

RAPID COMMUNICATION

## Gastrointestinal tract distribution of *Salmonella* enteritidis in orally infected mice with a species-specific fluorescent quantitative polymerase chain reaction

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### Abstract

**AIM:** To identify and understand the regular distribution pattern and primary penetration site for *Salmonella* enteritidis (*S. enteritidis*) in the gastrointestinal tract.

**METHODS:** Based on the species-specific DNA sequence of *S. enteritidis* from GenBank, a species-specific real-time, fluorescence-based quantitative polymerase chain reaction (FQ-PCR) was developed for the detection of *S. enteritidis*. We used this assay to detect genomic DNA of *S. enteritidis* in the gastrointestinal tract, including duodenum, jejunum, ileum, cecum, colon, rectum, esophagus and stomach, from mice after oral infection.

**RESULTS:** *S. enteritidis* was consistently detected in all segments of the gastrointestinal tract. The jejunum and ileum were positive at 8 h post inoculation, and the final organ to show a positive result was the stomach at 18 h post inoculation. The copy number of *S. enteritidis* DNA in each tissue reached a peak at 24-36 h post inoculation, with the jejunum, ileum and cecum containing high concentrations of *S. enteritidis*, whereas the duodenum, colon, rectum, stomach and esophagus had low concentrations. *S. enteritidis* began to decrease and vanished at 2 d post inoculation, but it was still present up to 5 d post inoculation in the jejunum, ileum and

cecum, without causing apparent symptoms. By 5 d post inoculation, the cecum had significantly higher numbers of *S. enteritidis* than any of the other areas ( $P < 0.01$ ), and this appeared to reflect its function as a repository for *S. enteritidis*.

**CONCLUSION:** The results provided significant data for clarifying the pathogenic mechanism of *S. enteritidis* in the gastrointestinal tract, and showed that the jejunum, ileum and cecum are the primary sites of invasion in normal mice after oral infection. This study will help to further understanding of the mechanisms of action of *S. enteritidis*.

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**Key words:** Fluorescence-based quantitative polymerase chain reaction; Gastrointestinal tract; *Salmonella* enteritidis

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### INTRODUCTION

*Salmonella* is an enteric pathogen that colonizes the intestinal tract of a variety of animals, especially humans and poultry, and accounts for millions of cases of gastroenteritis and food-borne illness each year<sup>[1,2]</sup>. *Salmonella* enteritidis (*S. enteritidis*) can be transmitted to humans through the food production chain, and undercooked or raw eggs and poultry meat are a particularly high risk for humans<sup>[3]</sup>. In the last few decades, *S. enteritidis* has emerged as a major cause of food-borne illness worldwide. As a result of the increased prevalence of *S. enteritidis* and its complex life cycle, identifying the regular distribution pattern of *S. enteritidis* in the gastrointestinal tract will help to understand its mechanism of action.

Previous studies have shown that orally introduced *S. enteritidis* has a rapid transit time through the intestine, and a small proportion of the inoculum establishes itself within the walls of the small intestine and cecum several

days before systemic infection<sup>[4,5]</sup>. However, the primary site of infection and the route taken by the organism to reach the heart, liver and spleen, are unclear. Infection with *Salmonella* is usually started by oral ingestion of the pathogen, and is followed by bacterial colonization of the gut and invasion of internal tissues. Therefore, knowledge about the mechanisms leading to invasive infections may ultimately lead to new insights into prevention and therapy.

We evaluated the specific fragment (Sdf I) reported by Agron<sup>[6]</sup>, which was screened for using the Suppression Subtractive Hybridization method. Sdf I appears to only be found in serovar enteritidis strains, which includes a wide range of clinical and environmental species. Here, based on the specific DNA sequence of serovar enteritidis, a specific fluorescent quantitative polymerase chain reaction (FQ-PCR) for detection of serovar enteritidis was developed and applied to the study of the gastrointestinal tract distribution of *S. enteritidis*.

