E. coli P4	P	>7.3	>7.7	0	>8.0	>7.7	6.3	2.6	1.0

[a] C, chicken; P, pig; B, cattle.
[b] Values are $\log_{10}$ LD_{50}s calculated from mortality occurring in a 2-week period postinoculation except for the values for chickens inoculated orally at day 0, for which mortalities for 20 chickens calculated 3 weeks after inoculation with 10^8 organisms (expressed as percentages) are shown.

and then washed three times in PBS prior to further treatment as described above.

RESULTS

The virulence of *Salmonella* serotypes for mice, chickens, and other experimental animals. The virulence of representative strains of a number of host-adapted and non-host-adapted *Salmonella* serotypes and a strain of *E. coli* when inoculated into mice and chickens is shown in Table 1. The results obtained by oral inoculation were as follows.

Neither *S. gallinarum* nor *S. pullorum* produced ill effects in mice, whereas the former produced typical fowl typhoid (36) in 3-week-old chickens and a high mortality following an acute infection in newly hatched chicks. In newly hatched chicks, but not older birds, *S. pullorum* produced diarrhea typical of pullorum disease (51) accompanied by mortality. Very similar results were obtained with three other *S. gallinarum* strains and one other *S. pullorum* strain.

The *S. choleraesuis* and *S. dublin* strains behaved similarly to each other in that they produced clinically typical murine salmonellosis, with animals showing a hunched appearance, rough coats, and anorexia and dying 4 to 8 days after infection. The two strains represented produced no apparent effects in chickens. In general, three other strains of both serotypes behaved similarly. However, two of the *S. dublin* strains produced 6 and 12% mortality in newly hatched chicks.

The *S. typhimurium* strain for which results are shown produced disease in mice and newly hatched chicks. No ill effects were produced in 3-week-old chickens by this or any of the other 14 strains studied. However, considerable variation in the virulence of these strains for mice and newly hatched chicks was seen. The results for six of these strains are shown in Table 2.

The four non-host-adapted serotypes, represented by *S. infantis*, and the *E. coli* strain produced no mortality or morbidity in mice or chickens.

The representative strains from Table 1 were assessed for virulence by different parenteral routes. In general, for the host-adapted strains, a high level of virulence by oral inoculation was associated with lower LD_{50}s by intramuscular or intravenous routes. Following intracranial inoculation of chickens, all the strains were highly virulent, with the *E. coli* strain less so in 3-week-old birds. Chickens inoculated intracranially with the *S. typhimurium* and *S. infantis* strains at this age died within 48 and 24 h, respectively, while the other serotypes killed the birds after 5 or 6 days. Very few *Salmonella* organisms were isolated from the livers of chickens killed by the *S. infantis*, whereas profuse growth was obtained from the livers of chickens killed by the other serotypes and from the brains of all infected birds.

The representative strains were assessed for oral virulence in rats, rabbits, and guinea pigs (two animals each in every case). *S. choleraesuis* was the only serotype tested which produced mortality in all three host species, although this serotype and *S. dublin*, *S. typhimurium*, and *S. gallinarum* all produced small necrotic areas in the walls of the ceca and the colons of rabbits.

Validation of criterion used for assessing invasiveness. The numbers of bacteria in early stages of infection of newly hatched chicks by *S. typhimurium* F98 Nal^r and *S. infantis* Spc^r and the commensal *E. coli* population already present in the gut are shown in Table 3.

The criterion for an association of bacteria with the mucosa was that the mucosal count should equal or exceed the luminal count (6, 45). This was already evident for the *S. typhimurium* strain in the ileum 6 h after infection and was extended to more-proximal regions of the alimentary tract as the experiment progressed and *Salmonella* organisms began to be isolated from the spleen and liver. The bacterial counts in the cecal mucosa never equalled the counts in the cecal contents, although they exceeded the mucosal counts for the other regions of the gut. However, the cecal-content count was also

TABLE 2. Virulence of *S. typhimurium* strains for mice and chickens by oral and parenteral inoculation

Strain	Source[a]	Virulence when inoculated by the indicated route into[b]:					
		Mice		Chickens			
				Day 21		Day 0	
		Oral	i.v.	Oral	i.m.	Oral	i.m.
Bangor	B	5.0	1.0	>7.1	5.4	40	1.2
116	C	6.3	1.6	>7.6	6.5	100	1.8
Wharton	C	7.3	<1.0	>7.8	5.5	25	1.7
2 Acres	B	>7.3	3.2	>7.8	6.6	20	2.4
1040	C	>7.7	3.2	<7.7	6.7	10	1.6
Swindon	H	>7.3	5.5	>7.3	6.0	5	0.4

