Prevalence of the Virulence Plasmid in *Salmonella Typhimurium* Isolates from Pigs

Takanori NAMIMATSU1), Tetsuo ASA13*, Takayuki OSUMI1), Yasuo IMAI1) and Shizuo SATO1)

1) Zen-noh Institute of Animal Health, 7 Ohja-machi, Sakura, Chiba 283–0043, Japan

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**ABSTRACT.** To determine the prevalence of the virulence plasmid in *Salmonella Typhimurium* isolates from pigs in Japan, a total of 106 porcine isolates were subjected to PCR amplification for the detection of the virulence plasmid. Out of the isolates of *S. Typhimurium*, 38 (35.8%) harbored the virulence plasmid. The presence of the virulence plasmid was widely observed in the isolates from systemically infected pigs (92.0%, 23/25), compared with diarrheic (18.8%, 12/64) and apparently healthy pigs (17.6%, 3/17) (P<0.01).

**KEY WORDS:** *Salmonella Typhimurium*, swine, virulence plasmid.

*Salmonella enterica* subsp. *enterica* serovar Typhimurium is a causative agent of gastroenteritis and systemic infection, including bacteremia in humans [3, 4, 7]. *S. Typhimurium* infection in pigs has generally manifested as diarrhea, resulting in severe economic loss [21]. Our previous investigation demonstrated that *S. Typhimurium* is a dominant serovar that can be isolated from the diarrheic feces of pigs in Japan [1]. It has also been isolated from several internal organs of dead pigs or pigs with severe symptom [21]. Fedorka-Cray *et al.* [6] experimentally reproduced this systemic infection of *S. Typhimurium* in pigs.

*S. Typhimurium*, *S. Choleraesuis*, *S. Enteritidis*, and *S. Dublin* harbor the virulence plasmid specific to each serovar involving in lethal systemic infection in mice [12, 14, 18, 22]. Previous investigations have suggested that the plasmid is associated with severe systemic infection due to *Salmonella* serovars in humans and livestock [5, 7, 17]. However, the presence of virulence plasmid of *S. Typhimurium* did not always correlate the bacteremia in humans [3, 4]. The present study was conducted to determine the prevalence of the virulence plasmid in porcine isolates in Japan.

A total of 106 isolates were collected from 25 systemically infected pigs, 64 diarrheic pigs and 17 apparently healthy pigs between 1997 and 2002 in Japan. Identification of the isolates was performed biochemically and serologically using standard methods [19]. All isolates were stored in 10% skim milk at –80°C until used.

Polymerase chain reaction amplification was applied to detection of virulence plasmid of *S. Typhimurium*. The total DNA was extracted from the isolates using an InstaGene Matrix (Bio–Rad Laboratories, Inc., CA, U.S.A.), following the manufacturer’s instructions. The DNA preparations were subjected to PCR amplification in the presence of taq polymerase (Takara Ex Taq, Takara Shuzo, Co., Ltd., Japan). A part of the *pefA* gene of the virulence plasmid of *S. Typhimurium* was amplified using primer pair STPeF-A1A (5'-TAA CCA GCC GGG TAA TTT TG-3') and STPeF-A-4B (CGC TTT CAA CCA GTA CTT TG-3'). The sequences of the primers were chosen from well-conserved regions in the virulence plasmid of *S. Typhimurium* and not from the regions of *S. Enteritidis* or *S. Choleraesuis*, which are known to possess the virulence plasmid [8, 10]. The putative size of the PCR products was 300 bp. The preparations were amplified for 35 cycles, denatured at 94°C for 60 sec, and annealed at 60°C for 60 sec. They were then electrophoresized on a 3.0% (W/V) agarose gel, stained with ethidium bromide, and photographed under UV light. The results were conducted using the Chi-square test or Fisher’s Exact test.