## MATERIALS AND METHODS

### Bacterial strains

A total of 14 *Salmonella* strains were included in this study. Most strains were purchased from the National Center for Medical Culture Collection, including *S. enteritidis* (Human, No. 50041), *S. enteritidis* (Human, No. 50040), *S. enteritidis* (Mouse, No. 50338), *Salmonella* Choleraesuis (No. 50191-1), *Salmonella* Typhi (No. 50013), *Salmonella* Typhimurium (No. 50115-13), *Salmonella* Paratyphi (No. 50001-24), *Salmonella* Pullorum (No. 50047-2), *Salmonella* Anatum (No. 50083-4), *Salmonella* Gallinarum (No. 50770), *Salmonella* Dublin (No. 50761). Three strains were isolated and maintained by the Research Center of Poultry Diseases, College of Animal Science and Veterinary Medicine of Sichuan Agricultural University, including *S. enteritidis* (Duck, No. MY<sub>1</sub>), *S. enteritidis* (Duck, No. SC<sub>1</sub>) and *S. enteritidis* (Chicken, No. CD<sub>1</sub>).

### Preparation of bacterial samples and generation of standard templates

For the bacterial samples, 5 mL of an overnight culture grown in Luria-Bertani broth was prepared, and then the *S. enteritidis* cells were harvested by centrifugation. The pellet was resuspended in lysozyme solution, followed by lysis using 10% SDS at 60°C for 1 h. DNA was purified by extraction with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). Then, a 1/10 volume of 3 mol/L sodium acetate and 2 vols absolute ethanol were added, and the nucleic acid was then pelleted by centrifugation, washed with 70% ethanol, and dried under vacuum. The DNA genomic pellet was resuspended in 40 µL TE buffer, and stored at -20°C until use.

A conventional PCR was carried out using a template from *S. enteritidis* (Mouse, No. 50338), with primers F<sub>1</sub> and R<sub>1</sub> (designed with Sdf I, GenBank Accession No. AF370707.1, generated by TakaRa Biotech, Dalian, China). The primer sequences from 5' to 3' were as follows: F<sub>1</sub>, TGTGTTTATCTGATGCAAGAGG; and R<sub>1</sub>, CGTTC TTCTGGTACTTACGATGAC. Amplification was carried out in a total volume of 50 µL, containing 1 µL each

primer (25 µmol/L), 1 µL dNTPs (10 mmol/L), 2.5 U Taq DNA Polymerase (TaKaRa Taq; TakaRa Biotech), 5 µL 10 × PCR buffer (with Mg<sup>2+</sup>, 25 mmol/L) and 2 µL templates, which were then made up to a volume of 50 µL with deionized water. An initial denaturation at 95°C for 5 min was followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 52.5°C for 30 s, and extension at 72°C for 40 s. Finally, an additional extension was achieved for 10 min at 72°C. The product size was 293 bp.

Finally, the product was gel-excised and quantified with appropriate standards. Its concentration was determined spectrophotometrically using the Bio-Rad Smartspec-3000, according to the manufacturer's instructions. The standards were diluted, divided into aliquots, and frozen before use.

### Specific verification of FQ-PCR and its products

The FQ-PCR assay, including volume, Mg<sup>2+</sup> concentration, probe and primer concentrations, and annealing temperature were optimized. Subsequently, the sensitivity of the assay, the linear range and standard curve were determined by using known amounts of purified template DNA (generated as described above). The primers (F<sub>2</sub> and R<sub>2</sub>) and TaqMan-probe (FP) of FQ-PCR were designed using an internal region of the 293 bp sequences (described above, generated by TakaRa Biotech), and were used as follows, from 5' to 3': F<sub>2</sub>, TTGATGTGGTTGGTTCGTCCT; R<sub>2</sub>, TCCCTGAA TCTGAGAAAGAAAACTC; and TaqMan-probe (FP), FAM-TGCAGCGAGCATGTTCTGGAAAGC-TAMRA. Amplification was carried out in a total volume of 25 µL, containing 0.6 µL each primer (10 µmol/L), 0.75 µL dNTPs (10 mmol/L), 1.25 U Ex Taq DNA Polymerase (TaKaRa Ex Taq Hot Start Version; TakaRa Biotech), 5 × PCR buffer (free Mg<sup>2+</sup>) 5 µL, 0.8 µL TaqMan probe (5 µmol/L), 0.5 µL Mg<sup>2+</sup> (250 mmol/L) and 1 µL templates, then made up to a volume of 25 µL with deionized water. Each run consisted of a 95°C 5 min hot start, which activated the conjugated polymerase, followed by 45 cycles (50 cycles for sensitivity experiments) with 94°C denaturation for 30 s, 55°C annealing for 30 s, and reading the fluorescent signal at this step.

