Isolation of *Haemophilus pleuropneumoniae* from the Nasal Cavities of Healthy Pigs

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ABSTRACT. Isolation of *Haemophilus pleuropneumoniae* (Hpn) from nasal cavities of healthy pigs was investigated. The organisms produced typical transparent and smooth colonies with iridescence on the S agar medium. Isolation was done according to the growth characteristics. A total of 293 Hpn strains (47.3%) was isolated from 619 pigs housed on 87 farms in different geographic districts of Japan during 1982/83. The organisms were isolated from all ages of pigs, and they were distributed in varying rates (20% to 100%) among the 87 farms. The organisms appeared to be one of common bacteria present in the upper respiratory tract of pigs. All the isolates were identified as serotype 2, therefore, the serotype 2 strains of Hpn were distributed widely among pigs in recent Japanese field as a clinically inapparent carrier state. Significant correlation \(P<0.01\) was observed between the presence of Hpn in nasal cavities and positive complement-fixation (CF) antibodies in pigs. The prevalence of Hpn among live pigs can be estimated by the CF test instead of the bacterial isolation test. The CF test might be useful for detection of the carrier pigs.—*Key words: carrier pigs, Haemophilus pleuropneumoniae, nasal cavity.* —Jpn. J. Vet. Sci. 46(5): 641–647, 1984

INTRODUCTION

Pleuropneumonia of swine caused by *Haemophilus pleuropneumoniae* (Hpn) is well known to be distributed in many parts of the world during the last 20 years [2, 5, 11, 17, 19, 21, 34]. The disease occurred both in naturally and experimentally infected pigs has been characterized by acute extensive and fibrinohemorrhagic chronic pneumonia localized and necrotizing with pleuritis [7, 34]. There are many data concerning to isolation of Hpn and the disease induced by Hpn, as reviewed by Sebynaya and Saunders [32], however, the ecology of this organism is poorly understood.

For the study of experimental infection with Hpn, the intranasal, intratracheal, or aerosol routes of exposure have been commonly employed [3, 12, 20, 24, 33, 34], since the disease is transmissible by the respiratory route. Inoculated bacteria were recovered from various organs or tissues, however, isolation rate of Hpn from the upper respiratory tract was significantly lower than that from the lower tract, especially than that from the lung lesions [11–13, 24, 25, 33]. Pigs recovered after the exposure with Hpn experimentally were able to transmit the infection to susceptible animals [27]. Therefore, Nielsen et al. [27, 28] suggested that healthy carrier pigs played an important role in the spread of pleuropneumonia caused by Hpn, though the organisms were not recognized by them [25, 27] as a normal resident of the upper respiratory tract. The organisms were rarely isolated from the nasal cavities of slaughterhouse pigs [3].

The serologic responses occurring after natural infections and as a result of experimental infections have been studied, using the comple ment-fixation (CF) test [4, 14, 16, 20, 23, 24, 27, 28, 31]. The CF test has been favored as the best routine method for serodi-
Table 1. Isolation of *H. pleuropneumoniae* (Hpn) from the nasal cavities of apparently healthy conventional pigs housed on 82 farms in Japan during 1982/83

<table>
<thead>
<tr>
<th>District</th>
<th>No. of farms examined</th>
<th>No. of pigs with positive isolation of Hpn/No. of pigs examined</th>
<th>Isolation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tohoku</td>
<td>15</td>
<td>43/83</td>
<td>51.8</td>
</tr>
<tr>
<td>Kanto</td>
<td>35</td>
<td>153/354</td>
<td>43.2</td>
</tr>
<tr>
<td>Chubu</td>
<td>11</td>
<td>23/49</td>
<td>46.9</td>
</tr>
<tr>
<td>Kinki</td>
<td>4</td>
<td>8/18</td>
<td>44.4</td>
</tr>
<tr>
<td>Chugoku</td>
<td>9</td>
<td>21/40</td>
<td>52.5</td>
</tr>
<tr>
<td>Kyushu</td>
<td>8</td>
<td>19/35</td>
<td>54.3</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>272/579</td>
<td>47.0</td>
</tr>
</tbody>
</table>

