Original Article

Prevalence of Leptospiral Antibody in Sows in Japan

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Summary

The prevalence of leptospiral antibodies in sows was investigated throughout Japan using microscopic agglutination test (MAT). A total of 1,121 sows raised in 140 farms in 20 prefectures between 2001 and 2004 were randomly selected for serological survey. Sows positive for leptospiral antibodies were found in 98 (70.0%) of the 140 farms. Of the 1,121 sows examined, 281 (25.1%) were positive. The positive rate for serovar Bratislava was the highest and was detected in 202 sows (18.0%) in 87 farms (62.1%), followed by Australis in 84 sows (7.5%) in 45 farms (32.1%), Autumnalis in 25 sows (2.2%) in 25 farms (17.9%), and Canicola in 25 sows (2.2%) in 14 farms (10.0%). The wide prevalence of leptospirosis antibody in sows in Japan has been confirmed by MAT in this study, and serovar Bratislava was the major serovar detected.

Keywords : Japan, Leptospira, serological survey, sows

Introduction

Leptospirosis is a zoonosis caused by pathogenic Leptospira species, such as Leptospira interrogans1-3. This zoonotic disease has been reported worldwide, and has been shown to be pathogenic in a broad range of animal species4-6. In swine, leptospirosis leads to the development of fever, anorexia, and inertia; however these symptoms are mild and may be overlooked. In pregnant sows, abnormal delivery (including complications such as abortion and stillbirth), and delivery of immature and infirm piglets may occur and cause large economic losses4-6. In Japan, a serological survey on swine leptospirosis was performed between 1956 and 1958 and 3,060 serum samples from 43 prefectures showed a positive rate of 10.9%7. The major serovar detected was Icterohaemorrhagiae, followed by Autumnalis, Australis, Pomona, and Canicola. Following this survey, prevalence of leptospirosis with accompanying premature birth and stillbirth have been reported in sows in Aichi8, Chiba and Gunma9,10 and Okinawa11. Moreover, antibodies against Leptospira were detected in sows and fattening pigs in Hokkaido, Kagoshima and Okinawa12. Furuya et al.8 investigated the prevalence of leptospirosis in swine by PCR in premature and stillborn fetuses, and found that it is present in Japan. These studies, however, were performed in limited regions, and no nationwide serological survey has been conducted since the 1956-1958 survey mentioned above. Therefore, the current state of this disease in Japan is unclear.

In this study, we performed a nationwide serological survey of the prevalence of leptospirosis in sows for breeding.

Materials and methods

Sows

The survey was performed in 20 of 47 prefectures of Japan from 2001 to 2004, and the prefectures were divided into 4 areas (Hokkaido/Tōhoku, Kanto, Hokuriku/Chubu, and Shikoku/Kyushu/Okinawa). From about 7,500 farms in Japan, 140 (1.9%) were randomly selected for serological survey. Serum was collected from 2 to 18 sows per farm, and a total of 1,121 samples were used for serological testing. No swine leptospiral vaccine was available in Japan; therefore, no sows received the leptospiral vaccine in the present study. Clinical findings of the sows were unclear.

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Table 1 Detection of antibodies against leptospiral serovars in farms and sows

<table>
<thead>
<tr>
<th>Species</th>
<th>Serovars</th>
<th>No. of positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Farms (n=140)</td>
</tr>
<tr>
<td><em>L. interrogans</em></td>
<td>Bratislava</td>
<td>87 (62.1)</td>
</tr>
<tr>
<td></td>
<td>Australis</td>
<td>45 (32.1)</td>
</tr>
<tr>
<td></td>
<td>Autumnalis</td>
<td>24 (17.1)</td>
</tr>
<tr>
<td></td>
<td>Canicola</td>
<td>14 (10.0)</td>
</tr>
<tr>
<td></td>
<td>Hebdomadis</td>
<td>6 (4.3)</td>
</tr>
<tr>
<td></td>
<td>Icterohaemorrhagiae</td>
<td>4 (2.9)</td>
</tr>
<tr>
<td></td>
<td>Pomona</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td></td>
<td>Hardjo</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td><em>L. kirschneri</em></td>
<td>Grippotyphosa</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Tatal&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td>97 (69.3)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Absolute number and rate (%) of positive cases.

Serological testing

Antibodies against *Leptospira* were detected by the microscopic agglutination test (MAT)<sup>2</sup>. The bacteria used as antigens were *Leptospira interrogans* serovars Bratislava, Australis, Autumnalis, Canicola, Hebdomadis, Icterohaemorrhagiae, Pomona and Hardjo, and *L. kirschneri* serovar Grippotyphosa (Table 1). The serovars that cause leptospirosis in pigs were chosen for this study. Serovar Bratislava was included because it represents a major source of swine leptospirosis in foreign countries. After the bacteria were cultured in EMJH medium at 30°C for 6–9 days, the bacterial count was adjusted to 1–2×10<sup>5</sup> cells/ml and used as an antigen. A screening test was performed before antibody titer measurement. The serum sample was diluted 1 : 12.5 and 1 : 25, added to 96-well plates with an equivalent volume of cultured bacterial suspension, mixed well, sealed with a tape, and reacted at 30°C for 3 hours. One loop of reaction solution from each well was observed by dark-field microscopy. When a 50% or higher agglutination was observed compared with the control, the sample was considered positive. Serum that agglutinated at 1 : 50 dilution was further diluted to 1 : 100–1 : 1,600, and MAT was similarly performed. The titer was defined as the highest dilution with 50% agglutination, and 1 : 100 or higher titers were regarded as positive.