The primers of FQ-PCR (F<sub>2</sub> and R<sub>2</sub>) were used for conventional PCR with *S. enteritidis* (Mouse, No. 50338) DNA templates, in order to verify the specific amplification. Amplification was carried out in a total volume of 50 µL, containing 1 µL each primer (25 µmol/L), 1 µL dNTPs (10 mmol/L), 2.5 U Taq DNA polymerase (TaKaRa Taq; TakaRa Biotech), 10 × PCR buffer (with Mg<sup>2+</sup>, 25 mmol/L) 5 µL, and 2 µL templates, then made up to a volume of 50 µL with deionized water. Initial denaturation at 95°C for 5 min was followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 49°C for 30 s, and extension at 72°C for 40 s. Finally, an additional extension was achieved for 10 min at 72°C. A 10 µL aliquot of PCR product was electrophoresed on a 1.5% agarose gel for 40-50 min at 80 V, and visualized and photographed under UV illumination.

### FQ-PCR standard curve

Based on previous studies, the standard curve was generated as follows<sup>[7-9]</sup>: Standard DNA from *S. enteritidis*

was used to establish a standard curve. The standard DNA contained amplified target DNA in different quantities which was included in each LightCycler run. The Primers F<sub>2</sub> and R<sub>2</sub> were used for this amplification, and a range of  $9 \times 10^8$  -  $9 \times 10^3$  gene copies was used. Concentrations of the standards were measured by fluorometric analysis, and an analysis of key LightCycler measures was performed after each run to verify identical amplification efficiencies and conditions between runs. Finally, these data were used to generate the standard curve through the iCycler IQ Detection System (Bio-Rad, USA) software.

### Reproducibility

To evaluate the variability between experiments, four different known concentrations of DNA were amplified by performing the assay described above in triplicate. For each experiment, the crossing point, average crossing point, standard deviation, and coefficient of variation for each assay were calculated.

### Sensitivity of FQ-PCR

To determine the detection limit of this FQ-PCR assay, different quantities of standard DNA and different cell numbers of *S. enteritidis* were introduced in the FQ-PCR. A range of  $4 \times 10^3$  -  $4 \times 10^{-1}$  copies of standard DNA of *S. enteritidis* was added. Then, the results of each concentration were measured by fluorometric analysis. Bacterial cell limit detection was as follows: *S. enteritidis* (Mouse, No. 50338) grown aerobically at 37°C for 18 h in 5 mL Luria-Bertani broth. Subsequently, 1 mL of overnight culture was harvested, and the pellet was resuspended in 500 µL TE buffer (pH 8.0), from which the number of *S. enteritidis* cells was obtained by conventional viable count method. Then, a 10 fold serial dilution was performed on the overnight culture liquor, and the phenol/chloroform/isoamyl alcohol method was used to extract the DNA template serially from  $10^{-6}$  -  $10^{-10}$  serial dilutions of bacterial liquor. Finally, 1 µL aliquot per concentration of the DNA template was subjected to a test of bacterial cell sensitivity, and the result of the viable count was used to obtain the final result. The FQ-PCR was performed and analyzed as described above.

### Specificity of the FQ-PCR

All 14 bacterial strains were used to assess the specificity of the FQ-PCR. The boiling method was used to prepare the DNA template, and 4 µL of this was used in FQ-PCR.

