Bacteræmia assays in chickens as a model for the evaluation of the virulence of Salmonella enterica serovars Typhimurium and Enteritidis strains

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SUMMARY

The virulence of twelve Salmonella enterica isolates, seven serovar Typhimurium and five serovar Enteritidis was evaluated in vivo after oral, intramuscular and intravenous inoculation of chickens. It was assumed that the bacteria would exhibit different degrees of virulence according to their origin. The experiments were conducted in order to obtain a method to evaluate the virulence of field isolates of Salmonella in the chick, via a method, which is more rapid than conventional models. Results were obtained within 21 days for the oral model, considered to be the reference, and within 7 days for the intramuscular model. The classification achieved with the intravenous model, comparing either mortality results until seven days or bacteraemia levels at 48-72 hours, was identical. Thus, a bacteraemia model using 12-day-old chicks inoculated intravenously with Salmonella Typhimurium isolates allowed rapid assessment of their relative virulence.

Moreover, the bacteraemia assays were used to test invA mutant strains of both serotypes and appeared useful in the differentiation between a Salmonella Typhimurium wild-type strain from the isogenic invA mutant.

Keywords : Salmonella - virulence - chicken - bacteræmia - model.

Introduction

Salmonella enterica serovars Typhimurium and Enteritidis are among the major aetiologic agents of human gastro-enteritis in France and are more frequently isolated in poultry than other types of products.

Salmonella strains are frequently isolated in poultry farms from healthy carrier birds as well as from birds that show clinical signs of illness. Infection in chickens with Salmonella during the first days of life can be followed by clinical signs and high mortality [6, 8]. It may be important to determine rapidly the virulence of a Salmonella field isolate for chickens in order to evaluate the possible health consequences of the infection. The pathogenesis of salmonellosis is complex and a number of in vivo and in vitro models have been developed to study the different stages of infection (for a review see [14]).

Our experiments were carried out in order to develop a rapid evaluation method of the virulence of Salmonella field isolates for the chick, by intravenous inoculation. We compared different strains of Salmonella Typhimurium and Salmonella Enteritidis in three experimental poultry models based on different conditions of inoculation. Oral challenge, the first model, mimics the natural route of infection [13]; it is routinely used in our laboratory, it gives a result within 21 days, based on different percentages of mortality resulting from variations in the virulence of the respective strains. When contamination occurs via the intramuscular route, differences in virulence are observed within a week. This probably corresponds to differences in the ability of the bacterium to grow within the host’s tissues and to overcome the host’s defences and/or the ability of the bacterium to invade and survive in macrophages. We were interested in introducing a third model using intravenous inoculation, which is presumed to give a more rapid answer. In the case of intravenous inoculation, a survey of bacteraemia can be performed within 48-72 hours [11, 12]. Mortality is also recorded up to the sixth day after inoculation, allowing the classification of...
isolates according to their virulence. The latter model, which is helpful for *E. coli* and *Enterococcus faecalis* infections [11, 12], could become the model of choice in field situations for salmonellosis.

The infection experiments used eight strains of *Salmonella* selected for their supposed different degrees of virulence depending on their origins. The experiments were carried out in order to develop a method of evaluation of the virulence of *Salmonella* field isolates in the chick, which is more rapid than conventional models. Two pairs of Typhimurium and Enteritidis strains were also tested to evaluate the ability of bacteremia assays to distinguish between isogenic wild type and mutant invA isolates. Present in the vast majority of *Salmonella* isolates, virulence inv genes are responsible for an invasive phenotype [7, 18].

### Materials and methods

1- **SALMONELLA STRAINS AND CULTURE CONDITIONS**

Seven strains of serovar Typhimurium: F98, BN8301, BN9501, BN91C1, BN9401, SL1344, SB136invA and five strains of serovar Enteritidis: BN92R1, SEPT9b, SEPT14b, 7193, SB131invA, were compared for their virulence in chickens. These poultry strains were from our collection [15, 16] except for F98 [3, 4], SEPT9b and SEPT14b [12]. In addition, wild type strains of serovars Typhimurium (SL1344) and Enteritidis (7193) and their respective isogenic invA mutants (SB136: SL1344invA, SB131: 7193invA) were obtained from Jorge Galán [7].

