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Correlation between the Presence of Sequences Homologous to the *vir* Region of *Salmonella dublin* Plasmid pSDL2 and the Virulence of Twenty-Two *Salmonella* Serotypes in Mice

CHANTAL ROUDIER,[1]* MARTIN KRAUSE,[1] JOSHUA FIERER,[1,2] AND DONALD G. GUINEY[1,3]

Department of Medicine[1] and Center for Molecular Genetics,[3] University of California, San Diego, San Diego, California 92103, and V.A. Medical Center, San Diego, California 92061[2]

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Large plasmids encoding important virulence properties have been found in several *Salmonella* serotypes. We have studied the relationship between the presence of a highly conserved 4-kilobase (kb) *Eco*RI fragment from the plasmid virulence region and pathogenicity for mice of 53 isolates representing 22 serotypes of *Salmonella*. Only strains possessing the homologous 4-kb region were virulent for mice. In addition, we transferred the virulence plasmid from *S. dublin* into nine different serotypes, including *S. typhi* and *S. paratyphi* A, that lack a native virulence plasmid. Only *S. heidelberg* and *S. newport* were rendered mouse virulent by the introduction of the *S. dublin* plasmid. This study demonstrates that plasmid-mediated virulence sequences are required for *Salmonella* virulence in mice, but many strains, including the agents of human typhoid fever, also lack chromosomal genes necessary to produce lethal systemic disease in mice. Since all the major *Salmonella* strains that are host-adapted to animals carry virulence plasmids, it appears that these plasmids are important in mediating systemic infection in animals and may contribute to septicemic, nontyphoid salmonellosis in humans.

Plasmids encoding essential virulence traits are common among members of the family *Enterobacteriaceae*, and well-studied examples include enterotoxin production in *Escherichia coli* (7), invasion in *Shigella* spp. (31), and calcium-regulated outer membrane protein synthesis in *Yersinia* spp. (35). In *Salmonella* species, plasmids of 50 to 100 kilobases (kb) encoding traits essential for virulence have been reported for the nontyphoid serotypes *S. typhimurium*, *S. dublin*, *S. choleraesuis*, *S. enteritidis*, and *S. gallinarum* (1, 3, 11, 18, 25–27, 42). These plasmids have been shown to be related by restriction endonuclease analysis and hybridization (1, 5, 21, 27a, 34, 34a, 43). The ability to kill mice (or chickens, in the case of *S. gallinarum*) is the only phenotype consistently reported for all these plasmids. Curing plasmids from *S. typhimurium*, *S. choleraesuis*, *S. dublin*, and *S. enteritidis* markedly reduces virulence in mice, and reintroduction of the initial plasmid restores wild-type virulence (11, 18, 25, 27).

pSDL2 is an 80-kb virulence plasmid isolated from *S. dublin* Lane (11). This strain produces a lethal systemic infection in BALB/c mice when administered by either the oral or intraperitoneal route of inoculation (20). The isogenic, plasmid-free derivative, designated LD842, invades the intestinal mucosa when given orally and reaches the mesenteric lymph nodes and spleen, but is unable to establish a progressive, lethal infection (20). LD842 is likewise avirulent by the intraperitoneal route of inoculation. These findings suggest that plasmid-encoded virulence traits act to enhance infection of the reticuloendothelial system and are not necessary for invasion of the intestine.