[a] B, bovine; C, chicken; H, human.
[b] Values are $\log_{10}$ LD_{50}s calculated from the number of deaths occurring in a 2-week period postinoculation except for values for chickens inoculated orally on day 0, for which mortalities for 20 chickens calculated 3 weeks after inoculation with 10^8 organisms (expressed as percentages) are shown. i.v., intravenous; i.m., intramuscular.

TABLE 3. Mucosal association of *S. typhimurium*, *S. infantis*, and *E. coli* in newly hatched chickens

Organ	Sample	Log_{10} viable bacteria g of sample^{-1} at the following times (h) after infection[a]:											
		6			12			24			48		
		S. typhimurium	*S. infantis*	*E. coli*	*S. typhimurium*	*S. infantis*	*E. coli*	*S. typhimurium*	*S. infantis*	*E. coli*	*S. typhimurium*	*S. infantis*	*E. coli*
Crop	Contents	7.5	7.0	7.0	6.2	8.0	8.0	6.0	6.8	5.9	5.6	4.2	4.7
	Wall	5.6	4.0	N	4.7	4.6	4.1	6.1 (1)	4.9	N	5.6 (1)	3.9	N
Duodenum	Contents	3.5	N	2.7	2.9	4.9	6.2	3.0	3.3	3.7	2.6	2.0	3.1
	Wall	2.0	2.5	N	2.6 (1)	2.0	4.2	4.5 (1)	N	N	3.5 (2)	N	N
Jejunum	Contents	4.6	3.9	4.6	3.8	5.6	6.4	4.2	3.3	4.3	2.7	2.5	N
	Wall	2.7 (1)	2.9	2.0	4.8 (2)	2.6	4.0	5.2 (2)	2.7	N	4.3 (3)	N	N
Ileum	Contents	4.6	6.4	6.8	6.8	6.8	7.6	4.8	6.0	6.1	3.4	5.6	6.0
	Wall	4.7 (2)	3.3	2.3	6.2 (2)	6.1	5.1	5.8 (3)	4.5	4.8	5.4 (3)	4.8	2.7
Cecum	Contents	9.1	8.7	9.1	9.6	9.3	9.2	9.0	9.7	9.7	8.6	9.4	9.4
	Wall	7.2	5.8	5.6	7.2	7.1	6.2	7.2	6.9	6.9	6.6	5.5	6.2
Liver		N	N	N	2.3	N	N	3.0	N	N	4.2	N	N
Spleen		N	N	N	N	N	N	3.9	N	N	4.3	N	N
Cecum[b]	Contents	8.3	—[c]	—	9.7	—	—	9.7	—	—	8.4	—	—
	Wall	4.7	—	—	7.4	—	—	7.1 (1)	—	—	7.1 (1)	—	—

[a] Numbers in parentheses indicate the number of occasions (out of three) in which the viable count per gram of mucosa equalled or exceeded the count per gram of contents. N, $\log_{10} < 2.0$; counts are medians of three values.

[b] The results were obtained in a second experiment in which, after being washed, the intestinal walls were incubated with kanamycin sulfate (250 μg/ml) for 90 min before being washed three times prior to removal of the mucosa.

[c] —, not done.

higher than those for other parts of the gut. In a second experiment, after incubation with kanamycin to remove extracellularly attaching bacteria and those attached to mucus, the cecal-mucosa *S. typhimurium* count was initially much lower, but it increased rapidly to that seen in the first experiment.

At no time during the first experiment did the mucosal counts of *S. infantis* and *E. coli* equal or exceed those for the lumen.

There was no microscopical evidence for bacterial adhesion to mucosal tissue taken from any of the birds in these experiments (results not presented).

In other experiments in which kanamycin was not used, similar results were obtained with *S. gallinarum* and a strain of *S. enteritidis* not used in the study described here and with an *S. typhimurium* strain in mice.

It was appreciated that penetration from the intestine to the reticuloendothelial system is a multistage process involving at least survival in the gut, adhesion, invasion, and translocation to the spleen, etc. However, given the differences between strains observed in these experiments and the survival of most *Salmonella* strains for several days in the spleen following intravenous inoculation (see below), the qualitative measurement of intestinal invasiveness by counting *Salmonella* organisms in the spleen appeared valid.

