NESTED PCR AND IFAT IN THE DIAGNOSIS OF PORCINE PROLIFERATIVE ENTERITIS

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Abstract

Nested PCR was used in order to detect the presence of specific p78 fragment of genomic DNA of Lawsonia intracellularis in ileal and colon mucosa. The IgG serum antibodies against L. intracellularis were detected by the use of indirect immunofluorescent test. A total of 364 healthy pigs from different region of Poland, killed in slaughterhouses were investigated. L. intracellularis p78 genomic fragment was found from 194 out of 364 intestinal samples. Serological studies confirmed that over 88.5% (322 out of 364) of swine had antibodies reacting with L. intracellularis antigen. Two results were doubtful and 40 proved negative. The above findings have provided evidence of increasing problems of porcine proliferative enteritis in Poland.

Key words: swine, porcine proliferative enteritis, Lawsonia intracellularis, nested PCR, IFAT.

Porcine proliferative enteritis (PPE) (5), caused by Lawsonia intracellularis, has been found more often among pigs in Poland recently. This disease is also called ileitis (6) or proliferative enteropathy (7). For the first time PPE was found by Schwartz and Beister in 1931 in USA (11). It was when the authors described some cases of pigs’ deaths where the animals had characteristic proliferative changes in the ileum and colon mucosa. This disease causes a considerable decrease in efficiency of production in many herds in Poland and worldwide (9). Most often it occurs particularly in mass productions with high hygienic standards (8), where big amount of chemotherapeutics for preventive treatments are used. Disturbances and selection of intestine microflora, including L. intracellularis follows as a consequence. Diagnosis of PPE is difficult due to unspecific both clinical signs and post mortem findings. In the case of the acute form, the course of the disease is atypical with a decrease in weight gain but no signs of diarrhoea (3). In the study presented here two laboratory methods for the diagnosis of PPE, such as PCR based on L. intracellularis detection and IFAT based on antibodies detection were compared.

Material and Methods

Animals and samples. Randomly selected 364 healthy, slaughtered pigs of various races and sex, ranging from 90 to 100 kg and coming from different regions of the country were submitted to the research. Material used in the study included scrapings of the ileum mucosa taken from 354 pigs, scrapings of the colon mucosa taken from 10 animals and blood samples for serology taken from all above mentioned animals.

Detecting of p78 fragment of L. intracellularis. DNA isolation was performed using Genomic DNA Prep Plus® device (A&A Biotechnology, Gdynia) with accordance to the manufacturer’s instructions. Amplification of DNA, obtained in the course of the above described extraction, was conducted using one-tube nested PCR, modified by Pejsak et al. (10). In the first round outside primers (Table 1) were used to amplify p78 fragment of L. intracellularis genome.

The second round, called nested PCR, included applying two primers (Table 1) to the product of the first round amplification. Both reactions were conducted in thermocycler in tubes of 0.5 ml. The program of thermocycling for both rounds was as follows: initial denaturation (95°C/5 min) was followed by 35 consequent cycles of denaturation (94°C/40 s), hybridization of starters 55°C/40 s, synthesis of DNA (72°C/40 s) concluded by final elongation (72°C/7 min).
Table 1
Sequences of external and internal primers used for multiplication of p78 fragment of *L. intracellularis* genome

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences of primers</th>
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<tbody>
<tr>
<td>Law1</td>
<td>5'-TATGGCTGTCAAACACTCCG-3'</td>
</tr>
<tr>
<td>Law2 Outside</td>
<td>5'-TGAAGGTATTGGTATTCTCC-3'</td>
</tr>
<tr>
<td>Law3</td>
<td>5'-TTACAGGTGAAGTTATTGGG-3'</td>
</tr>
<tr>
<td>Law4 Inside</td>
<td>5'-CTTTCTCATGTCCATAAACG-3'</td>
</tr>
</tbody>
</table>

The products of DNA amplification were then separated through electrophoresis on 1.5% agarose gel, in TBE or TAE buffer at 90 V. The gel was stained with ethidium bromide. To designate the product of amplification, MassRuller™DNA Ladder, Low Range (Fermentas) was used. The band of 270 bp (Fig. 1) appearing in the light of transiluminator was accepted as a positive reaction.