Beninger et al. (5) described a physical and genetic map of pSDL2 and located separate regions encoding virulence (*vir*) and replication functions. By a combination of deletion analysis and transposon insertions with Tn*5-oriT* (17), the *vir* region was mapped within a 6-kb region of the 14-kb *Sal*I B fragment of pSDL2. These results correlate well with earlier transposon mutations isolated in an *S. dublin* plasmid by Baird et al. (1) and recent cloning experiments by Williamson et al. (44). The sites of some virulence mutations in pSDL2 also appear to correlate with a similar virulence region cloned by Gulig and Curtiss from the *S. typhimurium* plasmid (19). Beninger et al. (5) showed that putative virulence plasmids from *S. enteritidis*, *S. choleraesuis*, and a Vi antigen-producing *S. dublin* strain (Vi^+) differed in size and restriction digestion pattern, but all contained a 4-kb *Eco*RI fragment homologous to the virulence region of pSDL2. Furthermore, plasmids of the Vi^+ *S. dublin* and *S. enteritidis* were able to restore the virulence of *S. dublin* LD842, demonstrating both structural and functional conservation in the virulence region of these plasmids. Williamson et al. (43) were also able to restore virulence to a cured *S. dublin* strain by introduction of plasmids from *S. typhimurium* or *S. dublin* and found that plasmids from 11 *Salmonella* serotypes have a homologous virulence region. However, representatives of 22 other serotypes lacked these virulence sequences.

In the present study, we have correlated the mouse virulence of 53 isolates of *Salmonella*, representing 22 different serotypes, with the presence of sequences homologous to the 4-kb *Eco*RI fragment from the virulence region of pSDL2. In addition, we have tested the ability of pSDL2 to enhance the mouse virulence of wild-type isolates of serotypes that lack a virulence plasmid.

MATERIALS AND METHODS

Strains. *S. dublin* Lane, LD842, and the virulence plasmid pSDL2 have been described previously (11). The sources of the other strains used in this study are listed in Table 1. The strains supplied by the California Department of Health Services were originally isolated from extraintestinal sites,

* Corresponding author.

TABLE 1. Hybridization to the 4-kb virulence probe from pSDL2 and mouse virulence of *Salmonella* isolates

Serotype	Serogroup (Kauffman-White)	No. of isolates	Source or reference[a]	Homology[b]	Virulence[c]
S. dublin Lane	D	1	8	+	+
S. dublin LD842	D	1	8	−	−
S. derby	B	1	Cal	−	−
S. hadar	C2	1	Cal	−	−
S. havana	C2	4	CDC	−	−
S. heidelberg	B	9	Cal	−	−
S. java	B	1	Cal	−	−
S. montevideo	C1	1	Cal	−	−
S. minnesota	L	1	9	−	−
S. newbrunswick	E2	1	Cal	−	−
S. newport	C2	5	Cal, VA	−	−
S. ohio	C1	1	VA	−	−
S. oranienburg	C1	2	Cal	−	−
S. paratyphi A	A	10	NAMRU	−	−
S. reading	B	1	Cal	−	−
S. saintpaul	B	2	Cal	−	−
S. sandiego	B	1	Cal	−	−
S. typhi	D	2	Switz	−	−
S. choleraesuis	C1	3	Cal, Switz	+	+
S. dublin	D	2	Switz	+	+
S. enteritidis	D	1	Switz	+	+
S. gallinarum	D	1	Switz	+	NT
S. naestved	D	1	Japan	+	+
S. typhimurium	B	2	Cal, Switz	+	+

[a] Numbers refer to references in Literature Cited. The other sources are as follows: Cal, California Department of Health Services, Berkeley, and San Diego Public Health Laboratory, San Diego, Calif.; CDC, Centers for Disease Control, Atlanta, Ga.; NAMRU, U.S. Naval Medical Research Unit, Egypt; V.A., Veterans Administration Medical Center, San Diego, Calif.; Switz, University Hospital of Zurich, Switzerland; Japan, Nuboyuki Terakado, Tsukuba, Japan.

[b] A sequence homologous to the pSDL2 probe was detected in whole-cell DNA by Southern blot (+), or no sequence homology was detected (−).

[c] Mouse virulence was assayed as described in the text: +, virulent; −, nonvirulent; NT, not tested.

