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

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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.

*S. 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 symptoms [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 STPeFA1A (5'-TAA CCA GCC GGG TAA TTT TG-3') and STPeFA1B (CGC TTT CAA CCA GTA CTT TG-3').

<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><strong>Total</strong></td>
<td><strong>23/25</strong></td>
<td><strong>12/64</strong></td>
<td><strong>3/17</strong></td>
</tr>
</tbody>
</table>

a) Number positive/tested.
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. Choleraesuis 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. Choleraesuis [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 0 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 transhoxic 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