Pathogenicity of *Haemophilus parasuis* Serovars 4 and 5 in Contact-Exposed Pigs

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**ABSTRACT.** The pathogenicities of *Haemophilus parasuis* strains SW124 (serovar 4) and Nagasaki (serovar 5) were examined by contact-exposure of specific pathogen-free (SPF) pigs. Ten pigs were divided into three groups. Two of four pigs in the first group were inoculated intranasally (IN) with $2 \times 10^6$ CFU of strain SW124, and the other two pigs were mingled with these IN-exposed ones. All the four pigs were subclinically infected in this group. The four pigs of the second group were likewise exposed to strain Nagasaki (two IN-inoculated pigs with $3 \times 10^6$ CFU of strain Nagasaki). All four pigs in this group died of Gläsrer’s disease. Two pigs kept as controls showed neither abnormality nor positive *H. parasuis* isolation. — Key words: contact-exposure, *Haemophilus parasuis* infection, pathogenicity.

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*Haemophilus parasuis* is the etiologic agent of porcine polyserositis, polyarthritides, and meningitis (Gläsrer’s disease). The disease usually affects young pigs in conventional herds and its occurrence is sporadic, and may be aggravated by stress induced by, for example, unfavorable environmental and management conditions [10]. The epidemiologic picture of the disease in specific pathogen-free (SPF) herds, however, may show very serious enzootic characteristics with very high morbidity [10, 12]. We presume that the communicability by contact-exposure to *H. parasuis* causes enzootic occurrence in SPF pig herds. Communicability by experimental contact-exposure to *H. parasuis* would be analogous to natural infection, however, there are few reports regarding experimental contact-exposures to confirm this.

Some investigations have indicated differences in virulence which may be related to phenotypic features such as serovars [3], or be associated with phenotypic features by PAGE types (peptide-patterns by polyacrylamide gel electrophoresis with whole-cell proteins) [5, 7, 9]. Although these indicators of potential pathogenicity of the organism are important, this remains to be confirmed by further studies.

Fifteen distinct serovars of *H. parasuis* strains have been reported so far [3, 8]. Of these, serovars 4 and 5 have been isolated with high frequency in Germany [3], North America [12], and Japan [unpublished data]. We have reported that strain Nagasaki (serovar 5) was highly pathogenic and strain SW124 (serovar 4) was rarely pathogenic for SPF pigs when they were inoculated intranasally [1]. The present study was undertaken in order to examine whether both *H. parasuis* strains could be transmitted among SPF pigs by contact-exposure and also to confirm the differences in pathogenicity of these two strains.

*H. parasuis* reference strains SW124 (serovar 4, PAGE type I) and Nagasaki (serovar 5, PAGE type II) were used [8]. Inocula for pigs were prepared as described previously [1]. Briefly, the organisms grown on chocolate agar at 37°C for 18 hr were harvested and suspended in 0.01 M phosphate-buffered saline, pH 7.2 (PBS), after washing twice with PBS. The bacterial suspensions were carefully kept below 4°C, for about 1 hr, just before inoculation.

Thirteen-week-old, SPF Large White pigs, known to be free of *H. parasuis*, were obtained from the Swine and Poultry Experimental Station, Shizuoka Prefecture. The ten pigs were divided into three groups. Two of the four pigs in strain SW124-exposed (SW124) group were inoculated intranasally (IN) with $2 \times 10^6$ CFU/5 ml of strain SW124, and the remaining two pigs were mingled with these IN-exposed ones for contact-exposure. In the same way, two of the four pigs in strain Nagasaki-exposed (Nagasaki) group were inoculated with $3 \times 10^6$ CFU/5 ml of strain Nagasaki, and the remaining two pigs were mingled with these IN-exposed ones for contact-exposure. The two remaining pigs served as non-inoculated controls. Each group of pigs was housed in isolated rooms under sanitary conditions. They were observed daily for clinical signs. Dead pigs and pigs killed at 13 days post-exposure (PE) were subjected to histopathological examination and recovery of the organisms.

Serum samples for serological tests and swab samples from the nasal cavity for isolation of *H. parasuis* were collected: prior to exposure, at the morbund stage and at the time of killing. Samples from the liver, spleen, kidney, lung, heart, brain, intestines, tonsil, main lymph nodes, trachea, and body fluid were also collected for bacterial isolation from the dead or killed pigs. These samples were inoculated onto chocolate agar containing 7% defibrinated sheep blood [1]. The cultures were incubated at 37°C for 24 hr in an atmosphere containing 5% carbon dioxide. Isolates identified as *H. parasuis* by biochemical tests [4] were classified serologically by the agar gel precipitation test [8]. Antibodies were detected in sera by the complement fixation (CF) test [2]. The two CF antigens were prepared from the cultures of strains SW124 and Nagasaki as described previously [6]. The avidin-biotin-complex immunoperoxidase technique (ABCIT) was performed as described previously [1].

There were neither *H. parasuis* isolation nor CF antibody response in any of the samples collected from pigs prior to exposure.