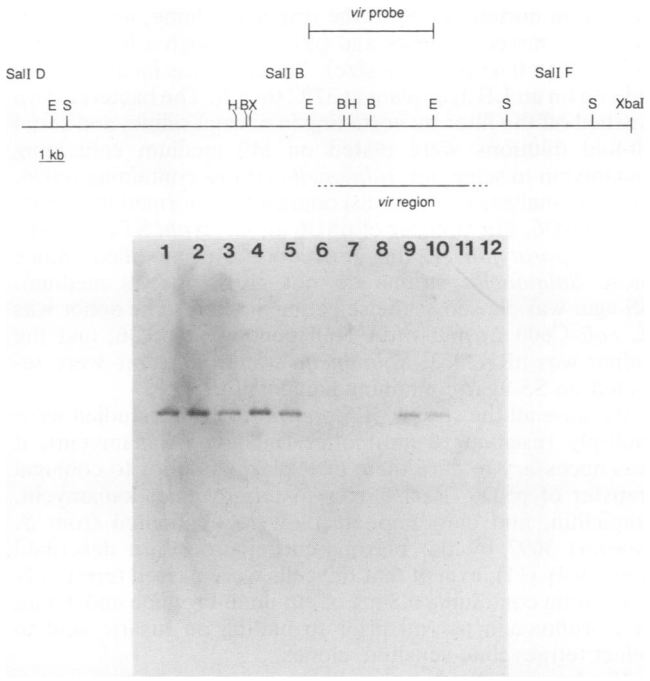

FIG. 1. Hybridization of DNA from *Salmonella* strains to the virulence region of pSDL2. The location of the *Eco*RI fragment used as the hybridization probe is shown on the restriction map of the *Sal*I B fragment of pSDL2 (5). The solid bar marked *vir* region shows the limits of Tn*5-oriT* inserts that abolish virulence in mice (5), and the dotted lines indicate that the exact ends of *vir* are not known. The *vir* probe was hybridized to *Eco*RI digests of whole-cell DNA prepared from *S. choleraesuis* SCH1 (lane 1), *S. dublin* SD2 (lane 2), *S. dublin* SD4 (lane 3), *S. enteritidis* SE1 (lane 4), *S. gallinarum* SGP1 (lane 5), *S. paratyphi* A SPA1 (lane 6), *S. paratyphi* B SPB1 (lane 7), *S. typhi* ST1 (lane 8), *S. typhimurium* STM3 (lane 9), *S. dublin* Lane (lane 10), and *S. dublin* LD842 (lane 11). Lane 12 is *Hin*dIII-digested λ DNA.

usually blood, and there was no epidemiological evidence for a common source except for the *S. newport* isolates (41). Some of the *S. havana* strains from the Centers for Disease Control were from an outbreak in Georgia that was characterized by multiple cases of extraintestinal infection. Strains from the Public Health Laboratory, San Diego County, were primarily gastrointestinal isolates. *S. minnesota* was a laboratory strain (14). The *S. typhi* and *S. paratyphi* A strains were isolated from blood cultures. *S. naestved* was a bovine isolate from Japan. *Escherichia coli* strains used as donors for matings were *E. coli* JA221 (*leuB trpE5 lacY recA hsdR*$^-$ *hsdM*$^+$) containing pSD6 (11) or *E. coli* C600 Δ*trpE5 recA* Nal^r (22) containing pSD6. pSD6 is a derivative of pSDL2 obtained by insertion of Tn*5-oriT* (11, 45) and restores wild-type virulence to the plasmid-free *S. dublin* strain LD842. The transfer-proficient helper plasmid pRK2073 was maintained in *E. coli* C600 Δ*trpE5 recA* (45).

Growth media. Bacterial strains were grown in LB (1% tryptone, 0.5% yeast extract, and 0.5% NaCl), tryptic soy broth (TSB), or Mueller-Hinton medium. For nutritional selection, M9 medium containing 0.5% casamino acids and 0.2% glucose (32) and *Salmonella-Shigella* agar (SS-agar; BBL Microbiology Systems, Cockeysville, Md.) were used. Antibiotics were added as indicated for selection at the following concentrations: kanamycin, 50 μg/ml; trimethoprim, 100 μg/ml; and nalidixic acid, 25 μg/ml.

DNA isolation. Rapid isolation of plasmid DNA for clone analysis of *Salmonella* and *E. coli* strains was performed by the alkaline lysis procedure (30). Whole-cell DNA for Southern hybridization was obtained by the technique described by Maniatis et al. (30).