Intestinal invasiveness of *Salmonella* strains for mice and chickens. The invasiveness of Nal^r mutants of selected *Salmonella* strains and *E. coli* P4 for mice and chickens, as indicated by their isolation from the liver and spleen following oral inoculation, is shown in Table 4.

With the exceptions of *S. pullorum* 3 in mice, *S. choleraesuis* 195 in 3-week-old chickens, and the *E. coli* strain in both mice and chickens, all the strains tested were found to be invasive to some degree. The level of invasiveness did not always correspond to the level of cecal carriage.

The invasiveness of *S. choleraesuis* in 3-week-old chickens was retested with their feed containing the antibiotic avoparcin at 100 μg g^{-1}. *S. pullorum* was retested in mice with the antibiotic dissolved at the same concentration in the drinking water. Barrow (2) has shown that avoparcin increases the colonization of chicken ceca by *S. choleraesuis* and, to a lesser extent, by *S. pullorum*. The $\log_{10}$ median counts of *S. choleraesuis* per gram in the livers and spleens of three chickens 4 days after infection were <2.00 and 2.69, respectively, with a $\log_{10}$ median cecal count of 7.65, and the corresponding $\log_{10}$ counts for *S. pullorum* in mice were <2.00, <2.00, and 7.38. This indicated at least for *S. choleraesuis* that its apparent noninvasiveness may be a result of a poor ability to colonize rather than an innate inability to invade.

Survival and proliferation of *Salmonella* strains in the organs of mice and 3-week-old chickens. The survival and multiplication of selected *Salmonella* strains and of *E. coli* P4 in the organs and tissues of mice and 3-week-old chickens following intravenous inoculation were assessed. Representative results for mice and for chickens are shown in Tables 5 and 6, respectively.

One hour after inoculation, most bacterial cells had been removed from the blood by the reticuloendothelial system. Thereafter, the strains behaved in different ways.

S. choleraesuis, *S. dublin*, and *S. typhimurium* in mice and the *S. gallinarum* strain in chickens proliferated rapidly in the liver and spleen and were soon isolated from the blood, myocardium, and voluntary muscle samples in similar numbers. The viable counts continued to increase until the animals died.

A second group of strains, comprising *S. gallinarum* and *S. infantis* in mice and *S. typhimurium*, *S. choleraesuis*, *S. pullorum*, *S. infantis*, and *S. dublin* in chickens, showed various degrees of persistence in the liver and spleen, with, generally, gradually decreasing viable counts until the organisms could not be detected at between 2 and 3 weeks postinfection.

A third pattern of behavior was observed with *S. pullorum* in mice and the *E. coli* strain in both hosts. In these cases, the bacteria did not persist in the liver and spleen for more than a

TABLE 4. Numbers of *Salmonella* and *E. coli* organisms in the spleen and liver following oral inoculation

Strain[a]	Day after oral inoculation[b]	Log_{10} viable bacteria g^{-1} in tissues after oral inoculation[c]								
		Mice			Chickens					
					Day 21			Day 0		
		Liver	Spleen	Cecal contents	Liver	Spleen	Cecal contents	Liver	Spleen	Cecal contents
S. gallinarum 9	1	N[d]	N	2.9	N	N	3.0	N	N (3.8)	9.2
	4/2	3.0	3.6	N	4.8	4.8	N	4.3	5.0	6.4
S. pullorum 3	1	N	N	N	N	N	2.30	N	N	9.0
	4/2	N	N	N	2.7	2.6	N	N	2.7	8.5
S. choleraesuis 195	1	N	N	3.5	N	N	5.4	N	N	9.2
	4/2	5.9	6.8	3.2	N	N	N	N (4.0)	N (4.0)	8.2
S. dublin 188	1	N	N	5.2	N	N	5.9	N (3.5)	N (4.7)	8.3
	4/2	6.9	6.7	4.9	N	2.3	3.0	N (2.0)	3.9	8.2
S. typhimurium Bangor	1	N	N	5.5	N	N	4.3	3.5	4.4	9.0
	4/2	6.0	6.4	5.0	2.0	3.1	6.5	3.9	4.3	9.1
S. infantis 1326/28	1	N	N	4.9	N	N	6.0	N	N	9.8
	4/2	N	N (2.0)	2.3	N (2.0)	N (3.2)	4.7	N	N (3.0)	9.5
E. coli P4	1	N	N	4.8	N	N	6.4	N	N	9.6
	4/2	N	N	4.1	N	N	6.2	N	N	9.4