### Experimental infection of mice

Our infection model was based on previous studies, which showed that orally introduced *S. enteritidis* had a rapid transit time through the intestine and establishes itself within the walls of the gut for more than 3 d<sup>[4,5,10-12,20]</sup>. Twenty-eight mice (age 9 wk, specific-pathogen-free) were purchased from the Animal Center of Sichuan University, China. In brief, a group of 14 white mice were orally infected with a virulent *S. enteritidis* strain (Mouse, No. 50338), at  $2.0 \times 10^4$  cells per mouse. Another group of 14 white mice was treated with an equal volume of water as a control. Duodenum, jejunum, ileum, cecum, colon,

rectum, esophagus and stomach were analyzed by FQ-PCR at different post-inoculation time points, at 30 min, 1, 2, 4, 8, 12, 16, 18, 24 and 36 h, and 2, 3, 4 and 5 d.

One mouse from each group was sacrificed at each time point and its organs were aseptically removed and immediately placed in 1.5 mL labeled snap-cap tubes. The contents were gently removed by lightly squeezing the excised organ and washing twice with 0.85% NaCl. Finally, the tissue samples, without contents in lumens, were placed in 1.5 mL labeled snap-cap tubes and frozen before use.

DNA extraction from the tissue samples was as follows. 0.3 g tissue samples from different segments of the gastrointestinal tract were washed with 0.85% NaCl twice in order to confirm the removal of contents in lumens. Then, the samples were ground in 1.5 mL Eppendorf tubes using a conventional method. The pellet was resuspended in 500 µL TE buffer (pH 8.0) with 10 µL proteinase K (30 mg/mL) and incubated at 37°C for 3 h. Finally, a conventional phenol/chloroform/isoamyl alcohol method (preparation described above) was used to extract the genomic DNA of *S. enteritidis* from tissue, and 1 µL aliquot of the sample DNA template was used for FQ-PCR detection.

## RESULTS

### Specific verification of FQ-PCR products

The primers of FQ-PCR were used for conventional PCR with *S. enteritidis* (Mouse, No. 50338) DNA templates, in order to verify the specific amplification. Results showed that the PCR produced an intense band with the expected 130 bp for *S. enteritidis*, which indicated 100% specificity.

### FQ-PCR standard curve

One of the main advantages of FQ-PCR is the ability to quantitate unknown samples. With this assay, it is possible to carry out a rapid quantitative analysis of DNA over a wide linear range, with an unknown template. By using a standard template containing from  $9 \times 10^8$  -  $9 \times 10^3$  copies, accurate results for a series of samples were obtained, based on the data used to generate the standard curve with the iCycler IQ Detection System. The correlation coefficient for the associated standard curve was 1.000 and PCR efficiency was 97.3%, indicating that the crossing threshold values for the standards fell within an acceptable range. Using the following formula, we could quantitate the number of DNA copies of *S. enteritidis* for unknown samples:  $Y = -3.389X + 44.276$  (where Y is the threshold cycle, and X the log of the starting quantity) (Figure 1).

