Prevalence of Porcine Proliferative Enteropathy and Its Control with Tylosin in Korea

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ABSTRACT

Porcine proliferative enteropathy (PPE) is an enteric disease been caused by Lawsonia intracellularis. It has become one of the critical problems in the pig industry. To investigate the prevalence of PPE in Korea, serum samples of 828 pigs from 65 herds were tested using indirect immunofluorescence antibody technique (IFA). The infection rate in individual pigs varied from 44 to 60%, whereas 100% in pig farms. The infection frequency was 57, 44.9, and 50.4% according to age respectively. Administration of tylosin in feed at a concentration of 110 ppm for 14 days reduced the infection rate of the farms. These data indicated that the high prevalence of PPE may be controlled by tylosin.

KEY WORDS: Lawsonia intracellularis, Porcine Proliferative Enteropathy, Prevalence of PPE in Korea

PPE is an enteritis of pigs, and is caused by Lawsonia intracellularis (L. intracellularis). The bacteria cause a proliferation of the mucosa of ileum and proximal part of the colon [5, 9]. The major clinical symptoms of PPE can be classified into chronic and acute hemorrhagic types. The former shows anorexia, a mild diarrhea and growth retardation, whereas the latter shows an acute intestinal hemorrhage, anemia, and sudden death [8]. These symptoms occur in pigs between 6 and 20 weeks and between 4 and 12 months of age, respectively. PPE has been recognized as one of the critical problems in pig production. It has been claimed that PPE causes an annual economic loss of $20 million in the pig industry of the USA [10].

The rapid diagnosis of PPE in pig herd is of importance in the management of pig production. Once PPE is found in a pig herd, proper treatment should be considered before the bacteria are transmitted to other pigs, otherwise the costs associated with pig production will be greatly increased. PPE can be diagnosed by postmortem examination of the pigs, which reveals typical mucosal proliferation of the ileum. Although this examination is definitive in the identification of the disease, it is not applicable to all pigs showing clinical signs since the high cost results from the sacrifice of the pigs. An alternative is to detect the infective agent of PPE from feces using the polymerase chain reaction (PCR) [2]. PCR has become one of the most reliable methods for the diagnosis of PPE. PCR has the advantage of sensitivity (because as few as 10 individual L. intracellularis in samples can be detected [2, 6]) and samples for examination can easily be obtained. (e.g. pig feces can be used as the sample). The most important fact is that there is a strong correlation between the results of the PCR and the incidence of PPE. Because of these merits, the PCR has long been utilized for the diagnosis of PPE.

Kim et al. investigated the prevalence of PPE in Korea by PCR, and reported that infection rate of pigs and herds were 3.3% and 20%, respectively [3]. This value is somewhat lower than that in other countries such as USA (12% in pigs, 29% in herds) [10] and Taiwan (5.5% in pigs, 30% in herds) [1]. Even though their report may

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reflect the PPE prevalence rate in Korea, we cannot neglect the possibility that this may be an underestimation due to the intrinsic features of PCR in that the pathogenic agent itself should be detected in samples in order to decide upon the final diagnosis. It is worthy to note that because *L. intracellularis* is an obligate intracellular bacteria, there is a possibility that some pigs with PPE do not discharge the agent into feces. In this case, *L. intracellularis* would not be detected by PCR, and in turn the infection rate would be underestimated [4]. After considering these defects of the PCR method, we investigated the PPE prevalence in Korea using a serologic diagnostic method that depends on the presence of antibody against *L. intracellularis* in pig sera.

An indirect immunofluorescence assay was performed using an IleTestR kit according to the manufacturer's instructions (Elanco), as previously described [4]. Briefly, the serum samples from pigs were diluted 30 times with 1% skim milk in PBS (pH 7.0), and 5 µL of the diluted samples were dispensed into each well of the slide precoated with Lawsonia antigen. The reaction was incubated at 37 °C in a humidified chamber for 1 hr. After washing three times with PBS, the slides were incubated with FITC-labeled anti-pig IgGs (Sigma) at 37 °C for 30 minutes, followed by three washes with PBS. After staining, the slides were observed under a fluorescence microscope. To verify the staining result, positive and negative sera, which are supplied by the manufacturer, were applied on every slide. The positive sera showed bright and green fluorescent color of the well, whereas negative samples were dark. (Fig. 1).