[a] All strains were used as nalidixic-acid resistant mutants inoculated in the following volumes: 0.1 ml (0-day-old chickens), 0.3 ml (3-week-old chickens), or 20 μl of a broth culture concentrated 10-fold (mice).
[b] Samples were taken from mice and 3-week-old chickens at 1 and 4 days and from newly hatched chickens at 1 and 2 days postinoculation.
[c] Values are median counts for three animals, with the highest count shown in parentheses where appropriate.
[d] N, $log_{10} < 2.0$.

few days and were clearly destroyed fairly rapidly in these tissues.

The oral administration of mixtures of *S. gallinarum*, *S. choleraesuis*, *S. typhimurium*, and *S. infantis* to mice and chickens. The different virulence characteristics of *S. gallinarum* 9, *S. choleraesuis* 195, *S. typhimurium* Bangor, and *S. infantis* 1326/28 were displayed when mixtures of these organisms, each resistant to a different antibiotic, were administered orally to mice and to both 3-week-old and newly hatched chickens. The counts of the viable organisms of each strain present in the organs of these animals at a single time point after inoculation are shown in Table 7.

In mice, *S. choleraesuis* and, to a lesser extent, *S. typhimurium* showed evidence of invasion and proliferation in the liver and spleen. The animals had been killed relatively early in infection, and no other organs were affected at this stage. *S. gallinarum* was not isolated at all, and *S. infantis* was isolated only in very small numbers from the ceca. The mice also received a smaller inoculum (ca. 2×10^7 CFU) than was used in initial experiments, which may also explain the relatively low

TABLE 5. Survival and multiplication of *Salmonella* strains in the tissues of mice following intravenous inoculation[a]

Strain inoculated	Organ sampled	Log_{10} viable bacteria g^{-1} in tissues at the following times (days) after inoculation					
		0	2	4	10	17	24
S. choleraesuis 195[b]	Blood	N	3.2	5.9	D[c]	D	D
	Liver	2.9	5.6	7.3	D	D	D
	Spleen	3.4	6.0	8.4	D	D	D
	Myocardium	N	3.6	6.3	D	D	D
	Gastrocnemius	N	N	6.2	D	D	D
S. gallinarum 9[d]	Blood	N	N	N	N	N	N
	Liver	2.8	2.9	2.0	N	N	N
	Spleen	3.7	4.2	4.0	3.5	2.7	N
	Myocardium	N	N	N	N	N	N
	Gastrocnemius	N	N	N	N	N	N
S. pullorum 3[e]	Blood	2.0	N	N	—[f]	—	—
	Liver	3.2	N	N	—	—	—
	Spleen	4.0	N	N	—	—	—
	Myocardium	N	N	N	—	—	—
	Gastrocnemius	N	N	N	—	—	—

[a] All animals were inoculated intravenously with approximately 10^4 organisms in 0.1 ml. See the footnotes to Table 4 for further details. N, $log_{10} < 2.0$.
[b] Similar counts obtained with *S. dublin* 188 and *S. typhimurium* Bangor.
[c] D, all animals dead.
[d] Similar counts obtained with *S. infantis* 1326/28.
[e] Similar counts obtained with *E. coli* P4.
[f] —, no further samples taken.

TABLE 6. Survival and multiplication of *Salmonella* strains in the tissues of 3-week-old chickens following intravenous inoculation[a]

