NOTE  Public Health

Survey of Porcine Proliferative Enteritis in the Tohoku District of Japan

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ABSTRACT. A survey of proliferative enteritis (PE) in pigs at a meat processing plant was conducted using polymerase chain reaction (PCR) testing methods. During the investigation period, 227 of 83,717 pigs brought to the meat processing plant from Iwate, Fukushima, Miyagi, Niigata, and Yamagata Prefectures displayed characteristic general pathological features in terminal ileum, including mucosal hyper trophy and reticulation of serosal surface. Of these, 179 cases were further examined in the laboratory. All cases displayed characteristic histopathological features, and the specific band of the Lawsonia intracellularis (Li) causative agent of PE in pigs was detected in 155 cases by PCR testing methods. These results suggested a general infiltration of Li in the Tohoku district.

KEY WORDS: Lawsonia intracellularis, PCR, porcin proliferative enteritis.


Porcine proliferative enteritis (PPE) is a transmissible gastroenteric disease caused by Lawsonia intracellularis (Li). Clinically, PE causes hemorrhagic diarrhea and sometimes death in growing pigs, but when the disease progresses to a chronic phase, the infected pig no longer displays significant symptoms. Chronic PE is usually detected by examination of intestinal tissue samples at meat processing plants [1, 6, 9]. However, epidemiological surveys of PE have focused only on selected farms or farms with an incidence of pig diarrhea or deaths [5, 11]. The purpose of the present paper is to report the findings of an epidemiological investigation of PE using the polymerase chain reaction (PCR) and nested PCR method on tissue samples from pigs processed by the Shonai Shokuniku Ryutu Center (SSRC) slaughterhouse, in Yamagata Prefecture, Japan.

Pig ileum tissue samples displaying any two or all three pathological signs (sub-serosal edema, mucosal hypertrophy, and reticulation of the serosal surface; Fig. 1), were identified during the examination period at SSRC from September to December 2002.

Paraffin-embedded tissue sections were prepared from approximately half of the lesional ileum samples by general methods after fixing in neutral buffered 20% formalin. These sections were stained using hematoxylin and eosin (HE) and Warthin Starry (WS) staining techniques.

The remaining half of the lesional ilea samples were stored at –80°C for PCR. The Cryptosporidium detection method [2] was used to obtain template DNA from mucosa samples treated with phenol/chloroform and alcohol [12]. The reactions were implemented with four primers cited by Jones et al. [4]. These primers were as follows: for PCR, primer A: 5'-TATGGCGTGTCAAAACTCCG-3' and primer B: 5'-TGAGGATTATTGTACTTCC-3'; and for nested PCR, primer C: 5'-TATAGGTTAGTATTGCTCCGT-3' and primer D: 5'-TTCCTCAGCTGCTCCAT-3'. The reactions were carried out in a Parkin Elmer DNA Thermal Cycler with a cycling profile as summarized in Table 1. For samples with negative PCR results, nested PCR was then performed on 1 µl of each amplification product, using primers C and D under the same reaction conditions for PCR. The results for PCR and nested PCR products were electrophoresed on 2% agarose gels and visualized using ethidium bromide staining.

At the time of the first general inspection, PE lesions were mostly found in the terminal ileum area, but in some cases also in the proximate and/or middle area. PE was found in 227 of the 83,717 (0.27%) pigs, and at 36 of the 172 (20.9%) farms. Except for one that was 2 years old, all of the pigs with PE were 6 months old and were just finish-
ing their growth stage. Of the PE-infected group, samples from 179 were brought to our laboratory for further testing.

Histopathologically, all 179 cases demonstrated characteristic PE features (Fig. 2). In 89 samples, the existence of curved, rod-shaped bacteria within the apical cytoplasm of the epithelial cells was clearly confirmed by WS staining (Fig. 3).

PCR showed a specific band at the 319 base pair (bp) (Fig. 4) in 116 of the samples, and nested PCR showed a specific band at 260 bp (Fig. 5) in a further 39, for a total of 155 samples (86.6%) in which the existence of Li was certified either by PCR or nested PCR (Table 2).

Our investigation is the first report on the incidence of PE at a meat processing plant in the Tohoku district of Japan. Pigs with two or all three pathological signs were given a diagnosis of PE on the basis of histopathological findings. In 89 (49.7%) of the 179 cases, intracellular bacteria were detected using WS staining. The number of Li-specific DNA fragments was consistently high, with detection rates of 86.6%. As a finding consistent with previous reports [4, 10], diagnostic PCR testing for Li in the present study also demonstrated a higher rate of Li detection than did histopathological examination.

