Introduction. *L. intracellularis* is widely spread swine enteric pathogen and it is mainly diagnosed in groups of weaners and fatteners [1, 2]. The disease caused by *L. intracellularis* in pigs is called proliferative enteropathy (PE). PE is regarded as one of the most serious enteric diseases affecting pigs after weaning and in all phases of finishing [2].

*L. intracellularis* is a Gram negative obligate intracellular bacterium that is cultivable only in cell culture. *L. intracellularis* is not culturable with a standard bacteriologic culture [2, 3]. Infection of pigs with this bacterium is consistently linked with the presence of proliferative lesions of the mucosa of the ileum and large intestine, hyperplasia of crypt enterocytes along with a decrease in goblet cells in association with the presence of intracellular, curved or S-shaped *L*. bacteria [3, 4].

On dead animals diagnosis of *L. intracellularis* is generally based on necropsy of clinically affected samples and histological examination. Clinical observations generally include diarrhoea, with weight "variations" in growing pigs [2, 3]. Some immunofluorescence tests using a monoclonal antibody directly on faeces are described but lack sensitivity. Therefore, detection of these microorganisms with the use of nucleic acid-based assays is not affected by viability of bacterial cells. The bacterial viability is always a concern of standard cultivation procedures due to e.g. antimicrobial residues present in intestinal specimens. These methods are time consuming and laborious [4]. That’s why different PCR methods are used very widely for diagnosis of *L*. infections in pigs [5 – 7].

Pathology of PE is diagnosed in many Lithuanian pig farms, however the confirmation of diagnosis requires to choose the most efficient sampling method. The aim of this study was to compare the efficiency of two sampling methods to provide material for the detection of *L. intracellularis* by nested PCR.

Material and methods. Samples of pathological material were taken from dead (scrapings of the ileum mucosa (im)) and alive (faeces (fc)) diarrhoeic pig during the outbreaks of enteric diseases in weaners and fatteners in Lithuanian pig farms. A total of 328 samples were tested with respect to *L. intracellularis* by means of nested PCR.
DNA isolation. DNA isolation was performed using 3 methods: Roti®-Phenol/Chloroform/Isoamyl alcohol (Carl Roth GmbH and Co, Germany), Genomic Mini (A&ABiotechnology, Poland) and Genomic DNA purification (Fermentas, Lithuania) kits with accordance to the manufacturer’s instructions.

Nested PCR. Amplification of DNA, obtained in the course of the above described extraction, was conducted using one-tube nested PCR, modified by Pejsak et al. [7]. A (5’-TATGGCTGTCACACACTCCG-3’) and B primers (5’-TGAAGGTATTGTTATTCCTCC-3’) were used for the first PCR [7], [8].

Nested PCR, included applying C (5’-TTACAGGTGAAGTTATTGGG-3’) and D primers (5’-CTTTCTCATGTCCATAAGC-3’) to the product of the first round amplification [9]. Both PCR were conducted in thermocycler (Mastercycler personal, Eppendorf, Germany) in tubes of 0.2 ml. The program of thermocycling for both PCR 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). The band of 270 bp appearing in the light of transiluminator was accepted as a positive reaction.

Statistical analysis was performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, USA). A z-test to compare proportions was conducted to identify significance.

Results. Altogether 49 outbreaks of porcine enteric diseases were studied. PE was diagnosed in 26.5% (13/49) cases. It was found that at least 26.7% (4/15) outbreaks of enteric diseases in weaners and 26.5% (9/34) ones in fatteners were associated with *L. intracellularis* infections.

Altogether 328 faecal samples (n=262) and 1 mucosal samples (n=66) were tested with respect to *L. intracellularis* by nested PCR (Table 1). *L. intracellularis* was detected in 7.2% (n=19) faecal samples of diseased pigs and in 21.2% (n=14) mucosal samples of dead ones.

*L. intracellularis* was detected in 3.2% (n=2) faecal samples of diseased weaners, and in 40.0% (n=4) mucosal scrapings from dead ones (P<0.0001). Also *L. intracellularis* was detected in 8.5% (n=17) faecal samples of diseased fatteners, and in 17.9% (n=10) mucosal scrapings from dead ones. Faeces and mucosal scrapings from weaners were positive for *L. intracellularis* in 8.3% (n=6) cases, and in fatteners in 10.5% (n=27) ones (P>0.05).

Table 1. Detection of *L. intracellularis* by nested PCR in pigs with evidence of enteric diseases

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Samples tested, n</th>
<th>Positive, n/%</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaners</td>
<td>Faeces</td>
<td>62</td>
<td>2/3.2</td>
<td>-1.18 - 7.58</td>
</tr>
<tr>
<td>Weaners</td>
<td>Ileum mucosa</td>
<td>10</td>
<td>4/4.00</td>
<td>9.64 - 70.36</td>
</tr>
<tr>
<td>Fatteners</td>
<td>Faeces</td>
<td>200</td>
<td>17/8.5</td>
<td>4.63 - 12.37</td>
</tr>
<tr>
<td>Fatteners</td>
<td>Ileum mucosa</td>
<td>56</td>
<td>10/17.9</td>
<td>7.86 - 27.94</td>
</tr>
</tbody>
</table>
Conclusions. The results of this study demonstrated that *L. intracellularis* is an important pathogen in swine farms. We assure that ileum mucosa sampling is a very suitable method for rapid *L. intracellularis* detection.

References

Comparison of Two Sampling Methods for Detection of *Lawsonia intracellularis* in Industrial Pig Farms
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The aim of this study was to compare the efficiency of two sampling methods to provide material for the detection of *L. intracellularis* by nested PCR. Prevalence of *L. intracellularis* was evaluated in diseased and dead diarrhoeic weaners and fatteners in 49 cases of outbreaks of enteric diseases. Faecal (n=262) and ileum mucosal scrapings (n=66) were tested. Proliferative enteropathy was diagnosed in 26.5% (13/49) cases of outbreaks of enteric diseases: 26.7% (4/15) ones in weaners and 26.5% (9/34) ones in fatteners. *L. intracellularis* was detected in 7.2% (19/262) faecal samples and in 21.2% (14/66) ileum mucosal. The results of this study demonstrated that *L. intracellularis* is an important pathogen in swine farms. We assure that ileum mucosa sampling is a very suitable method for rapid *L. intracellularis* detection.