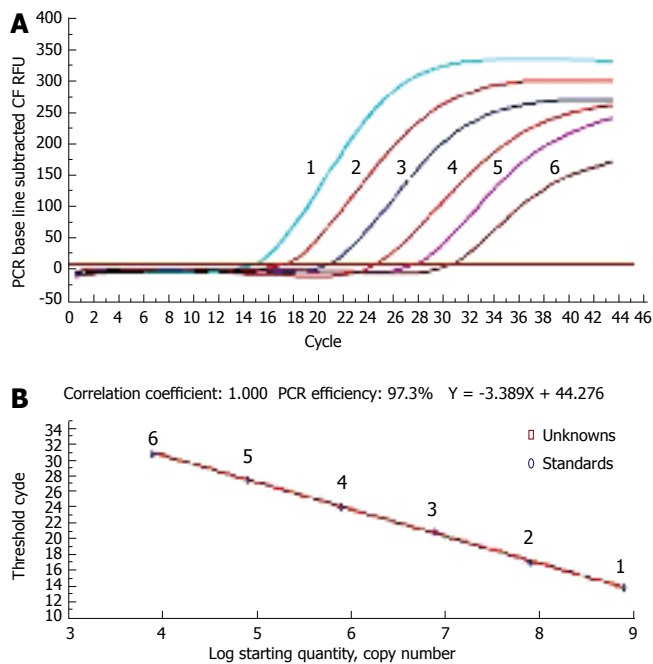
### Sensitivity of PCR

A range of  $4.0 \times 10^3$  -  $4.0 \times 10^{-1}$  copies of the *S. enteritidis* standard template was used, and the limit of detection was 4 copies/µL. A sensitivity of 6 cfu/mL was obtained when 10-fold serial dilutions of bacterial cell cultures were used as the PCR template (Figure 2).

### Reproducibility

Four different, known concentrations of DNA ( $1.2 \times 10^9$  -  $1.2 \times 10^6$  copies/µL) were amplified by performing the





**Figure 1** Standard DNA template with 10 fold serial dilutions used to develop the standard curve. 1:  $9 \times 10^8$  copies/ $\mu$ L; 2:  $9 \times 10^7$  copies/ $\mu$ L; 3:  $9 \times 10^6$  copies/ $\mu$ L; 4:  $9 \times 10^5$  copies/ $\mu$ L; 5:  $9 \times 10^4$  copies/ $\mu$ L; 6:  $9 \times 10^3$  copies/ $\mu$ L.

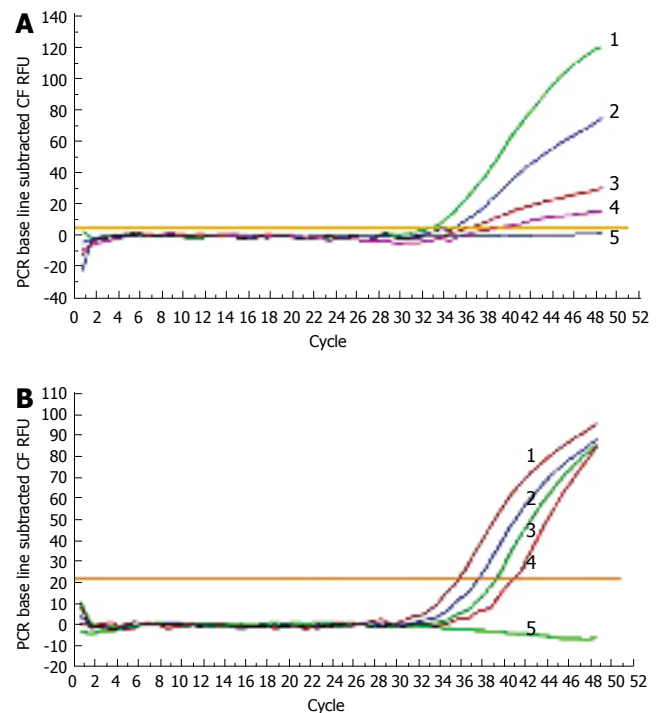
assay described above in triplicate. Analysis of these values proved that the assay was reproducible, as the coefficient of variation was statistically low, at  $< 1.5\%$ . The threshold cycle for each concentration ranged from  $1.2 \times 10^9$ - $1.2 \times 10^6$  copies/ $\mu$ L and was different between 0.1-0.3 cycles and highly reproducible (Figure 3).