Strain inoculated	Organ sampled	Log_{10} viable bacteria g^{-1} in tissues at the following times (days) after inoculation						
		0	2	4	6	9	13	17
S. gallinarum 9	Blood	N	N	3.8	3.4	D[b]	D	D
	Liver	2.9	5.4	6.5	6.3	D	D	D
	Spleen	4.8	5.6	6.3	5.5	D	D	D
	Myocardium	N	3.7	4.5	6.5	D	D	D
	Breast muscle	N	N	3.0	3.5	D	D	D
S. typhimurium Bangor[c]	Blood	2.0	N	N	N	N	N	N
	Liver	2.6	2.6	2.3	2.0	N	N	N
	Spleen	4.7	4.5	5.0	4.5	4.6	3.9	N
	Myocardium	N	N	2.5	N	N	N	N
	Breast muscle	N	N	N	N	N	N	N
S. infantis 1326/28[d]	Blood	N	N	N	N	N	N	N
	Liver	2.5	N	N	N	N	N	N
	Spleen	4.4	4.3	4.1	3.6	3.7	3.3	N
	Myocardium	N	N	N	N	N	N	N
	Breast muscle	N	N	N	N	N	N	N
E. coli	Blood	N	N	N	N	—[e]	—	—
	Liver	3.6	N	N	N	—	—	—
	Spleen	4.3	2.0	N	N	—	—	—
	Myocardium	N	N	N	N	—	—	—
	Breast muscle	N	N	N	N	—	—	—

[a] All animals were inoculated intravenously with approximately 10^5 organisms in 0.1 ml. For other details, see the footnotes to Table 4. N, $\log_{10} < 2.0$.
[b] D, all animals dead.
[c] Similar counts obtained with *S. choleraesuis* 195.
[d] Similar counts obtained with *S. pullorum* 3 and *S. dublin* 188.
[e] —, no further samples taken.

bacterial recovery. In 3-week-old chickens, *S. gallinarum* showed extensive invasion and proliferation, being isolated from all the organs sampled. Invasion was also seen with *S. typhimurium*. In the ceca, much higher numbers of *S. typhimurium* than of *S. gallinarum* were isolated. *S. infantis* was isolated from the ceca only, albeit in high numbers, whereas *S. choleraesuis* was not isolated from any sample. It was perhaps not surprising that *S. infantis* and *S. typhimurium* were present in the ceca in the highest numbers, since in comparison with the other serotypes used here, these two colonize the chicken gut well and are shed in the feces extensively (6). In the more susceptible newly hatched chicks, multiplication of *S. gallinarum* and *S. typhimurium* was greater than in the older birds, and higher numbers were also found in the ceca. *S. choleraesuis* was present in the ceca and showed invasion of the liver and spleen in some chicks while *S. infantis* colonized the ceca well but had not invaded at the time of observation.

DISCUSSION

Following oral inoculation, the *Salmonella* serotypes generally regarded as host specific or host adapted showed a considerable degree of specificity for either mice or chickens. Thus, *S. choleraesuis* and *S. dublin*, which are normally associated with disease in pigs and cattle, respectively, and are rarely isolated from birds, were more virulent for mice than chickens. By contrast, *S. gallinarum* and *S. pullorum*, which typically produce systemic disease of poultry and other birds but rarely affect mammals, were pathogenic in chickens but not mice. *S. choleraesuis* was the only serotype which was able to kill other experimental animal species, as recorded previously (46). Epidemiological evidence suggests that in some cases the potential host range of these serotypes is wider than simply individual genera or related genera (9). Experimental evidence also indicates that some serotypes may produce typical disease in host species other than the one with which they are usually associated, for example, *S. choleraesuis* in calves (46). It is thus unclear at the moment whether host specificity is expressed primarily at the taxonomic family level with respect to the host, which would differentiate pigs, cattle, and mice within mammals, or whether it is expressed at the class level, which divides mammals from birds. The situation is obviously more complicated than this, since *S. typhi* appears to be more host restricted than other mammalian serotypes and does not produce significant disease in mice whereas *S. choleraesuis* shows a greater capacity to produce disease in several other genera.

Even if *Salmonella* host restriction is apparently expressed in much broader groups than generally considered, the isolation in the past of typical mammalian serotypes such as *S. choleraesuis*, *S. dublin*, *S. abortus equi*, and *S. typhi* from poultry and other birds and *S. pullorum* and *S. gallinarum* from mammals, including humans (9), requires some explanation. In the past, in western countries, these serotypes were much more prevalent and so would both enter the human and animal food production system and also result in greater cross-infection on mixed farms than would be possible today. In addition, some isolations from healthy animals were made, which may have arisen from feeding on offal from condemned or diseased animals. In the early years of the Kauffmann-White scheme, some confusion may also have been possible in serological differentiation between *S. enteritidis* and *S. dublin* and between the kunzendorf variant of *S. choleraesuis*, supposedly isolated from poultry, and a variety of other H_2S-positive serotypes which were monophasic in their flagellar antigens.