DNA hybridization. The standard procedures for agarose gel electrophoresis and transfer of DNA fragments to nitrocellulose were performed as described previously (5). The virulence region probe consisted of the 4-kb *Eco*RI fragment contained within the *Sal*I B fragment of pSDL2 (Fig. 1). The probe was purified by agarose gel electrophoresis from an *Eco*RI digest of pSD15.2, which was constructed by ligating the *Sal*I B fragment of pSDL2 into the *Sal*I site of pBR322. The probe was labeled with biotin-7 dATP by using a nick translation kit from Bethesda Research Laboratories (BRL). Hybridization and washes were done under stringent conditions, and color development reactions were performed according to a protocol supplied by BRL (BluGene System). *S. dublin* Lane was used as a positive control, and LD842 was used as a negative control.

Conjugal transfer of pSD6 into *Salmonella* serotypes. Transfer of pSD6 into *S. derby*, *S. havana*, *S. heidelberg*, *S. minnesota*, *S. saintpaul*, and *S. newport* was done as described previously for transfers into *S. dublin* (11). Strain *E. coli* JA221(pSD6) was grown in LB broth with kanamycin, strain *E. coli* C600 Δ*trpE5 recA*(pRK2073) was grown in Mueller-Hinton broth with trimethoprim, and the *Salmonella* strains were grown in LB broth. Strains were harvested in late log phase, washed once in normal saline, and sus-

pended in normal saline to the original volume, and 1 ml of each was mixed together and passed through a filter (Millipore Corp.; 0.45-μm pore size). The filter was incubated cell side up on an LB agar plate at 37°C for 1 h. The bacteria were washed off the filter by vortexing in normal saline, and serial 10-fold dilutions were plated on M9 medium containing kanamycin to select for *Salmonella* strains containing pSD6. Plasmid analysis of the transconjugants confirmed the transfer of pSD6. For transfer of pSD6 into *S. typhi* ST1 and ST2 and *S. paratyphi* A, this procedure was modified. Since these *Salmonella* strains do not grow on M9 medium, SS-agar was chosen as the selecting medium. The donor was *E. coli* C600 Δ*trpE5 recA* Nal^r containing pSD6, and the helper was pRK2073. *Salmonella* transconjugants were selected on SS-agar containing kanamycin.

Because all the strains of *S. newport* that we studied were multiply resistant to antibiotics (including kanamycin), it was necessary to cure them of R plasmids prior to conjugal transfer of pSD6. Resistances to tetracycline, kanamycin, ampicillin, and chloramphenicol were eliminated from *S. newport* 3097 by the plasmid-curing procedure described previously (11), except that the cells were passed repeatedly in medium containing 0.8 mg of ethidium bromide and 0.3 μg of ciprofloxacin per ml prior to plating on fusaric acid to select tetracycline-sensitive clones.

Virulence studies in mice. Salmonellae were grown overnight in TSB, washed once in normal saline, and suspended in saline to an optical density that corresponded to 10^9 CFU/ml. Salmonellae containing pSD6 were grown overnight in TSB with kanamycin (50 μg/ml) to ensure preservation of the plasmid in the inoculum. Each strain was diluted in saline to approximately 10^6 CFU/ml, and 0.1 ml was injected intraperitoneally. We tested each strain in three to five female BALB/c mice (15 to 18 g). The mice were observed for 14 days or were killed after 10 days in order to enumerate bacteria in their spleens as described previously (20). All mice infected with *S. dublin* Lane (10^2 CFU) died between 7 and 14 days after infection.

All experiments involving *S. typhi* and *S. paratyphi* A were approved by the Institutional Biosafety Committee of the University of California, San Diego. Mice infected with these strains were housed in isolators. All potentially contaminated material, including dead mice, was autoclaved prior to disposal.