**Detecting of *L. intracellularis* antibodies.**
Serologic examination was conducted with the use of indirect immunofluorescence test (IleiTest, Elanco). Collected blood samples were left to clot for 18 h at room temperature. The samples were then centrifuged and serum was taken for further analyses. Serum was stored at -20°C if the test could not be carried out subsequently. For the test, slides with fixed *L. intracellularis* cells and 15 pits were used. *L. intracellularis* came from *in vitro* culture. Each slide test included 13 experimental samples, positive and negative serum control. Five µl of serum prior inactivated in 1:30 dilution in PBS buffer, was put into each pit. The slides were then incubated in a humid chamber at 4°C for 12-14 h. Next the slides were washed for 5 min with PBS buffer four times and dried. Five µl of the diluted conjugate (anti-rabbit IgG directed against anti-swine IgG, marked with fluorescein isothiocyanate, Sigma or ICN, at 1:30 dilution in PBS) was then added to each pit and the incubation at 37°C for 30 min succeeded. Finally, the slides were washed for 5 min with PBS four times. The results were read under the immunofluorescence microscope (Nicon) at 20x to 40x magnification. Samples with clearly fluorescent bacteria were considered positive (Fig. 2).

![Fig. 1. Results of p78 fragment of *L. intracellularis* amplification received by one-tube, modified nested PCR. Positive result is visualized by 270 bp stripe. Lane M = marker MassRuller™DNA Ladder, Low Range (Fermentas), lane L+ = positive control; lane L- = negative control; lanes 1-7 =test results from ileum mucus samples.](image)

![Fig. 2. Fluorescent *L. intracellularis* observed under immunofluorescence microscope. x400.](image)
Results and Discussion

The results of \textit{L. intracellularis} p78 fragment detection in scrapings of the ileum mucus and evaluation of blood samples, coming from healthy pigs, are presented in Table 2. Using nested PCR \textit{L. intracellularis} specific fragment of DNA was found in 194 mucus scrapings out of 364 tested what constitutes 53.3%.

<table>
<thead>
<tr>
<th>number of samples tested</th>
<th>nested PCR</th>
<th>IFAT</th>
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<tbody>
<tr>
<td></td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>188</td>
<td>134</td>
<td>134</td>
</tr>
<tr>
<td>364</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>total</td>
<td>194</td>
<td>170</td>
</tr>
<tr>
<td>%</td>
<td>53.29</td>
<td>46.70</td>
</tr>
</tbody>
</table>

Serology conducted with the use of IFAT for the same swine revealed the presence of antibodies in 322 (88.46%) out of 364 animals tested. A negative result was obtained for 40 swine (10.99%), as for 2 animals the outcome was doubtful. The analysis of the obtained data showed that in 188 cases specific antibodies against \textit{L. intracellularis} in blood were detected together with \textit{L. intracellularis} in the ileum. This can be a proof that the infection had been lasting for some weeks prior to taking the samples. In 134 cases a p78 sequence of \textit{L. intracellularis} was not detected unlike the specific antibodies. It may result from the contact of such animals with \textit{L. intracellularis} or from the infection that healed. In 5 cases IFAT did not confirm positive result of \textit{L. intracellularis} detection by PCR. In such cases the infection have probably taken place several days before collecting the samples, therefore any specific IgG could not be found yet. Negative results for both tests were obtained in 35 animals out of 364 tested (9.62%) and in 2 cases the result was doubtful. Data presented here confirm the common occurrence of \textit{L. intracellularis} antibodies in pigs from herds of different location in Poland (88.46%). Presumably PPE, recently found in traditional swine breed, had in most cases various, sometimes atypical, clinical forms. Therefore the disease is probably mistaken with spirochetics, swine dysentery, colibacillosis or alimentary intoxication. Moreover, preventive and protective use of chemotherapeutics can cause the masking of clinical picture of the disease. On the basis of the research we can conclude that porcine proliferative enteritis is a one of the most frequent gastroenterical diseases in piglets and in growing and finishing pigs.

Nested PCR tested in the study is essential and the most useful for the detection and identification of \textit{L. intracellularis} and also for early diagnosis of infection in particular animals, whereas IFAT seems to be more useful for determining immunological status of a herd and for monitoring herds in different stages of infection. The results of serological tests can be more valuable for assessing the health status of a herd rather than for individual animals as the presence of antibodies not always results from ongoing disease process.

References