Three strains were isolated from pathological cases, F98 from chicken, BN8301 from pigeon and BN9401 from turkey. The strains BN8501, BN91C1 and BN92R1 isolated from healthy animals were considered as carriage strains. The strains SEPT9b and SEPT14b could not be classified as pathological or carriage isolates.

The strains were characterized as already described [15, 16]: they could be distinguished on the basis of ribotypes, IS200 types and phage types (Table I).

All strains were conserved in 20% glycerol at -80°C. After a 24 h preculture at 37°C in 5 mL Brain Heart Infusion (BHI) broth in a shaking water bath, all cultures were incubated in 5 mL BHI broth at 37°C for 18 h. These cultures contained approx. 10^9 viable bacteria per ml. *Salmonella*-Shigella agar (Sanofi Diagnostics Pasteur, France) was used for the enumeration of bacteria. Decimal dilutions of inoculum were made in sterile phosphate-buffered saline (10 mM sodium phosphate, 140 mM sodium chloride, pH 7.4).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin</th>
<th>Antibiotic resistance®</th>
<th>Plasmids (kb)</th>
<th>Ribotype</th>
<th>IS200 type</th>
<th>RAPD-type</th>
<th>ERIC-PCR profile</th>
<th>Phage type b</th>
</tr>
</thead>
<tbody>
<tr>
<td>F98</td>
<td>Chicken - pathology</td>
<td>NaI, OxO</td>
<td>90</td>
<td>12</td>
<td>IX</td>
<td>2-5-10</td>
<td>1</td>
<td>111 atyp</td>
</tr>
<tr>
<td>BN8301</td>
<td>Turkey - pathology</td>
<td>Tet</td>
<td>90, 3.5</td>
<td>16</td>
<td>IX</td>
<td>2-4-9</td>
<td>II</td>
<td>96</td>
</tr>
<tr>
<td>BN91C1</td>
<td>Pigeon - septicaemia</td>
<td>Sm</td>
<td>90, 60, 3</td>
<td>13</td>
<td>X</td>
<td>2-5-10</td>
<td>I</td>
<td>405</td>
</tr>
<tr>
<td>BN8501</td>
<td>Duck - carriage</td>
<td>-</td>
<td>90, 2.8, 2.5</td>
<td>6</td>
<td>II</td>
<td>2-5-10</td>
<td>I</td>
<td>209</td>
</tr>
<tr>
<td>SL1344</td>
<td>Galán and Curtiss, 1991</td>
<td>Sm</td>
<td>90</td>
<td>21</td>
<td>ND</td>
<td>2-7-10</td>
<td>I</td>
<td>ND</td>
</tr>
<tr>
<td>SB136</td>
<td>Galán and Curtiss, 1991</td>
<td>Km, Sm</td>
<td>90</td>
<td>21</td>
<td>ND</td>
<td>2-7-10</td>
<td>I</td>
<td>ND</td>
</tr>
<tr>
<td>BN92R1</td>
<td>Guinea fowl - carriage</td>
<td>-</td>
<td>54</td>
<td>10</td>
<td>VIII</td>
<td>a-c</td>
<td>II</td>
<td>33</td>
</tr>
<tr>
<td>SEPT9b</td>
<td>Poultry - Denmark</td>
<td>-</td>
<td>100</td>
<td>20</td>
<td>XV</td>
<td>a-c</td>
<td>II</td>
<td>9b</td>
</tr>
<tr>
<td>SEPT14b</td>
<td>Poultry - Denmark</td>
<td>Tet</td>
<td>54</td>
<td>10</td>
<td>VIII</td>
<td>a-c</td>
<td>II</td>
<td>14b</td>
</tr>
<tr>
<td>7193</td>
<td>Galán and Curtiss, 1991</td>
<td>-</td>
<td>54</td>
<td>20</td>
<td>ND</td>
<td>f-g</td>
<td>II</td>
<td>ND</td>
</tr>
<tr>
<td>SB131</td>
<td>Galán and Curtiss, 1991</td>
<td>Km</td>
<td>54</td>
<td>20</td>
<td>ND</td>
<td>f-g</td>
<td>II</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Table I**—Origin and characteristics of the twelve *Salmonella* isolates [15, 16]

°: susceptible. Km: Kanamycin; NaI: Nalidixic acid; OxO: Oxolinic acid; Sm: Streptomycin; Tet: Tetracycline.

b: Strains were phage-typed by F. Grimont (Institut Pasteur), except SEPT9b and SEPT14b phage-typed by J. E. Olsen, Ward scheme. atyp.: atypical. ND: not done.