Results

Detection of leptospiral antibodies

Leptospiral antibodies were detected in sows from 19 out of 20 prefectures surveyed. Sows raised in 97 of the 140 farms nationwide were positive for at least 1 of the 9 leptospiral serovars, and 281 of the 1,121 sows were positive (Table 1). The positive rate for Bratislava was the highest; the antibody was detected in 16 out of 20 prefectures. Of the 1,121 sows in 140 farms, 202 in 87 farms were positive for Bratislava; the next most common serovars detected were Australis, Autumnalis, and Canicola. The positive rates for the other 5 serovars were low.

Of the 202 sows positive for Bratislava, 139 tested positive only for this serovar (Table 2). Fifty-three sows tested positive for both Bratislava and Australis, and 10 showed a reaction to other serovars.

Distribution of antibody titers against the serovars

The antibody titer was 1 : 100 in about half of the positive sows. However, the antibody titers against serovars Bratislava and Australis were high, and the titer was 1 : 1,600 or higher in many sows (Table 3). The titers against serovars for which the positive rates were low were 1 : 800 or higher in some sows, particularly the anti-Grippotyphosa antibody titers (1 : 800 and higher than 1 : 1600 in 2 sows).

Regional distribution of positive farms and sows

The regional positive rates in the farms and sows surveyed were investigated (Table 4). In all areas, the positive rate for...
Bratislava was the highest, but showed variation among the farms. Some farms showed a high positive rate (7-8 of 10 sows) in a farm that was positive for Bratislava. The positive rates of sows and farms for serovar Canicola were higher in Shikoku/Kyushu/Okinawa than in other prefectures.

**Discussion**

Ninety-seven of the 140 farms and 281 of the 1,121 sows were positive for at least 1 of the 9 serological strains, demonstrating that leptospirosis is widely prevalent nationwide in sows in Japan. In the nationwide survey reported in 1960, the antibody titer was 1 : 300 or higher in 334 (10.9%) of 3,060 sows tested. Although it cannot be simply compared because 1 : 100 or higher was considered positive in our survey, the values may have been similar to those in the previous survey based on the distribution of the antibody titer. Thus, the prevalence of *Leptospira* in sows may have not markedly changed. However, major changes were observed on comparison of the positive serovars. Serovars Icterohaemorrhagiae, Canicola, and Hebdomadis were the major serovars in previous surveys, but the most widely prevalent serovars in the current survey were Bratislava, Australis, and Autumnalis. Naito et al. reported the detection of antibody against Bratislava in pigs in Japan. The survey, however, was performed in limited regions, and the positive rate for Bratislava was unclear. The antibody titre was considered positive in Japan, compared because 1 : 100 or higher was considered positive in America and Germany. In Japan, however, no report has been available on the swine leptospirosis due to serovar Bratislava, and almost all previous serological surveys of sows had not detected serovar Bratislava. Thus, it is difficult to identify whether this serovar has been present long-term or has been recently disseminated. However, the high positive

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**Table 3** Distribution of antibody titers against leptospiral serovars in sows

<table>
<thead>
<tr>
<th>Serovars</th>
<th>Antibody titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50±</td>
</tr>
<tr>
<td>Bratislava</td>
<td>919</td>
</tr>
<tr>
<td>Australis</td>
<td>1037</td>
</tr>
<tr>
<td>Autumnalis</td>
<td>1096</td>
</tr>
<tr>
<td>Canicola</td>
<td>1096</td>
</tr>
<tr>
<td>Hebdomadis</td>
<td>1112</td>
</tr>
<tr>
<td>Icterohaemorrhagiae</td>
<td>1116</td>
</tr>
<tr>
<td>Pomona</td>
<td>1118</td>
</tr>
<tr>
<td>Hardjo</td>
<td>1117</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>1119</td>
</tr>
</tbody>
</table>