RESULTS

Correlation between mouse virulence and the presence of a sequence homologous to the virulence region of pSDL2. We studied 53 isolates from 22 *Salmonella* serotypes. The results of hybridization of *Salmonella* whole-cell DNA to the virulence region of pSDL2 and the studies of mouse virulence are summarized in Table 1, and representative strains are shown in Fig. 1. *S. dublin* Lane was used as a positive control, and its cured derivative, LD842, served as the negative control. If no deaths occurred within 14 days of infection, the strain was considered avirulent for mice. Surviving mice were autopsied and their spleens were cultured. None of them contained greater than 10^3 CFU, confirming the lack of virulence in mice. All strains classified as virulent killed the majority of mice, and most killed all the infected mice within 14 days. Six serotypes (10 isolates) contained a 4-kb *Eco*RI fragment homologous to the virulence region of pSDL2 by Southern hybridization, and all these strains except *S. gallinarum* were virulent in mice: *S. choleraesuis* SCH1, *S. choleraesuis* SCH2, *S. choleraesuis* var. Kunzendorf, *S. dublin* SD2, *S. dublin* SD4, *S. typhimurium* var. Copenhagen, *S. typhimurium* STM3, *S. enteritidis* SE1, and *S. naestved*. The *S. gallinarum* isolate hybridized to the probe but was not tested in mice because this serotype is host-adapted to fowl and nonvirulent for mice. None of the other 16 serotypes (43 isolates) hybridized to the probe, and none of these were virulent in mice. The results were the same for all isolates within a serotype and demonstrate that only those serotypes that contain the highly conserved 4-kb *Eco*RI homologous fragment are virulent in mice.

TABLE 2. Mouse virulence of wild-type and isogenic pSD6-containing *Salmonella* strains

Serotype	No. of isolates	Virulence[a]	
		Wild-type	With pSD6
S. derby	1	−	−
S. havana	2	−	−
S. heidelberg	3	−	+
S. minnesota	1	−	−
S. newport	1	−	+
S. ohio	1	−	−
S. paratyphi A	1	−	−
S. saintpaul	1	−	−
S. typhi	2	−	−

[a] Mouse virulence was assayed as described in the text: +, virulent; −, nonvirulent.

Transfer of pSD6 and virulence in mice. To determine whether nonvirulent serotypes could be converted to virulent strains, we transferred pSD6 into 13 *Salmonella* isolates from nine different serotypes that lacked a native virulence plasmid: *S. derby*, *S. havana*, *S. heidelberg*, *S. minnesota*, *S. newport*, *S. saintpaul*, *S. ohio*, *S. typhi*, and *S. paratyphi* A. Transfer and stability of pSD6 in transconjugants were confirmed, before and after infection of mice, by rapid isolation of plasmid DNA and agarose gel electrophoresis. The inocula were adjusted to 5×10^3 CFU/mouse, except that 10^5 CFU/mouse was the inoculum used for *S. typhi* and *S. paratyphi* A serotypes (both wild-type strains and pSD6-containing transconjugants). Table 2 gives the virulence of wild-type strains (pSD6$^-$) and of their isogenic derivatives containing pSD6 (pSD6$^+$). Only two serotypes, *S. heidelberg* and *S. newport*, were rendered virulent by the introduction of pSD6, and all three *S. heidelberg* isolates tested expressed virulence in conjunction with pSD6. None of the seven other serotypes were rendered virulent by pSD6.

The virulence of pSD6 in *S. heidelberg* was investigated further. Table 3 shows the results of the virulence testing of two *S. heidelberg* strains in groups of 10 mice, with an intraperitoneal inoculum of 10^3 organisms. Mice surviving 5 days after infection were killed, and the number of bacteria in the spleen was determined as a measure of virulence. Both strains 2883 and 3360 were capable of multiplying in infected animals but did not reach lethal levels of infection. In contrast, introduction of pSD6 into these strains dramatically increased the virulence as measured by the number of deaths and the bacterial counts in the spleens of survivors after 5 days.