### Specificity of the PCR

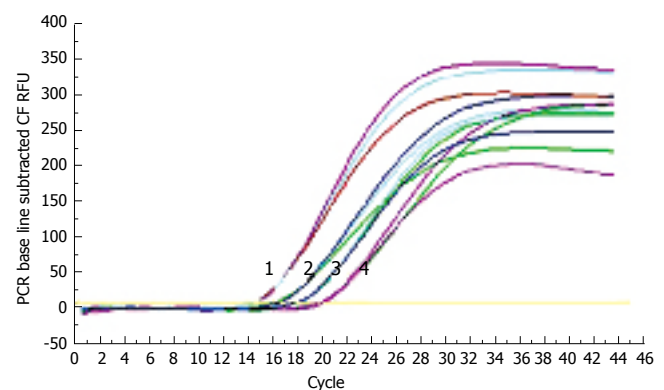
All 14 bacterial strains used to assess the specificity of the PCR indicated that only *S. enteritidis* genomic strains showed positive results, while there was no amplification with non-*S. enteritidis* strains (data not shown).

### Distribution of *S. enteritidis* in the gastrointestinal tract

The distribution of *S. enteritidis* within the gastrointestinal tract after oral infection was determined by means of FQ-PCR at intervals on separate segments of gut over a 5-d period. Results showed that the jejunum and ileum were positive at 8 h postinoculation, with approximately 200 copies/g. Then, *S. enteritidis* was consistently detected in all segments of the gastrointestinal at 8 h post inoculation, and the last organ to show a positive result was the stomach at 18 h post inoculation. The copy numbers of *S. enteritidis* in each tissue reached a peak at 24-36 h, with the jejunum, ileum and cecum containing high concentrations of *S. enteritidis*, whereas the duodenum, colon, rectum, esophagus and stomach had low concentrations. Numbers of bacteria decreased at 2-3 d, and by 4 d the level of *S. enteritidis* had clearly decreased, with the duodenum, colon, esophagus and stomach not having a positive result. The rectum had about 10 copies/g at 4 d post inoculation, and then the bacteria vanished. The rectum carried the *S. enteritidis* for up to 5 d in others without causing apparent symptoms. Importantly, with respect to the number of



**Figure 2** **A:** Sensitivity of FQ-PCR detection using 10 fold serial diluted *S. enteritidis* (Mouse, 50338) standard template. 1:  $4.0 \times 10^3$  copies/ $\mu$ L; 2:  $4.0 \times 10^2$  copies/ $\mu$ L; 3:  $4.0 \times 10^1$  copies/ $\mu$ L; 4:  $4.0 \times 10^0$  copies/ $\mu$ L; 5:  $4.0 \times 10^{-1}$  copies/ $\mu$ L; **B:** Sensitivity of FQ-PCR detection used 10 fold serial diluted *S. enteritidis* (Mouse, 50338) cell number. 1:  $6.0 \times 10^3$  CFU/mL; 2:  $6.0 \times 10^2$  CFU/mL; 3:  $6.0 \times 10^1$  CFU/mL; 4:  $6.0 \times 10^0$  CFU/mL; 5:  $6.0 \times 10^{-1}$  CFU/mL.



**Figure 3** Reproducibility of FQ-PCR. 1:  $1.2 \times 10^9$  copies/ $\mu$ L; 2:  $1.2 \times 10^8$  copies/ $\mu$ L; 3:  $1.2 \times 10^7$  copies/ $\mu$ L; 4:  $1.2 \times 10^6$  copies/ $\mu$ L.

*S. enteritidis* cells at 5 d postinoculation, compared to the other organs the cecum had significantly higher numbers of *S. enteritidis* than all of the other regions, with  $10^{3.3}$  copies/g, whereas the jejunum had  $10^{1.5}$  copies/g, and the ileum had  $10^{1.3}$  copies/g ( $P < 0.01$ ). The control group did not have any positive results at any time in any location. The details are given in Table 1.

