Characterisation of *Yersinia pseudotuberculosis* isolates from tonsils and faeces of pigs

Taina T. Niskanen, Maria Fredriksson-Ahoma, Riikka Laukkanen and Hannu Korkeala

Department of Food and Environmental Hygiene, Faculty of Veterinary, Medicine, P.O. Box 57, 00014 University of Helsinki, Finland, Phone: +358 9 19149706, Fax: +358 9 19149718, E-mail: taina.niskanen@helsinki.fi

**Abstract:** In this study pheno- and genotypic methods were used to characterise *Yersinia pseudotuberculosis* isolates from pigs. All isolates belonged to bioserotype 2/O:3 and were pathogenic. PCR was a rapid and convenient assay to confirm the pathogenicity when salcin, pyrazinamidase, and esculin tests were inefficient. Variations between pulsed-field gel electrophoresis (PFGE) patterns were small, indicating genetic homogeneity among pig strains of serotype O:3.

**Keywords:** *Yersinia pseudotuberculosis*, pig, tonsil, faeces, pulsed-field gel electrophoresis (PFGE)

**Introduction:** *Yersinia pseudotuberculosis* is an uncommon cause of human yersiniosis (Naktin et al. 1999). The infections are mostly sporadic but between 1990 and 1999 five outbreaks of serotype O:3 were reported in Finland (Anonymous 2000). The bacterium has sporadically been isolated from clinically healthy pigs in some countries (Fukushima et al. 1989, Shiozawa 1991). The epidemiology of *Y. pseudotuberculosis* is still obscure. In this study pheno- and genotypic methods were used to characterised the *Y. pseudotuberculosis* isolates from pigs. Serotyping is the most commonly used method for characterisation of *Y. pseudotuberculosis*. Pathogenicity of the isolates has been confirmed in some studies using phenotypic tests, such as autoagglutination, calcium dependence, and Congo red uptake (Fantasia et al. 1991, Martins et al. 1998). Pulsed-field gel electrophoresis is a genotyping methods which have been previously used for characterization of *Y. pseudotuberculosis* isolates from different sources (Iteman et al. 1995, Martins et al. 1998). However, a need exists for subtyping of isolates belonging to one serotype to obtain more information about the epidemiology.

**Materials and Methods:** A total of 14 isolates from 425 tonsils and 17 isolates from 200 faeces samples of pigs were characterised. The biotypes were determined
using biochemical reactions with raffinose, melibiose and citrate. The isolates were biotyped and serotyped with slide agglutination O:1-O:6 antisera. The pathogenicity was determined by PCR targeting the virF gene located on the virulence plasmid and on CR-MOX agar. The strains were also tested for pyrazinamidase activity, esculin hydrolysis and salicin fermentation. For genotypic characterisation, pulsed field gel electrophoresis (PFGE) with NotI and SpeI enzymes were used.

Results: All the isolates were serotype O:3 and were pathogenic. They were virF positive and showed calcium dependence and Congo red absorption on CR-MOX agar. Isolates belonged to biotype 2. The isolates hydrolysed esculin but salicin and pyrazinamidase reactions varied. Eleven different genotypes were obtained when the isolates were characterised by PFGE by combining the results of SpeI and NotI enzymes. Variations between PFGE patterns were small, indicating genetic homogeneity among pig strains of serotype O:3.

Table 1: Characteristics of Yersinia pseudotuberculosis isolates recovered from pigs in Finland and different genotypes with SpeI enzymes.

<table>
<thead>
<tr>
<th>Isolation no</th>
<th>Sal</th>
<th>Esk</th>
<th>PYZ</th>
<th>VirF gene</th>
<th>SpeI profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>I/III/IV/VII</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>III/IV</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td>I/II/V/VI/VII</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>I/III/IV</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>III/IV</td>
</tr>
</tbody>
</table>

Discussion and conclusions: PFGE was shown to be efficient in subtyping strains of the same serotype. The differences between the PFGE patterns were small indicating a limited genetic diversity among pig strains belonging to serotype O:3. All isolates were pathogenic. PCR was a rapid and convenient assay to confirm the pathogenicity when pyrazinamidase and esculin tests were unreliable and also easier to interpret compared to calcium dependence and Congo red absorption on CR-MOX agar plates.

Acknowledgments: We wish to thank Raija Keijama and Sirkka Ekström for their technical assistance. This work was supported by the Finnish Foundation of Veterinary Sciences.
   KTL B4 / 2000, National Public Health Institute, Helsinki, Finland.
Fantasia, M., A. Martini, E. Filetici. and Anastasio M. P. 1991. Plasmid mediated characteristics of
Yersinia pseudotuberculosis strains isolated in Italy from humans, cattle and swine.
of pigs in fecal Yersinia contamination of pig carcasses at slaughter.
   Fleischwirtsch 69: 369-372.
pseudotuberculosis isolated from animals in Brazil.
tonsils of swine by Yersinia enterocolitica.