The introduction of pSD6 into *S. newport* for virulence testing presented a problem because all isolates available to us were resistant to ampicillin, tetracycline, sulfonamides, kanamycin, and chloramphenicol. Therefore, *S. newport* 3097 was first cured of these markers, facilitating the transfer of pSD6 into the cured derivative, 3097-1. When tested for virulence, 3097-1 containing pSD6 killed four of five mice

TABLE 3. Virulence of *S. heidelberg* strains with and without pSD6

Strain	pSD6 present	No. of mice dead/no. tested	No. of bacteria in spleens of survivors[a]
S. heidelberg 2883	−	0/10	4.5 ± 0.2
	+	9/10	8.0
S. heidelberg 3360	−	0/10	4.5 ± 0.3
	+	4/10	6.9 ± 0.6

[a] Log_{10} ± standard deviation measured 5 days after inoculation.

after 14 days, while 3097 and 3097-1 without pSD6 did not result in any deaths.

Previous work has shown that *S. typhi* and *S. paratyphi* A lack virulence for mice (10, 33). Our hybridization results indicate that both serotypes are missing the plasmid-mediated virulence sequences. We investigated whether introduction of pSD6 into *S. typhi* and *S. paratyphi* A would increase the ability of these strains to proliferate in mice. Two strains of *S. typhi* and one strain of *S. paratyphi* A containing pSD6 were compared with their wild-type parents by intraperitoneal inoculation of 10^5 organisms in groups of five mice. When no deaths occurred after 10 days in any of the mice, all the animals were killed and organism counts in the spleens were determined. None of the strains could establish a significant infection, since the number of bacteria per spleen was less than 200 in every animal. The lack of virulence was not due to plasmid instability in vivo, since the spleen isolates were shown to contain pSD6 by plasmid analysis of representative colonies.

DISCUSSION

We screened 53 clinical isolates of *Salmonella*, representing 22 serotypes in seven O antigen groups, using a 4-kb probe made from the virulence region of pSDL2 (5), which enabled us to correlate the presence of the virulence region with pathogenicity in mice. None of the 43 *Salmonella* isolates (16 serotypes) that lacked this plasmid-encoded sequence were virulent in mice. Conversely, the five serotypes that contained the virulence region were virulent in mice. We did not test *S. gallinarum* for virulence because this serotype is highly host-adapted to fowl and is known not to be mouse virulent (12). In each strain that hybridized to the probe, an identical 4-kb *Eco*RI homologous fragment was found. Using a larger 8-kb *Sal*I-*Xho*I fragment probe from a similar region of the *S. typhimurium* plasmid, Williamson et al. (43) found differences in the sizes of the hybridizing *Sal*I-*Xho*I fragments in some isolates of *Salmonella*. In both studies, *S. choleraesuis*, *S. dublin*, *S. enteritidis*, *S. gallinarum*, and *S. typhimurium* hybridized, whereas *S. derby*, *S. heidelberg*, *S. java*, *S. montevideo*, *S. newport*, and *S. saintpaul* did not. Furthermore, several serotypes (including *S. derby*, *S. heidelberg*, *S. minnesota*, and *S. newport*) which failed to hybridize do harbor plasmids of unknown phenotype (43) (data not shown). The presence of a virulence plasmid within a given serotype may vary with isolates (i.e., *S. dublin*, *S. enteritidis*, and *S. naestved*) (11, 43; this paper). Our results indicate that the 4-kb *Eco*RI fragment is present in several serotypes of *Salmonella* and that this conserved region is required for *Salmonella* species to express virulence in mice. However, we also clearly established that the virulence plasmid is not sufficient to make all wild-type *Salmonella* spp. virulent for mice. We had previously shown that a virulence plasmid from *S. dublin*, pSD6, could restore virulence to *S. enteritidis* that had been cured of its virulence plasmid (5). In this study, we showed that while two nonvirulent serotypes (*S. newport* and *S. heidelberg*) could be made mouse-virulent by introducing pSD6, seven other serotypes of *Salmonella* were not made mouse virulent by pSD6. Because we tested only one to three isolates of each serotype, we cannot be certain that the virulence potential of all strains of a given serotype is the same. However, whenever we tested multiple strains of a single serotype, there was complete concordance of results.