As shown in Table 1, the four IN- and contact-exposed
Table 1. Clinical, bacteriological, and pathological findings of pigs inoculated intranasally with *H. parasuis* and pigs contact-exposed to the organism

<table>
<thead>
<tr>
<th>Strain exposed (serovar)</th>
<th>Pig No.</th>
<th>Clinical sign</th>
<th>Histopathological lesion</th>
<th>Isolation of <em>H. parasuis</em></th>
<th>CF° antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW124</td>
<td>IN°</td>
<td>No</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>(4) Contact</td>
<td>1</td>
<td>No</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>No</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>No</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>No</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nagasaki (5)</td>
<td>IN°</td>
<td>Pyrexia (2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Nervous distress (5)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Pyrexia (8)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Nervous distress (7)</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Non-exposed</td>
<td>9</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

All surviving pigs were killed at 13 days post-exposure.

a) The organism was isolated from the liver, spleen, kidneys, lung and heart.
b) Complement fixation test.
c) Intranasal route.
d) - Negative, +: Positive.

Pigs in the SW124 group exhibited neither clinical signs during the observation period nor gross lesions at necropsy. *H. parasuis* was recovered from the nasal cavities of all the pigs in this group, but not from the visceral organs and the brain at 13 days PE.

In the Nagasaki group, the two IN-exposed pigs showed pyrexia at 1 day PE; one pig (No. 5) died at 2 days PE, and the remaining pig (No. 6) showed nervous distress (recumbency, padding and convulsion) and died at 5 days PE. The two contact-exposed pigs also showed pyrexia at 5 (No. 8) and 7 (No. 7) days PE; one pig (No. 7) died at 8 days PE, and the other pig (No. 8) showing nervous distress died at 7 days PE. The histopathological and immunopathological findings were similar to those described previously [1]. Briefly, two of the four dead pigs of the Nagasaki group had septicemic lesions, and fibrinous thrombi in the kidney, lung, and liver; and the other two pigs had fibrinopurulent meningitis. *H. parasuis* antigen, as demonstrated by ABCIT, was found mainly in the small vessels of many organs in the two septicemic pigs, and in the cytoplasm of infiltrating cells in the two pigs with meningitis. In this group, *H. parasuis* was recovered from the nasal cavities of the two IN-exposed pigs, and from the visceral organs or the brain of one of each of the IN- and contact-exposed pigs (Table 1). However, this organism was not recovered from the nasal cavities of the two contact-exposed pigs. No bacteria other than *H. parasuis* were isolated from the visceral organs.

*H. parasuis* isolated from pigs in SW124 and Nagasaki groups was classified into serovars 4 and 5, respectively, by the agar gel precipitation test.

All the pigs in SW124 group developed detectable CF antibodies (titers, 1:16 or 1:32) at 13 days PE. No CF antibodies were detected in serum samples collected from three moribund pigs of the Nagasaki group.

The control pigs displayed no clinical signs, and were negative for *H. parasuis* isolation, lesion formation, and CF antibody response throughout the experimental period.

Our results indicate that there are differences in the pathogenicity of strains Nagasaki and SW124 by contact-exposure as well as by intranasal inoculation. In this study, strain Nagasaki was highly pathogenic for pigs through both methods of infection, intranasal inoculation and contact-exposure. The contact-exposed pigs did however live longer (died at 7 and 8 days PE) than the intranasally inoculated pigs (died at 2 and 5 days PE). It has been observed, under natural conditions, that susceptible pigs, which were introduced into a farm with an established *H. parasuis* infection, contracted a severe disease with high mortality about one to two weeks after introduction (unpublished data). The experimental infection by contact-exposure could be considered to replicate naturally-occurring Glässer's disease.

In all the pigs exposed to strain SW124, by both methods of infection, the organism colonized the upper respiratory tract and the CF antibody was well developed. Nevertheless, neither clinical signs nor lesion formation at necropsy were seen. Nielsen [11] reported similarly that strains Nagasaki and SW124 were pathogenic and apathogenic, respectively, for SPF pigs after intranasal inoculation. Although Kielenstein and Rapp-Gabrielson [3] observed mild virulence with strain SW124 when SPF pigs were inoculated intraperitoneally, the discrepancy in the pathogenicity of this strain may be attributed to the difference in host defense mechanisms dependant on the route of inoculation; the intraperitoneal inoculation easily caused systemic infection, while the inoculation via the airway resulted in localization of the organism in the respiratory tract through the action of the respiratory defense mechanism.

In this study, it was confirmed that strain SW124 caused...
subclinical infection in both IN- and contact-exposed pigs under stress-free conditions. In our previous study, however, intranasal inoculation with a high dose ($10^{10}$ CFU) of this strain caused death with polyserositis and arthritis in one of the SPF pigs tested [1]. Therefore, it remains to be seen whether strain SW124 causes Glässer’s disease in SPF pigs in cooperation with other microorganisms or through stress factors.

REFERENCES