## DISCUSSION

Attachment to host tissues is the first important step for establishing a bacterial infection<sup>[13,14]</sup>. The fimbria *Sef* 14 is found in a limited number of *Salmonella enterica*

**Table 1** Distribution and quantity of *S. enteritidis* in different time and segment of gastrointestinal tract in orally infection mice (lg copies/g)

Time	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum	Esophagus	Stomach
30 min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8 h	0.0	2.3	2.5	0.0	0.0	0.0	0.0	0.0
12 h	1.2	2.5	2.8	2.1	1.3	1.6	0.0	0.0
16 h	1.5	3.3	3.2	2.6	1.3	1.6	1.1	0.0
18 h	2.2	3.3	3.6	3.0	2.2	1.8	1.2	1.0
24 h	3.0	4.0	4.2	3.9	2.6	2.1	1.4	1.2
36 h	3.3	6.2	6.6	5.4	2.6	3.5	2.6	1.9
2 d	2.2	4.1	4.0	3.9	1.6	2.6	1.6	1.7
3 d	1.2	3.0	2.8	3.6	1.2	1.2	1.3	1.2
4 d	0.0	2.5	2.3	3.4	0.0	1.1	0.0	0.0
5 d	0.0	1.5	1.3	3.3	0.0	0.0	0.0	0.0

serovars, including enteritidis. This surface structure appears to be required for macrophage uptake and survival in intraperitoneal infections<sup>[15]</sup>, in contrast to other *Salmonella* fimbriae that promote binding to host epithelial cells<sup>[16]</sup>. There is also evidence that quorum sensing plays an important role in the life cycle of *Salmonella* serovar enteritidis<sup>[17]</sup>. Upon interaction with the intestinal epithelium, *Salmonella* can induce changes in the brush border known as membrane ruffles, survive and multiply there<sup>[18]</sup>. This study showed that *S. enteritidis* in orally infected mice very quickly reached the intestinal tract, especially the jejunum and ileum. We suggest that penetration by *S. enteritidis* occurs in the upper half of the gastrointestinal tract, which possibly indicates sites that are more easily penetrated by *S. enteritidis*. This finding is compatible with results obtained in previous studies, which showed that *S. enteritidis* has a rapid transit time through the normal mouse intestine; a small proportion of the inoculum establishes itself within the walls of the small intestine, before a systemic infection can be demonstrated<sup>[4,5,19]</sup>. However, these studies were unable to precisely establish the primary site of bacterial invasion in these animals. From our study, it seems reasonable that the jejunum and ileum are most likely sites for bacterial penetration that is responsible for systemic infection. First, the jejunum and ileum were positive at 8 h post inoculation. Second, the *S. enteritidis* copy number reached a maximum between 8 h and 36 h post inoculation, with copies of *S. enteritidis* being about 10-10000 times that of other regions. Finally, by 4 d post inoculation, the level of *S. enteritidis* clearly decreased, with no bacteria in the duodenum, colon, esophagus and stomach, but *S. enteritidis* remained in the jejunum, ileum and cecum for up to 5 d post inoculation. At 12 h post inoculation, the *S. enteritidis* in the cecum were increasingly obvious, but less so in the jejunum and ileum (about 10 times fewer) at 24-36 h post inoculation. By 5 d post inoculation, the cecum had significantly higher numbers of *S. enteritidis* than all the other areas, with  $10^{3.3}$  copies/g, whereas the jejunum had

$10^{1.5}$  copies/g, and the ileum had  $10^{1.3}$  copies/g ( $P < 0.01$ ). Therefore, this appeared to reflect the function of the cecum as a repository for *S. enteritidis* and a site for its penetration. By way of contrast, Ozawa<sup>[20]</sup> has concluded that the primary site of *Salmonella* infection involves the cecum and the large intestine.