All the pigs lacked any clinical abnormalities until this trial. Numbers of pigs investigated in each farm varied from four to ten. The organisms of Hpn were isolated from pigs housed on all farms, though isolation rates varied from 20% to 100% among them. Each strain originated from each pig. The organisms were isolated alone or in combination with *H. parassuis* (Hps) from the same pig. Isolation rate of Hps was 44% in total.

agnosis of the Hpn infections [14, 31]. The meanings of the “infection” employed in these reports included appearance of clinical signs, production of gross or histopathologic lesions, recovery of Hpn, and/or presence of the serum antibodies. Therefore, the data obtained by individual researchers can not be compared directly. Even in experimental infections, varying degrees of correlations between the CF test and gross or pathologic examinations, or bacterial recovery test at postmortem were reported in elthwhere. For control of the disease caused by Hpn, development of effective criteria for detection of carrier pigs [27, 28] among live animals would be desired. The present investigation deals with isolation of Hpn from the nasal cavities of apparently healthy pigs in Japanese field, and the data are discussed in relation to those obtained by the CF test.

MATERIALS AND METHODS

Source of the isolates: During 1982/83, we isolated 272 Hpn strains from the nasal cavities of 579 apparently healthy conventional pigs housed on 82 farms in different geographic districts of Japan (Table 1). Outbreak of the disease caused by Hpn has not been recognized in most of these farms (about 90%). Morbidity and mortality of the disease were less than 1% in the farms previously attacked by Hpn. All the pigs investigated lacked any clinical abnormalities until this trial, according to the informations of owners. Numbers of pigs investigated in each farm varied from four to ten. Ages of the pigs at investigation were shown in Table 2. Each strain was originated from each pig.

We also isolated 21 Hpn strains from the nasal cavities of 40 4-month-old healthy pigs housed on two grand parent (20 pigs) or three parent stock (20 pigs) farms (Table 4). Outbreak of the disease has not been recognized in these farms. Sera obtained were titrated by the complement-fixation (CF) test, and the data were compared with those of the bacterial isolation test.

Reference strains and culture medium: Strain SH-15 of Hpn previously identified as serotype 2 by Nakai et al. [18], according to the method described by Nicolet [20], was used as a reference. Strains used as antigens for the CF and rapid plate agglutination (RPA) tests and for preparation of rabbit antisera represented each of the five serotype strains of Hpn, viz, serotype 1 (4047), serotype 2 (1536), serotype 3 (1421), serotype 4 (M 62), and serotype 5 (K 17) [6, 9]. These
strains were provided by Dr. Nicolet, University of Bern, Switzerland. The S agar medium [10], composed of chicken meat infusion broth and chicken serum, was used throughout this work.

Isolation of Haemophilus spp.: Method for isolation of Haemophilus spp. from the nasal cavities of pigs was similar to that employed previously for the isolation of H. paragallinarum of avian origin [10]. Cotton swab samples were directly streaked on the S agar medium immediately after the collection, and the plates were transported to our laboratory mainly by mail. Generally, one to three days are necessary for the transportation. The plates were incubated in the presence of 5% CO₂. After 12 hr incubation at 37°C, the colonies grown on the medium were examined morphologically by a stereomicroscope under transmitted and obliquely reflected light according to the method described by Sawata and Kume [29]. Haemophilus-like colonies were selected from the cultures and spread on the S agar medium. Identification procedures were followed by the criteria described previously [1, 9] by using the organisms grown on the medium after 10 hr incubation at 37°C. The Hpn isolates were confirmed their serotype by the RPA test [15] with rabbit antisera. Preparation of the antisera was followed by the method described previously [29].

Complement-fixation (CF) test: Preparation of the whole cell antigens for the CF test was followed by the method described by Gunnarsson [4], except by using the orga-
organisms grown on the S agar medium after 10 hr incubation at 37°C. The CF test was carried out according to the method described previously [4] by using the sera collected from 119 conventional pigs housed on 18 farms and 40 pigs housed on five grand parent or parent stock farms.