**Table 4** Regional distribution of farms and sows positive for leptospiral antibody

<table>
<thead>
<tr>
<th>Serovars</th>
<th>Farms (%)</th>
<th></th>
<th></th>
<th></th>
<th>Sows (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tohoku/</td>
<td>Kanto/</td>
<td>Hokuriku/</td>
<td>Shikoku/</td>
<td>Tohoku/</td>
<td>Kanto/</td>
<td>Hokuriku/</td>
<td>Shikoku/</td>
</tr>
<tr>
<td></td>
<td>Hokkaido</td>
<td>(n=40)</td>
<td>Chubu (n=17)</td>
<td>Kyushu/</td>
<td>Hokkaido</td>
<td>(n=339)</td>
<td>Chubu (n=104)</td>
<td>Kyushu/</td>
</tr>
<tr>
<td>Bratislava</td>
<td>20 (51.3)</td>
<td>26 (65.0)</td>
<td>10 (58.8)</td>
<td>31 (70.5)</td>
<td>49 (14.5)</td>
<td>60 (18.2)</td>
<td>20 (19.2)</td>
<td>73 (20.9)</td>
</tr>
<tr>
<td>Australis</td>
<td>12 (30.8)</td>
<td>14 (35.0)</td>
<td>4 (23.5)</td>
<td>15 (34.1)</td>
<td>22 (6.5)</td>
<td>25 (7.6)</td>
<td>10 (9.6)</td>
<td>27 (7.7)</td>
</tr>
<tr>
<td>Autumnalis</td>
<td>7 (17.9)</td>
<td>7 (17.5)</td>
<td>2 (11.5)</td>
<td>8 (18.2)</td>
<td>7 (2.1)</td>
<td>7 (2.1)</td>
<td>2 (1.9)</td>
<td>9 (2.6)</td>
</tr>
<tr>
<td>Canicola</td>
<td>4 (10.3)</td>
<td>1 (2.5)</td>
<td>1 (5.9)</td>
<td>8 (18.2)</td>
<td>4 (1.2)</td>
<td>1 (0.3)</td>
<td>1 (1.0)</td>
<td>19 (5.4)</td>
</tr>
<tr>
<td>Hebdomadis</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (6.8)</td>
<td>0 (0.0)</td>
<td>3 (0.9)</td>
<td>0 (0.0)</td>
<td>6 (1.7)</td>
</tr>
<tr>
<td>Icterohaemorrhagiae</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (6.8)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (1.0)</td>
<td>4 (1.1)</td>
</tr>
<tr>
<td>Pomona</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (5.9)</td>
<td>1 (2.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>Hardjo</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (2.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (0.6)</td>
</tr>
</tbody>
</table>
rate suggests that Bratislava has been present for a long
time in Japan since its first introduction. It is hypothesized
that Bratislava was introduced in Japan by sows or boars
from foreign countries, and that carrier pigs might have
contaminated healthy pigs through direct or indirect contact.
Many recently reported cases of swine abortion and stillbirth
were caused by serovars Canicola, Pomona, and Icterohaemor-
rhagiae, and fewer cases were due to Bratislava,
suggesting that Bratislava has a weaker ability to cause
abortion and stillbirth than Canicola and Pomona in sows\(^4,\text{11,13,15}\). Whether or not Bratislava causes reproductive
disorders in sows has not yet been definitively confirmed.
The relationship between Bratislava and abnormal delivery
remains to be investigated.

Of the sows positive for serovar Bratislava, 25% was also
positive for Australis, but fewer cases were positive for the
other serovars. Since Bratislava and Australis belong to the
same serogroup, some of the cases may have cross-reacted.

It is known that abortion, stillbirth, haemoglobinuria, and
jaundice are clinical findings of swine leptospirosis, but many
other cases may show inapparent infection. Accordingly,
workers involved in sow production are likely to overlook
this disease, during which time infected sows are likely to
excrete the bacteria in urine, spread the infection, and cause a
hygiene problem. To control this disease, vaccination is
useful, and is presently being performed in America and
Europe\(^1,\text{3,7,14}\). To use the Leptospira vaccine, it is necessary
to know which types of leptospiral serovars are prevalent in
the region. Therefore, serological surveys are needed peri-
odically.

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原 著

わが国の繁殖雛豚におけるレプトスピラ抗体の浸潤

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要旨

わが国の繁殖雛豚を対象として、顕微鏡学的凝集反応（MAT）を用いてレプトスピラ抗体の検出を行い、その浸潤状況を調べた。MAT は病原性レプトスピラの 9 血清型の生菌液を抗原として行った。2001年から2004年にかけて、20 郡県の140農場に飼育されていた1,121頭について血清学的調査を実施した。その結果、19 郷県の98農場（70.0%）に飼育されている281頭（81.0%）がレプトスピラに対する抗体を保有していた。血清型別で最も陽性率が高かったのは血清型 Bratislava で、87農場（62.1%）の202頭（18.0%）から抗体が検出された。次いで血清型 Australis の45農場（32.1%）84頭（7.5%）、Autumnalis の25農場（17.9%）25頭（2.2%）、Canicola の14農場（10.0%）の25頭（2.2%）であった。以上のように、わが国の繁殖雛豚においてレプトスピラ抗体が全国的に広く浸潤していることが判明した。特に血清型 Bratislava が主要な血清型であることが明らかになった。

キーワード：日本、レプトスピラ、血清型検査、豚