Using the indirect immunofluorescence assay, we tested 828 pig sera from 65 herds, which is equivalent to 0.01% and 0.26% of the total number of pigs and herds in Korea respectively, between March and August 2000. The geographical regions in Korea along with number of herds used for sampling are depicted in Fig. 2. In the event that even one pig from a farm was positive, the infection rate of that farm was considered to be positive. Surprisingly, we found that all the herds tested were positive (Table 1). The positive rate of individual pigs in Gunggi-Do, Guungsgang-Do, Chungcheung-Do, Gangwon-Do, and Cholla-Do was 68.6% (105/153), 55.4% (153/276), 43.9% (29/66), 55.6% (59), and 54% (175/324), respectively (Table 1). In addition, we analyzed the positive rate of individual pig to determine whether age was related with susceptibility to infection. As shown in Table 2, the infection rate of weaning pigs (4 to 10 weeks old), growing pigs (11 to 13 weeks old), and finishing pigs (older than 14 weeks old) was 57%, 44.9% and 59.4%, respectively. This indicated that there is no significant relationship between age and susceptibility to the infection. The present study revealed that the infection frequency in pigs was an average of 53%, and 100% in herds. These data indicated that PPE is markedly prevalent and its infection widely distributed in individual pig and herd in Korea.

Our current data shows a higher infection rate than that of a previous report by Kim et al. [3], in which the average infection frequency of PPE in pigs and herds was 3.3% and 29%, respectively. We are not sure of the reason for this discrepancy. However, several explanations are possible. The first possibility is that there is a gap of three

Fig. 1. Representative photographs of the result of an indirect immunofluorescence assay. Serum was diluted 1:30 in PBS. The diluted serum was loaded into a well of a slide. The slide was incubated at 37 °C for 1 hour and washed with PBS. The slide was loaded with anti-pig antiserum labeled with FITC. The slide was washed 3 times and then observed under fluorescence microscope (A : Positive and B : Negative).
years between the previous and current studies: Kim et al.’s report dealt with investigations that had taken place from June to August 1997. As Kim et al. indicated, we found that prevalence of PPE in Korea tended to be increased, it is possible that PPE had been spread throughout the country during the interval of three years. The second one, is likely that the immunofluorescence assay may give us more accurate estimation of the prevalence than PCR. Even though these issues remain unclear until a blind comparison study is performed by both methods using the same pigs during same period, our data will be valuable in assessing the extent of the prevalence of PPE in Korea, and highlights the importance of developing a preventive as well as a therapeutic program against PPE.

Tylosin, which is a macrolide antibiotic, is known to be effective in the treatment of PPE [7]. To examine its therapeutic effect in barnyard situations, we used a feed medication for pigs from two farms that were heavily infected by *L. intracellularis*. Serum samples were taken from 13 pigs that were randomly selected from a group of 100 pigs per farm. The infection rate in two farms was 77% and 69%. Then the two groups of pigs were given a feed containing 110 ppm of tylosin (Elanco) for 14 days. Two weeks after the treatment, we randomly tested sera of 13 pigs from the same groups of herds. One herd showed that the infection rate was reduced from 77% to 28%. Even though there was no change in infection rate during the same period in the other herd, 2 months after the treatment the infection rate dropped from 69% to 46% (Fig. 3). These results strongly suggest that tylosin treatment may be a strategic medication for use in the treatment of PPE.

Table 1. Prevalence of PPE in pig farms and individual pigs in Korea between March and August 2000.

<table>
<thead>
<tr>
<th>Province</th>
<th>No. of farms</th>
<th>No. of pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Positive</td>
</tr>
<tr>
<td>Gunggi-Do</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Gungsim-Do</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Chungcheung-Do</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Gungwgon-Do</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Choll-Do</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>65</td>
</tr>
</tbody>
</table>

Table 2. Relationship of pig age with the infection rates of *L. intracellularis*.

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of pigs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning</td>
<td>42</td>
<td>24</td>
</tr>
<tr>
<td>Growing</td>
<td>49</td>
<td>22</td>
</tr>
<tr>
<td>Finishing</td>
<td>138</td>
<td>82</td>
</tr>
</tbody>
</table>

Fig. 3. The effect of tylosin treatment to PPE. Thirteen pigs were randomly selected from a group of 100 pigs in two pig herds (A : ■ and B : □), and were tested for the PPE using an indirect immunofluorescence method. After two weeks of treatment with 110 ppm of tylosin, 13 pigs randomly chosen from the same group of the herds were tested for PPE. Since group B did not show any change in the infection rate within one month, more tests were performed up to two months after treatment with tylosin.

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