RESULTS

The organisms of Hpn well grew on the S agar medium, and they produced smooth and transparent colonies with various grades of iridescence. *H. parasuis* (Hps) also grew on the medium, however, the organisms produced small colonies surrounding characteristic yellowish color. The organisms of Hpn were isolated alone or in combination with Hps from the nasal cavities of apparently healthy pigs, but selection of each bacterium on the medium can easily be done according to the above mentioned criteria. The organisms of Hpn were frequently isolated (47%) from the nasal cavities of healthy conventional pigs, and they distributed in varying rates (20% to 100%) among the farms (Table 1). There was no difference in prevalence of Hpn among the districts investigated. The organisms were isolated from all ages of pigs (Table 2). All the Hpn isolates were identified as serotype 2. The serotype 2 strains of Hpn were distributed widely among pigs in recent Japanese field as a clinically inapparent carrier state. The organisms of Hps were also isolated alone or in combinatin with Hpn from the same samples. Totally, the isolation rate reached 44%.

The CF antibodies (≥4) against the serotype 2 strain were detected in 52 (43.7%) out of the 119 healthy conventional pigs (Table 3). Range of the CF titers was from 4 to 128. The organisms of Hpn were isolated from 48 (92.3%) out of the 52 pigs with the CF antibodies.

Serotype 2 strains of Hpn also highly harbored (52.5%) in the nasal cavities of healthy pigs (40% to 80%) housed on grand parent or parent stock farms in different geographic districts (Table 4). The CF antibodies were detected in 52.5% of the pigs. Significant correlation (P<0.01) was observed between isolation of Hpn and the presence of the CF antibodies. None of the CF antibodies against the organisms belonging to the five serotype 1 through 5 were detected among the sera investigated, except for the CF antibodies against the serotype 2. The organisms of Hps were also isolated from 43% of the same samples.

DISCUSSION

Isolation attempts of Hpn from the nasal cavities of both experimentally or naturally infected pigs are impeded by the less fastidious and rapid growing normal bacterial flora [3, 12, 24, 33], except for one case reported by Harris et al. [8]. For example, Gilbridge and Rosendal [3] isolated only ten strains in total from the nasal swab samples of 244 slaughterhouse pigs, though each sample was cultured on four different kinds of media. In contrast, we obtained 293 serotype 2 strains of Hpn (47.3%) from the nasal cavities of 619 healthy live pigs. Significant correlation (P<0.01) was observed between the isolation of Hpn and presence of the CF antibodies against the organisms in these pigs. Therefore, we can conclude that Hpn is a normally commensal in the upper respiratory tract of pigs, and the serotype 2 strains were distributed widely in recent Japanese field. A high prevalence of Hpn and the presence of a clinically inapparent carrier pig are very important for control of the disease. The CF test might be useful for detection of the carriers.

Harris et al. [8] isolated *Haemophilus* spp. from about 70% of the nasal swab samples, but the isolates might involved Hps known as a nasal commensal of pigs. The results of Mitui et al. [16] and present authors showed that Hpn was frequently isolated alone or in combination with Hps from the nasal cavities.
of pigs, and the isolation rate of Hpn was similar to that of Hps. The observation also supports our present conclusion that Hpn is a normal resident in the upper respiratory tract of pigs.

Varying degrees of morbidity and mortality caused by Hpn were reported in elthwhere [7, 11, 17, 22], but the degrees are generally low, except for outbreaks of the disease in acute form. The organisms were widely distributed through the susceptible tissues or organs of pigs, however, the density of the organisms deposited in the lung lesions was generally higher than that in trachea or turbinates [3, 33]. Multiplication of Hpn in lungs may be essential for production of the lung lesions or appearance of clinical signs in pigs. Production of the lung lesions in the carrier pigs presented herein, however, remained to be clarified. Outbreaks of the disease were occasionally recognized in part of the farms investigated. Therefore, it may be reasonable to consider that the disease appears in part of the naturally infected pigs having Hpn in the upper respiratory tract as clinically inapparent carrier state, if a stress [17, 22] stimulates multiplication of Hpn in the host. The organisms may be opportunistically pathogenic at worst, but normally commensal in the upper respiratory tract of pigs.