Mucosal immunity provides the first line of protection following oral exposure to pathogens. In particular, the involvement of mucosal IgA in protection against salmonellosis has been reported<sup>[21]</sup>. Secretory IgA limits mucosal colonization by *S. enteritidis* by preventing adherence and subsequent invasion of the bacteria<sup>[22,23]</sup>. The environment of the gastrointestinal tract can also prevent invasion by *S. enteritidis* to some extent. The lowest concentration of *S. enteritidis* was found in the stomach, and this was the last area in which *S. enteritidis* was detected, which indicates that environmental factors, such as pH, can influence the survival and virulence of *S. enteritidis*. It has been reported that normal host defenses are capable of eliminating  $> 80\%$  of organisms from the gut within hours<sup>[20]</sup>. Most of the *Salmonella* in the challenge inoculum had no pathogenic significance, since only a few organisms passed through the mucosa of the ileum during the initial hours of infection. A previous study has shown that *S. enteritidis* colonization in the gastrointestinal tract can persist for as long as 18 wk post inoculation in hens<sup>[24]</sup>. Nevertheless, these few organisms have the potential to cause a lethal systemic infection<sup>[20]</sup>. Changes in the microenvironment in the gastrointestinal tract have important implications for understanding the gastrointestinal factors necessary for protection against *S. enteritidis* infection. *S. enteritidis* is distributed evenly along the intestinal tract, and the microenvironment may be a predictor for severity of *S. enteritidis* invasion and infection<sup>[25,26]</sup>.

FQ-PCR has become a potentially powerful alternative in microbiological diagnosis due to its simplicity, rapidity, reproducibility and accuracy. The specific primer-probe combination is a powerful tool for detecting the genetic content of closely related bacterial species<sup>[27,28]</sup>. A series of sensitivity experiments was performed and proved that the detection limit of this method was 4 copies/ $\mu$ L for standards template, and 6 cfu/mL for bacterial cell number. Four different, known concentrations of DNA were amplified by performing the assay described above in triplicate, and showed a coefficient of variation less than 1.5%. Also, one of the main advantages of FQ-PCR is the ability to quantitate unknown samples. With this assay, it is possible to carry out a rapid quantitative analysis of DNA over a wide linear range, with an unknown template. Simultaneously, variation in results may be due to the extraction efficiency of DNA, the PCR inhibitors, or a large amount of DNA from background organisms.

In conclusion, our results provide significant data for clarifying the pathogenic mechanism of *S. enteritidis* in the gastrointestinal tract, and show that the jejunum, ileum and cecum are the primary sites of invasion in normal mice after oral infection. This study will help to understand the mechanisms of action of *S. enteritidis*.

## COMMENTS

### Background

There are over 2500 serovars in the genus *Salmonella*. It has been a public health concern for over 100 years, and the incidence of *Salmonella* infections has risen dramatically, especially those caused by *S. enteritidis*. Therefore, knowledge about *Salmonella* infection could be an additional means for decreasing the incidence of infection. Infection with *Salmonella* is usually started by oral ingestion of the pathogen, and is followed by bacterial colonization of the gut and invasion of internal tissues. Therefore, it is necessary to understand its mechanisms of action in the gastrointestinal tract.

### Research frontiers

To date, the exact site of primary penetration of *S. enteritidis* in the gut has not been established. FQ-PCR, as a rapid, sensitive technique for precise quantitation of nucleic acid, will be an ideal method to study the distribution of *S. enteritidis* in the gastrointestinal tract.

### Innovations and breakthroughs

Previous studies have shown that *S. enteritidis* establishes itself within the walls of the small intestine before a systemic infection can be demonstrated. However, these studies have been unable to identify the regular distribution pattern and the primary site of *S. enteritidis* invasion. In this study, we offered a significant improvement over previous studies, and suggested that the jejunum, ileum and cecum are the primary sites of invasion after oral infection.

### Applications

This study will provide significant data for clarifying the pathogenic mechanisms of *S. enteritidis* in the gastrointestinal tract, and may ultimately lead to new insights in prevention and therapy.

### Terminology

Standard DNA template: The purified target DNA fragment for FQ-PCR amplification that is usually used to generate standard curves. It can be generated by gel excised directly after a conventional PCR amplification or after the target fragment is cloned using plasmid DNA.

### Peer review

This study detected FQ-PCR *S. enteritidis* in different segments of the gastrointestinal tract in mice after oral infection, and demonstrated the regular distribution pattern and primary penetration sites of *S. enteritidis*. The method is simple and accurate, and it may lead to new ways to prevent *S. enteritidis* infection.

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