The ubiquitous *S. typhimurium* showed considerable variation in virulence for mice and newly hatched chicks. The strains tested produced no disease in older chickens, which was not

TABLE 7. Numbers of *Salmonella* organisms in the ceca and tissues of mice and chickens given a mixture of four *Salmonella* strains orally[a]

Animals	Sample	Log_{10} viable bacteria g^{-1} in tissues[b]			
		S. gallinarum 9 Ap^r	*S. choleraesuis* 195 Nal^r	*S. typhimurium* Bangor Spc^r	*S. infantis* 1326/28 Rif^r
Mice[c]	Liver	N (N–N)	2.5 (N–3.6)	N (N–N)	N (N–N)
	Spleen	N (N–N)	3.0 (N–3.8)	N (N–2.3)	N (N–N)
	Cecal contents	N (N–N)	N (N–3.5)	2.0 (N–3.6)	N (N–2.0)
Three-week-old chickens	Blood	N (N–3.4)	N (N)	N (N)	N (N)
	Liver	5.8 (2.8–7.0)	N (N)	3.2 (3.1–3.4)	N (N)
	Spleen	5.5 (3.6–6.8)	N (N)	4.0 (3.9–4.3)	N (N)
	Kidney	4.1 (3.3–5.0)	N (N)	N (N)	N (N)
	Muscle	N (N–3.5)	N (N)	N (N)	N (N)
	Cecal contents	N (N–2.3)	N (N)	7.0 (4.8–8.2)	8.7 (6.9–9.0)
Newly hatched chickens	Blood	2.8 (N–3.3)	N (N)	N (N–3.2)	N (N)
	Liver	6.4 (5.9–6.6)	N (N–3.0)	4.9 (3.8–5.2)	N (N)
	Spleen	6.5 (5.1–6.7)	N (N–2.8)	5.6 (5.1–6.3)	N (N)
	Kidney	4.6 (4.0–5.3)	N (N)	3.6 (N–6.2)	N (N)
	Muscle	3.2 (N–3.5)	N (N)	2.3 (N–4.5)	N (N)
	Cecal contents	4.5 (2.9–5.0)	5.0 (3.4–6.0)	9.0 (7.0–9.6)	8.9 (8.7–9.0)

[a] The mixtures contained equal volumes of undiluted broth cultures of the four strains. The volumes inoculated were 0.1 (newly hatched chickens), 0.3 (3-week-old chickens), and 0.03 (mice) ml. Animals were killed 3 (mice), 4 (newly hatched chickens), or 6 (3-week-old chickens) days after inoculation. N, $log_{10} < 2.0$.
[b] Median counts for five animals, with ranges in parentheses.
[c] Blood, muscle, and kidney samples contained no detectable organisms.

surprising, since overt disease produced by this serotype in older birds is less common. It is likely that *S. enteritidis* would also belong to the same group as *S. typhimurium*, since it is ubiquitous and is capable of producing systemic disease in mammals, including mice, and birds (22). From this point of view, it was interesting to observe that two of the *S. dublin* strains produced some disease in very young chicks, since the general consensus of opinion is that this serotype and *S. enteritidis* are closely related phylogenetically (40).

None of the four non-host-adapted serotypes studied were virulent for either mice or chickens. However, it is well-known that individual strains of some serotypes are capable of producing disease in young chickens (22, 31).

With the exceptions of *S. pullorum* in mice and *S. choleraesuis* in the older chickens, all the *Salmonella* strains exhibited some intestinal colonization and penetration or invasiveness as far as the liver and spleen, two of the main sites of multiplication. The invasiveness of *S. choleraesuis* was demonstrated after its concentration in the gut was increased by antibiotic-mediated removal of inhibitory components of the gut flora. Invasion of the intestinal mucosa was not assessed, but given the results observed, together with the results of the initial validation experiment, it seems likely that it occurred in some form or other. Given the ability of nearly all of the strains tested here to invade through the intestinal tissues if they are able to colonize the alimentary tract sufficiently to do so, it is tempting to speculate that common mechanisms for both processes may exist throughout the *Salmonella* genus. Some invasion genes common to a wide range of serotypes have already been demonstrated (17). However, some variation is known to exist. Thus, in serotypes such as *S. typhi*, *S. typhimurium*, and *S. enteritidis*, intestinal invasiveness is chromosomally mediated (1, 14, 20, 22, 27). In contrast, in *S. gallinarum*, the virulence-associated plasmid contributes to intestinal colonization and invasion (7). Some quantitative differences in invasiveness between different serotypes were observed. Thus, *S. choleraesuis* and *S. dublin* appeared to be more invasive in mice than was *S. gallinarum*, whereas the situation was reversed in chickens. Differences between *S. typhimurium* and *S. infantis* in chickens were also apparent. How far these differences in numbers of bacteria in the tissues reflect differences in invasiveness or in multiplication in the tissues after invasion is very difficult to determine without a more detailed investigation involving larger groups of animals.