2 - CHICKENS

Specified pathogen free (SPF) chickens used in experiments were from the PA12 White Leghorn outbred line, maintained by the Unité BioAgresseurs, Santé, Environnement (INRA, Tours; agreement nb 37802). They were humanely treated and anesthetized, according to national guidelines [1a].

3 - IN VIVO EXPERIMENTAL MODELS

3.1 - Oral challenge

Oral inoculation of around 30 or 60 PA12 one-day-old chickens per strain was made by direct inoculation into the crop with 0.5 mL broth culture containing \(10^8\) salmonellae. Birds were maintained during 21 days in sterile isolators. They were given ad libitum sterile water and feed. The intestinal carriage was assessed twice by enumeration of the Salmonella in the faeces of 5 birds, at days 12 and 21 after inoculation, only in the first assay. All 321 chicks were observed daily for a period of 21 days, and mortality and clinical signs were recorded. This model, commonly used in our laboratory, was used as the reference.

3.2 - Intramuscular challenge

As already described [4], intramuscular inoculation of 5-6 one-day-old PA12 chickens per dose was performed in the pectoral muscle with 0.1 mL decimal dilutions of an overnight culture. Five doses ranging from \(10^4\) to \(10^8\) bacteria were used for each strain. All 385 chicks were reared in micro-isolators and observed daily for a period of 7 days.

3.3 - Intravenous challenge

Intravenous inoculation of 15 to 30 chickens per strain was performed by intravenous injection into the ulnar vein with 0.1 mL containing \(10^7\) salmonellae [6]. The chickens were inoculated at 12 days of age. The kinetics of elimination or multiplication of the bacteria were followed in capillary blood samples taken 30 min, 1 h, 3 h, 4 h, 24 h, and 48 h after inoculation. Two 50 µL samples were taken at each time point from all individuals. The first sample was used for direct plating and enumeration on Salmonella-Shigella agar. Decimal dilutions in sterile PBS of the second sample were spread on Salmonella-Shigella agar plates. All 192 chickens were observed for a period of 7 days and mortality recorded daily. Three successive assays were conducted with strains F98, BN8501 and BN9401, and two successive experiments with strains BN8301, BN91C1, BN92R1, SEPT9b and SEPT14b, SL1344, SB136, 7193 and SB131.

4 - Statistical analyses

Statistical analyses were performed using the software package SAS [20]. For oral and intravenous challenges, survival curves were plotted and differences between animals inoculated with different Salmonella strains were compared using the log-rank test (SAS Lifetest procedure). The LD50s (lethal dose 50%) after intramuscular challenge were calculated using a logistic model (SAS catmod procedure). Intravenous challenge data were analyzed using a mixed linear model to take into account the correlation between repeated measures in the same animal. The model also allowed the testing of an interaction between time and strains. When the interaction was not significant, strains were globally classified using the pairwise t test with a comparisonwise error rate equal to 5% of the number of comparisons. When this interaction was significant, we used a simple time by time Anova model. When differences were significant, strains were classified using a multiple Newman-Keuls test.

Results

1 - ORAL CHALLENGE

Table II presents the results of the first assay. No morbidity

<table>
<thead>
<tr>
<th>Strain</th>
<th>% mortality after oral challenge (Number of inoculated animals)</th>
<th>Median age on the day of death (mean ± SD)</th>
<th>Calculated logarithmic LD50** (Number of inoculated animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F98</td>
<td>38 a * (55)</td>
<td>5.5±1.43</td>
<td>5.19±0.38 ab (60)</td>
</tr>
<tr>
<td>BN9401</td>
<td>13 b (30)</td>
<td>4±2.16</td>
<td>4.84±0.32 ab (30)</td>
</tr>
<tr>
<td>BN91C1</td>
<td>5 bc (55)</td>
<td>4±1.00</td>
<td>4.59±0.22 b (60)</td>
</tr>
<tr>
<td>BN8501</td>
<td>3 c (60)</td>
<td>8±1.41</td>
<td>6.07±0.20 b (84)</td>
</tr>
<tr>
<td>BN8301</td>
<td>0 c (30)</td>
<td>-</td>
<td>5.97±0.26 b (60)</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BN92R1</td>
<td>17 b (30)</td>
<td>3±1.00</td>
<td>5.26±0.40 ab (40)</td>
</tr>
<tr>
<td>SEPT 9b</td>
<td>10 bc (30)</td>
<td>6±2.00</td>
<td>5.57±0.28 b (40)</td>
</tr>
<tr>
<td>SEPT 14b</td>
<td>8,5 bc (31)</td>
<td>2</td>
<td>4.42±0.31 b (40)</td>
</tr>
</tbody>
</table>