Culture media supplemented with blood are generally employed for isolation and propagation of Hpn [1–4, 16, 33]. The S agar medium [10] employed herein can be applied for the isolation purpose of Haemophilus spp. of pig origin without the supplement. Both Hpn and Hps grew on the medium, but Hps produced generally smaller colonies than those of Hpn, suggesting the different nutritional requirements of the two bacteria for their growth. Classification of Hpn or Hps can easily be done according to the growth characteristics of each bacterium. The medium and criteria for the selection described herein would offer great advantages to study on the rate of recovery of Hpn from the upper respiratory tract of pigs.

For serologic typing of Hpn, various procedures are reported [1, 4–6, 9, 15, 16, 19, 20]. Type-specific antigens of Hpn are thought to represent capsular substance [6]. The grades of iridescence were considered as a criteria to estimate the amount of capsule in H. paragallinarum of avian origin [29, 30]. All of our Hpn isolates were easily serotyped by the RPA test [15]. The Hpn isolates selected by the criteria [30] may have a sufficient amount of capsular substance for the typing.

At least five serotype strains have been well recognized among the isolates of Hpn on the agglutination test [5, 15]. The pattern of distribution of the serotype strains around the world varies from one country to another [32]. In 1974, Chan et al. [2] isolated 18 serotype 2 and one serotype 3 strains in Japanese field. Since then, the serotype 3 has not been obtained in Japan. All the isolates of Mitui et al. [16] and present authors were identified as the serotype 2. The prevalence of the serotype 3 strains have not been confirmed by the CF test. Therefore, the serotype 3 strain [2] was probably introduced from a foreign country with the host.

A high herd (farm) and an individual pig prevalence of the CF antibodies to Hpn were reported [14, 31]. Once the CF antibodies were produced experimentally in pigs, the titers persisted for a long period [20, 23, 24]. The organisms were recovered from various tissues, irrespective of the degree of CF titers [23–28]. The organisms also persisted in the nasal cavities of naturally infected pigs at least during two months after finding the seroconversion in the pigs [16]. The present investigation showed that the CF antibodies highly prevalenced on Japanese farms, and the organisms harbored in most of the nasal cavities of these seroconverting pigs, irrespective of the ages of pigs. Therefore, the seropositive pigs may be a potential carrier, as suggested by Nielsen and Mandrup [27]. It seems likely that many of the pigs with the CF antibodies
and the seropositive herds have the potential for developing pleuropneumonia or for spreading Hpn to other healthy pigs or to other seronegative herds. Persistence of Hpn in the upper respiratory tract of pigs may be necessary to maintain the CF antibodies. The factor(s) responsible for the nasal clearance of Hpn should be clarified.

Based on the present results, it would be possible to detect the inapparent infection caused by Hpn in a farm by testing a reasonably large numbers of pigs with the CF test. Positive rate of the antibodies in a farm might reflect exactly the degrees of the infection. If the farm is negative for the CF test, the farm should restock from other negative farms to maintain negative status. If a large proportion of the sampled pigs has the antibodies, the pigs should be kept in a good environmental condition to avoid multiplication of Hpn in the host. The CF test is recommended to use as an effective method for detection of the carrier pigs instead of the bacterial isolation test.

REFERENCES


要約

健康豚の鼻腔からの *Haemophilus pleuropneumoniae* 分離：久米勝巳・中井豊次・澤田涼（北里研究所附属家畜衛生研究所）——見上健康豚の鼻腔からの *H. pleuropneumoniae* (Hpn) の分離を試みた。Hpn は、S 天培地で、虹彩を有する特異的な集落を形成した。本特性を指標に、日本各地の 87 養豚場で飼育中の 619 頭の健康豚の鼻腔から計 293 株（47.3%）の Hpn が分離された。本菌は、調査全養豚場の異なった週齢の豚に存在（20%〜100%）し、分離率は、すべて血清型 2 に属した。したがって、Hpn は、不顯性感染豚の上部気道に常在し、かつ、その頻度はきわめて高率であった。本菌が鼻腔から分離された大部分の豚では、補体結合 (CF) 抗体の上昇が認められ、保菌と抗体保有は相関しており、CF 試験で保菌状況を推定しうることが示唆された。