In the preliminary examination, 24 strains of *S. Typhimurium* were used for estimating the degree of PCR. The presence of the virulence plasmid was confirmed by gel electrophoresis according to the methods of Kado and Liu [13]. Nine strains of *S. Typhimurium* harboring the virulence plasmid were positive for PCR, and 15 strains without the virulence plasmid were negative. A total of 130 strains of *Salmonella* serovars other than *S. Typhimurium*—including 21 of *S. Enteritidis*, 18 of *S. Choleraesuis*, six of *S. Pullorum*, and one of *S. Dublin*—were negative for PCR (Data not shown).

We investigated the presence of the virulence plasmid among porcine isolates of *S. Typhimurium* using PCR amplification. Out of 106 isolates of *S. Typhimurium*, 38 (35.8%) harbored the virulence plasmid (Table 1). It is known that all field isolates of *S. Choleraesuis*, *Enteritidis* and *Dublin* harbored the virulence plasmid [3, 11, 14]. As

<table>
<thead>
<tr>
<th>Systemically-infected pigs</th>
<th>Diarrheic pigs</th>
<th>Healthy pigs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sows</td>
<td>0/0†</td>
<td>2/2</td>
<td>0/0</td>
</tr>
<tr>
<td>Suckling pigs</td>
<td>0/0</td>
<td>0/1</td>
<td>0/0</td>
</tr>
<tr>
<td>Weaned pigs</td>
<td>20/21</td>
<td>1/10</td>
<td>3/6</td>
</tr>
<tr>
<td>Finisher pigs</td>
<td>3/4</td>
<td>9/51</td>
<td>0/11</td>
</tr>
<tr>
<td>Total</td>
<td>23/25</td>
<td>12/64</td>
<td>3/17</td>
</tr>
</tbody>
</table>

† Correspondence to and present address: ASAI, T., National Veterinary Assay Laboratory, Ministry of Agriculture, Forestry and Fisheries, 1–15–1 Tokura, Kokubunji, Tokyo 185–8511, Japan.
for human isolates in Taiwan, 80% or more of *S. Typhimurium* harbored the virulence plasmid [3, 4]. The present result showed the low prevalence of virulence plasmid in porcine origin.

The plasmid was detected in 23 (92.0%) of 25 isolates from systemically infected pigs, in 12 (18.8%) of 64 from diarrheic pigs and in 3 (17.6%) of 17 from apparently healthy pigs. The presence of the virulence plasmid was widely observed in systemically infected pigs, compared with diarrheic pigs and apparently healthy pigs (P<0.01). The presence of the virulence plasmid in *S. Typhimurium* is associated with lethal infection in mice [12]. Its virulence mechanism might be growth in the internal organs and resistance to microcidal activity [9]. An experimental *S. Choleraeuis* infection of pigs demonstrated that the presence of the virulence plasmid is associated with the severity of septicemia in pigs [5]. The corresponding open reading frames of the virulence plasmid are highly homologous between *S. Typhimurium* and *S. Choleraeuis* [10]. The presence of the virulence plasmid in *S. Typhimurium* may involve in the clinical severity of the infection in pigs.

Almost all of the pigs with systemic symptoms were infected with *S. Typhimurium* harboring the virulence plasmid. However, it was isolated from 2 of 2 sows, 1 of 10 weaned pigs, and 9 of 51 finisher pigs suffering from diarrhea. The infection was found in older pigs without systemic infection, suggesting that the resistance of pigs to *S. Typhimurium* infection may depend on their age. In Japan, weaned pigs are often suffered from variety of pathogens such as *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome virus and so on [2, 20]. Furthermore, Fedorka-Cray *et al.* [6] described the transthoracic route caused them to develop septicemia and systemic infection. Several factors may contribute to the development of systemic infection in weaned pigs.

A salmonella contamination in pork products can be caused by the improper separation of intestinal contents at slaughter. Systemic infection in pigs may increase the risk of contamination of pork products, thereby increasing the risk of foodborne disease [15, 16]. Thus, the prevalence among pigs of *S. Typhimurium* harboring the virulence plasmid is a significant concern for public health and the swine industry.

**REFERENCES**