Table II.—Mortality percentages after oral challenge and lethal dose 50% after intramuscular challenge.
*: within the same rank column, values followed by different letters are significantly different with the log rank test (p<0.05).
**: LD50 ± Standard Error (S.E.).
was observed with BN8301. With the other strains, clinical signs could be observed in birds, which were ill. They generally died within 24 to 48 h after onset: showing ruffled feathers, drooping wings, and signs of dehydration and loss of balance. With strain F98, signs of diarrhoea were noted in the majority of birds (Table II).

The level of intestinal carriage was equivalent in all birds tested independent of the strains used for inoculation, demonstrating their establishment in the intestinal tract of the chickens (data not shown).

Mortality was recorded during the first nine days of rearing for all the strains used with the exception of BN8301.

The eight strains studied were classified into three categories according to their virulence after oral inoculation. Only strain F98, with 36% mortality, could be considered as a very virulent isolate. Strains BN9401, BN92R1 and SEPT9b, with 13%, 17% and 10% mortality respectively, were of intermediate virulence. Strains BN8301, BN8501, BN91C1 and SEPT14b were less virulent, with less than 10% mortality.

2- INTRAMUSCULAR CHALLENGE (TABLE II)

Differences between the most and the least virulent strains were weak in this model either with serotype Typhimurium or with serotype Enteritidis. Before dying, animals showed clinical signs: ruffled feathers, droopy wings, and signs of malaise and dehydration could also be observed. No diarrhoea was observed whatever the strain used. Calculated LD50 values ranged from 104.42 to 106.07 bacteria.

The classification of the strains into three categories according to their virulence after intramuscular challenge did not mirror the classification obtained after oral challenge. The strains BN9401, a turkey clinical isolate, and BN91C1 isolated from carriage in a duck were the most virulent. F98 was of intermediate virulence. BN8301 isolated from a pigeon septicaemia and BN8501 isolated from ducklings were the least virulent with a LD50 over 106 bacteria. Among the Enteritidis isolates, SEPT14b was the most virulent with LD50 of 104.42 bacteria. SEPT9b and BN92R1 were of intermediate virulence with LD50 values over 105 bacteria.

3- INTRAVENOUS CHALLENGE: BACTERAEMIA STUDIES

Preliminary results using PA12 White Leghorn chickens indicated that the clearest discrimination was obtained with 12-day-old chicks inoculated with 107 bacteria per animal.

Successive experiments demonstrated the reproducibility of the results: mortality percentages as well as levels of bacteremia.

Among Typhimurium isolates, significant differences were detected for the first 4 hours. The bacteraemia was high with BN9401 showing good colonization of capillary blood (around 104 to 105 bacteria per ml), and about 1 log lower with F98 (Table III). Further 48 h samples demonstrated the persistence of BN9401 as well as F98 within capillary blood. (Table III). The kinetics of bacteremia were very similar for BN8301, BN8501 and BN91C1.

The mortality percentages 6 days after intravenous challenge ranged from 5 to 100% (Table IV). Among Typhimurium isolates, BN9401 was the most and BN8501 the least virulent strain (Table IV). Mortality results were reproducible with significant differences in the exact probability Fisher’s test. The increase in individual bacteremia when associated with clinical signs preceded the animal’s death by circa 24 h, probably corresponding to an ante mortem septicaemia. Individual bacteremia and mortality results allowed the virulent isolates BN9401 and F98 to be grouped in this model, as well as those of the least virulent strains BN8301 and BN8501. Mortality results with BN91C1 were intermediate, however BN91C1 could not be distinguished from BN8301 and BN8501 by the levels of bacteremia.