The reason neither PCR nor nested PCR methods detected Li specific bands in 3 samples that otherwise tested positive with WS staining may have been attributable to an insufficient number of DNA fragments stemming from technical error during the extraction of Li DNA. There seems therefore to be scope for further improvement in the DNA extraction procedure. As for the 21 samples that showed no intracellular bacteria nor 319 bp or 260 bp bands, a possible explanation for these negative results may be that the Li infection life cycle had already passed by the time of analysis [13]. Additional analyses, such as the immunofluorescence assay or serological examinations recognized as useful in detecting Li in subclinical-infected pigs [7, 8], may be required in order to accurately diagnose pigs that show no characteristic features and test negative with the PCR and nested PCR methods.

The incidence of PE reported by the present study (0.27%) was lower than that of a previous report (3.3%) by Kim et al. in Korea [5], and our average 20.9% PE-positive rate for farms was similar to their reported 20% [5], and lower than the 30% rate reported by McOrist et al. [9].

![Fig. 2. Thin section of lesional ileum. Mucosa was almost completely occupied by proliferative immature crypts. Few goblet cells were observed (HE stain).](image)

![Fig. 3. Intracellular bacteria. Black bacterial figures are observed in atypical epithelial cytoplasm (arrow)(WS stain).](image)

<table>
<thead>
<tr>
<th>Table 1. PCR and nested PCR cycling profile</th>
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<tbody>
<tr>
<td>Pre-Heat</td>
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<tr>
<td>Temperature (°C)</td>
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<tr>
<td>Time (minutes)</td>
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<tr>
<td>Cycle</td>
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</tbody>
</table>
Porcine PE was observed in pigs supplied to the SSRC from farms in Iwate, Miyagi, Niigata, and Yamagata Prefectures (Table 3), thus indicating a general infiltration of Li in the Tohoku district of Japan.

REFERENCE


Table 2. Histopathological findings and results of PCR and nested PCR

<table>
<thead>
<tr>
<th>WS</th>
<th>PCR positive</th>
<th>n-PCR positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>89</td>
<td>77</td>
<td>9</td>
</tr>
<tr>
<td>-</td>
<td>90</td>
<td>39</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td>116</td>
<td>39</td>
</tr>
</tbody>
</table>

a) Typical intracellular bacteria were observed (+) or not observed (−) with Warthin Starry stain.
b) Number of cases in which a 319 bp band was detected by PCR.
c) Number of cases in which a 260 bp band was detected by nested PCR (performed subsequently on PCR-negative samples).
d) Number of samples testing negative with both PCR and nested PCR.

Table 3. Occurrence of porcine PE in Tohoku district

<table>
<thead>
<tr>
<th>Aomori</th>
<th>Akita</th>
<th>Iwate</th>
<th>Yamagata</th>
<th>Miyagi</th>
<th>Fukushima</th>
<th>Niigata</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE pigs</td>
<td>NE</td>
<td>NE</td>
<td>4</td>
<td>209</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>NE</td>
<td>NE</td>
<td>9,931</td>
<td>70,079</td>
<td>1,621</td>
<td>1,120</td>
<td>966</td>
</tr>
<tr>
<td>PE farms</td>
<td>NE</td>
<td>NE</td>
<td>2</td>
<td>31</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>NE</td>
<td>NE</td>
<td>4</td>
<td>157</td>
<td>2</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

a) Number of PE-infected pigs brought to SSRC from each prefecture.
b) Number of pigs brought to SSRC.
c) Number of farms from each prefecture that supplied PE-infected pigs to SSRC.
d) Number of farms from each prefecture that supplied pigs to SSRC.
e) Not examined.
f) The source farms for 10 pigs were undetermined.

Fig. 4. Electrophoresis of PCR results. M: size marker, N: negative control without prepared samples, P: positive control, 1 to 5: prepared samples. Specific bands were observed at 319 bp in lanes 1 and 4.

Fig. 5. Electrophoresis of nested PCR performed on PCR negative products. M: size marker, N: negative controls without prepared samples, P: positive control, 1 to 5: prepared samples. Specific 260 bp bands were observed in lanes 1, 3, and 4.

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