The virulence of the host-specific serotypes following oral inoculation was similar to that seen following intravenous or intramuscular inoculation, supporting the contention that host specificity in the strains studied is expressed primarily in the differential ability to multiply in the tissues, particularly those of the reticuloendothelial system in the immunologically mature animal. The behavior of the representative strains after intravenous inoculation reflected this. With the exceptions of *S. pullorum* in mice and the *E. coli* strain, the two patterns of behavior observed were either multiplication in the spleen and liver followed by spread to other tissues and eventual death of the host or gradual elimination from those organs, with little multiplication or spread to other organs. The quicker elimination of *S. pullorum* than of *S. gallinarum* from the livers and spleens of mice has been observed previously (11).

Following intravenous inoculation, primary localization occurred in the organs of the reticuloendothelial system. Within these organs, the exact site of bacterial multiplication is currently controversial, since although macrophages are traditionally considered to be the main host cell type (32), there is now some evidence to suggest that at least in the initial stages of infection most *Salmonella* cells may be ingested by polymorphonuclear neutrophil leukocytes (12). Because under some experimental conditions a small amount of multiplication of avirulent salmonellae may occur in the liver or spleen for a few days prior to gradual elimination, it may be more important to determine the main type of host cell utilized at 2 to 4 days postinfection rather than those first encountered. The serum resistance of the strains tested here implies not only that serum sensitivity does not play a part in determining host specificity, but also that serum sensitivity would be no barrier to extracellular bacterial multiplication if it is involved.

The level of host resistance seen here is similar to that observed in Ity$^+$ mice and in chickens genetically resistant to salmonellosis (8, 41). It would be interesting to know whether the expression of host specificity is independent of Ity or whether the two act in concert. Whatever the exact nature of the expression of host specificity, it is known that the microbial

characteristics associated with it in *Salmonella* spp. are chromosomally mediated and not virulence associated plasmid mediated (5). These characteristics are not, however, related to lipopolysaccharide (LPS) structure. The big differences seen here between the virulence characteristics of strains within group B (*S. typhimurium*, *S. agona*, and *S. heidelberg*), group C (*S. choleraesuis*, *S. montevideo*, and *S. infantis*), and group D (*S. gallinarum-pullorum* and *S. dublin*) suggest that the structural and associated virulence differences between group B, C, and D LPS as observed in mice by Valtonen (55) were not involved in a major way.

The variations in virulence observed with the different serotypes when inoculated intravenously or intramuscularly were not seen following intracranial inoculation. The LD_{50}s, including that of *E. coli*, were all much lower. It may have been significant that the prototrophic *S. typhimurium* and *S. infantis* strains apparently multiplied faster and killed more quickly than the nutritionally more exacting host-specific serotypes. The rapid multiplication of bacteria in the brain may reflect the sometimes fulminating nature of meningitis.

It was interesting that in the mixed-infection experiment the different serotypes behaved virtually the same as when inoculated individually, despite the presence in some cases of very high numbers of other serotypes in the gut. This suggests that in vivo the greater invasion of one serotype does not normally promote or induce increased invasion of other serotypes. Evidence supporting this idea has been found for different serotypes and genera in vitro (2a, 15). However, an increase in invasiveness in the adhesive but less invasive *invA* mutants of *S. typhimurium* can be demonstrated by coinfection of cell monolayers with the fully invasive parent strain (18). The present results also indicate that, at the level of the reticuloendothelial system, susceptibility to a host-specific serotype does not normally induce susceptibility to other nonadapted *Salmonella* serotypes. It is not known whether this also occurs at the cellular level. This would be difficult to determine in vivo since it is unlikely, with the relatively small number of bacterial cells that reach the liver and spleen, that host cells are infected with more than one bacterial cell exhibiting different host specificities.

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