The three isolates of serovar Enteritidis were characterized by an initial increase in capillary blood as well as a good persistence, with high levels of bacteremia. Bacteraemia and mortality results were reproducible. SEPT14b and BN92R1 induced 100% mortality, within 6 days after inoculation.

<table>
<thead>
<tr>
<th>Strain</th>
<th>30 min.</th>
<th>60 min.</th>
<th>3 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>F98 (n=28)</td>
<td>4.12±0.59 <strong>a</strong></td>
<td>4.00±0.73 bc</td>
<td>3.27±0.24 a</td>
<td>3.27±0.48 ab</td>
<td>3.41±0.55 a</td>
<td>3.52±0.88 a (n=28)</td>
</tr>
<tr>
<td>BN9401 (n=31)</td>
<td>4.85±0.34 ab</td>
<td>4.63±0.51 a</td>
<td>3.91±0.70 a</td>
<td>3.83±0.75 a</td>
<td>3.22±0.71 ab (n=28)</td>
<td>3.07±1.23 ab (n=28)</td>
</tr>
<tr>
<td>BN8501 (n=22)</td>
<td>3.88±0.48 c</td>
<td>3.88±0.48 bc</td>
<td>3.62±0.51 ab</td>
<td>3.32±0.54 ab</td>
<td>2.71±0.85 b (n=21)</td>
<td>2.62±0.93 bc (n=21)</td>
</tr>
<tr>
<td>BN8301 (n=22)</td>
<td>4.45±0.52 bc</td>
<td>3.64±0.91 c</td>
<td>3.85±0.62 a</td>
<td>3.08±1.06 b</td>
<td>2.70±0.94 b (n=21)</td>
<td>1.91±0.89 bc (n=19)</td>
</tr>
<tr>
<td>BN91C1 (n=23)</td>
<td>4.46±1.09 bc</td>
<td>3.73±0.87 c</td>
<td>3.53±0.14 a</td>
<td>3.50±0.78 ab</td>
<td>2.56±1.45 b (n=21)</td>
<td>1.76±1.12 c (n=19)</td>
</tr>
</tbody>
</table>

Table III.—Bacteraemia kinetics in 12-day-old PA12 White Leghorn chicks inoculated intravenously with 10⁷ Salmonella Typhimurium

Mean bacteremia (in Log₁₀ CFU salmonellae per ml of capillary blood) ± Standard Deviation (S.D.).

*: (n): number of animals
**: within the same column, values followed by different letters are significantly different with the log rank/ANOVA tests (p<0.05).
SEPT9b was of intermediate virulence, with 31% mortality (Table IV).

Three successive experiments were conducted with strains SL1344, SB136 invA, 7193 and SB131 invA. In the first experiment, significant differences were detected in the first 4 hours only: the bacteraemia was high with SL1344 showing a colonization of capillary blood and low with SB136 invA (data not shown).

Cumulative 48 h bacteraemia data from the three experiments were significantly different (log-rank test and Anova) between SL1344 and its invA isogenic mutant SB136 invA, but not between 7193 and SB131 invA (data not shown). Yet, the mortality percentages 6 days after intravenous challenge were not found to be significantly different, even for Typhimurium isolates (Table IV).

### Discussion

#### CHOICE OF BACTERIAL STRAINS

The first eight isolates used in this study were selected because of their different origins and therefore genetic characteristics. Six of them were defined as pathological or carriage strains according to field information, although this is not a very certain basis for classification since such anamnestic data can be biased by environmental conditions and numerous other factors.

Differences in virulence were observed for the different inoculation routes: mortality following oral challenge ranged from 0 to 36%, depending on the Salmonella isolate. Our values are compatible with other studies, which compared different serogroups of salmonellae in SPF chickens [19]. Reproducible differences between isolates in mortality following intramuscular or intravenous inoculation were also obtained.

#### CHOICE OF THE CHICKEN PA12 WHITE LEGHORN LINE

The PA12 line, used as a reference at the Unité BioAgresseurs, Santé, Environnement, originates from a commercial White Leghorn line.