This study shows that chromosomal genes necessary for the expression of mouse virulence may be absent in the isolates of *S. derby*, *S. havana*, *S. minnesota*, *S. ohio*, *S. paratyphi* A, *S. saintpaul*, and *S. typhi* listed in Table 2. The *Salmonella* genome has been mapped for a large number of markers (37), and certain loci influencing virulence have been described, including auxotrophies (23, 40), metabolic defects (13), and the structure of O antigen side chains (28, 36). However, none of these factors can explain the inability of pSD6 to increase the mouse virulence of these strains. Other chromosomal virulence genes are known to be present in *S. typhimurium* (6, 15), but nothing is known about the distribution of these genes among the many serotypes of *Salmonella*. The close relationship between *S. heidelberg* and *S. typhimurium* demonstrated by Beltran et al. by isoenzyme analysis (4) may explain the ability of pSD6 to render *S. heidelberg* mouse-virulent. Except for this observation, there is no clear relationship between mouse virulence and genetic proximity among *Salmonella* species. It could be also possible that the virulence genes on pSD6 are not expressed in all *Salmonella* serotypes, even though plasmid stability and copy number are similar to the wild-type pSDL2 virulence plasmid of *S. dublin*.

It is particularly significant that neither *S. typhi* nor *S. paratyphi* A carries virulence plasmids (34), nor could we find evidence of a homologous genetic sequence in their chromosomal DNA. Nevertheless, these species are highly pathogenic in humans even though they are avirulent in mice. We postulated that the restricted host range of *S. typhi* and *S. paratyphi* A might be a consequence of not having a virulence plasmid, but we were unable to overcome this species barrier by transfer of pSD6. Apparently these two human-adapted *Salmonella* species lack chromosomal genes that are required for growth in the murine liver and spleen (33), and the function of these genes is not replaced by the virulence plasmid which determines whether or not *S. dublin* proliferates in the same organs (20). These observations seriously question the long-held belief that *S. typhimurium* infection in mice (mouse typhoid) is analogous to *S. typhi* infection in humans (29). It appears that all mouse-virulent *Salmonella* strains, including *S. typhimurium*, require plasmid-encoded genes in order to be fully virulent in mice. These genes are not required to produce typhoid fever in humans. Furthermore, *S. typhi* lacks chromosomal genes required for virulence in mice. Therefore, the virulence mechanisms important for *S. typhi* infection in humans and *S. typhimurium* disease in mice may differ substantially.

The evidence strongly suggests that virulence plasmids are important in the pathogenesis of naturally occurring systemic *Salmonella* infections in animals. All the major *Salmonella* species that are highly host-adapted to animals carry a virulence plasmid, including *S. choleraesuis* (27), *S. dublin* (1, 11, 42), *S. gallinarum* (3), *S. pullorum* (2), and *S. abortus ovis* (34, 43). The importance of the virulence plasmid is now firmly established in experimental murine infections. It is also likely that the plasmid contributes

substantially to the virulence of nontyphoid *Salmonella* species in human disease and is probably responsible for the septicemic form of salmonellosis (39). The two serotypes of *Salmonella* that most often cause this syndrome are *S. choleraesuis* (38) and *S. dublin* (16), and nearly all naturally occurring isolates of these bacteria carry the virulence plasmid. Furthermore, the two other *Salmonella* species that are commonly isolated from blood cultures, *S. typhimurium* and *S. enteritidis* (8), also contain virulence plasmids (25, 26), although they are not found in all clinical isolates of *S. typhimurium* (9). The absence of virulence plasmid sequences in certain extraintestinal isolates of *Salmonella* does not contradict the hypothesis that the plasmid contributes to virulence in human disease, since other important factors influence the invasiveness of *Salmonella* infection, including the inoculum size and immunocompetence of the host (24). We postulate that the *Salmonella* virulence plasmids enhance the ability of the bacteria to multiply in extraintestinal tissues of both animal and human hosts.

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