Oral challenge results in our study confirmed the virulence of the strain F98 and the relative resistance of the PA12 line to Salmonella infections, which has already been described [9]. The calculated LD50 for F98, $10^{5.2}$, was higher than values reported by Bumstead and Barrow [4] with different chicken lines (LD50 around $10^3$ to $10^4$): this confirms also the relative resistance of the PA12 line to Salmonella [6]. Comparison assays undertaken with another White Leghorn poultry line: B13 chickens (data not shown), confirmed the greater susceptibility of B13 poultry line to infections by Salmonella [9]. As the results obtained with both poultry lines led to the same conclusions and since B13 chickens are more susceptible to infection and more difficult to obtain, we chose to use PA12 chickens in our experiments.

#### USEFULNESS OF THE BACTERAEMIA MODEL

The oral challenge was used as the reference method. The differences were higher between Typhimurium than between Enteritidis isolates, as has already been published [2, 19].

F98 was indeed the most virulent isolate after oral inoculation but of only intermediate virulence in the intramuscular model. The absence of a correlation between results obtained after oral and intramuscular challenges underlines the multifactorial feature of Salmonella virulence.
In the intravenous tests on 12-day-old PA12 animals, a classification of the strain virulence could be deduced from both bacteraemia levels and mortality percentages, resulting in the same grouping of the strains studied. Moreover, the mortality after intravenous inoculation can be predicted by the level of bacteraemia measured between 24 to 48 hours after inoculation.

The similarity of the classification of the virulence of the Typhimurium strains after oral and intravenous inoculation must be underlined. The two most virulent strains in the reference model are also the most virulent with the bacteraemia model. The two least virulent isolates in the reference test, BN8301 and BN8501, were also the least virulent after intravenous inoculation.

It is generally accepted that virulence is affected by three factors, namely adhesion to intestinal epithelium, invasion through the intestinal mucosa and finally intracellular survival. For instance, it has been demonstrated that Enteritidis flagella play an important role in pathogenesis in the chick [1]. The intravenous model theoretically skips the first two factors while the oral model tests all three. It is thus surprising that the intravenous model led to the same discrimination between isolates than the oral model, with the advantage of a more rapid response. This could mean that intracellular survival in internal organs is an important step in the virulence of Salmonella Typhimurium for the chick. Therefore, this intravenous model could be recommended for studying this particular step, occurring after the invasion process.

By contrast, results obtained with Enteritidis isolates were not concordant between the two models used. The intravenous model does not appear useful for the discrimination between Enteritidis isolates. This could also mean that critical steps in the virulence for chickens of either Typhimurium or Enteritidis isolates are different.

COMPARISON OF ISOGENIC WILD-TYPE AND INV A MUTANT STRAINS

The comparison of isogenic wild-type and mutant inv A strains of serotypes Typhimurium and Enteritidis was useful to evaluate the bacteraemia model after intravenous inoculation. The results obtained with strains SL1344 and SB136 might underline the potential of this particular model to discriminate between a laboratory mutant strain from an original wild-type isolate. However, 7193 and SB131 were not seen to be different, suggesting that SB131 may be not as attenuated as SB136, and that our experimental model needs to be refined.

Porter and Curtiss have stressed the importance of inv genes (invA, invB, invC) in the virulence of Salmonella Typhimurium and Enteritidis isolates for White Leghorn chicks, notably after oral inoculation [18]. Nevertheless, they failed to observe any effect of inv mutations on the virulence after intra-peritoneal inoculation. Our results contrast with this work, but only for Typhimurium isolates.

The present results need to be confirmed using additional isogenic mutants, in order to evaluate more precisely the potential of the bacteraemia model for the evaluation of the virulence of such strains.

Conclusion

In conclusion, experiments of bacteraemia allowed a rapid evaluation of the virulence of five field isolates of Salmonella Typhimurium. Indeed, in the bacteraemia model with 12-day-old PA12 animals, the level of bacteraemia between 24 and 48 hours after infection allows the evaluation of the virulence of strains of serovar Typhimurium.

The comparison of isogenic wild type and mutant invA strains of serovar Typhimurium and Enteritidis was also performed, using the bacteraemia model after intravenous inoculation. Surprisingly, the wild type Typhimurium strain SL1344 and its isogenic mutant, SB136 invA, were discriminated between in this model. This may suggest that these strains differ in other properties. On the contrary, the bacteraemia model did not discriminate between Enteritidis isolates 7193 and SB131 invA.

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