Detection of *Actinobacillus pleuropneumoniae* (App) serotype 1 in pigs by real-time quantitative PCR

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Diagnostic Service

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Introduction

- *Actinobacillus pleuropneumoniae* = swine pleuropneumonia
- Still clinically important in many countries
- Historically, serotypes 1, 5 and 7 mostly recovered from clinical outbreaks in Canada
- USA and Canada: sub-clinical infections
- In these two countries, $$ and effort:
  - To keep herds free from subclinical infection with virulent serotypes
  - To monitor absence of App in breeding herds
- Laboratory testing: key issue; main concern
Diagnosis of sub-clinically infected herds

• Serology
  – Excellent results in routine cases
  – Unexpected positive reactions are rarely observed; however: these reactions are difficult to interpret for breeder farms
  – Indirect diagnosis

• Detection of App in tonsils
  – Direct diagnosis of the presence of the pathogen
    • Isolation by standard culture
    • Isolation by magnetic beads
    • PCR
Detection of App in tonsils

- Isolation by standard culture
  - Fastidious; many contaminants
  - Poorly sensitivity
- Isolation by magnetic beads
  - Better sensitivity
  - Some contaminants are still present
  - Expensive and time consuming
- PCR
  - Good sensitivity, mainly with after-culture samples
  - Fast
  - More difficult when done directly from tonsils
• Some conventional PCR tests for qualitative detection of App species directly from tonsils: results difficult to interpret
• One real-time PCR for direct quantitative detection of App species from tonsils
• For serotype 1, only conventional PCR tests (for CPS) to be used with pure cultures
  – not tested for direct App serotype 1 detection from tonsils
Goal of the project

• Since no sensitive, fast and affordable test is available for detection of App serotype 1 in sub-clinically infected animals, the goal of this project was to develop a quantitative real-time PCR test for the direct detection of App serotype 1 in sub-clinically infected animals.
## qPCR conditions

<table>
<thead>
<tr>
<th>Gene</th>
<th>LPS1-9-11 PCR</th>
<th>CPS1 PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene</strong></td>
<td>ORF17 of LPS-O antigen biosynthesis region of serotypes 1, 9 and 11</td>
<td>CPS1c of capsular polysaccharide biosynthesis region</td>
</tr>
<tr>
<td><strong>Product length</strong></td>
<td>148 bp</td>
<td>200 bp</td>
</tr>
<tr>
<td><strong>Primers</strong></td>
<td>200 nM</td>
<td>200 nM</td>
</tr>
<tr>
<td><strong>Probe</strong></td>
<td>160 nM Cy5-BHQ1 (quencher)</td>
<td>200 nM FAM-BHQ1 (quencher)</td>
</tr>
<tr>
<td><strong>Annealing Temperature</strong></td>
<td></td>
<td>58°C</td>
</tr>
</tbody>
</table>
Efficiency

CPS1 with serotype 1

Efficiency: 87.7%; $R^2$: 0.996; slope: -3.656

LPS1-9-11 with serotype 1

Efficiency: 95.4%; $R^2$: 0.997; slope: -3.437

Same results for serotypes 9 and 11

Good primers and probes
• Reference strains of App serotypes 1 to 15
  
  CPS1
  positive:
  only serotype 1

  LPS1-9-11
  positive:
  serotypes 1, 9 and 11

Other bacterial species:
  - *Actinobacillus porcitonsillarum* (3)
  - *Actinobacillus suis* (2)
  - *Actinobacillus lignieresii* (1)
  - *Actinobacillus porcinus* (1)

CPS1 positive:
only serotype 1

LPS1-9-11 positive:
serotypes 1, 9 and 11

All negative with both PCR
## Analytical sensitivity

<table>
<thead>
<tr>
<th>PCR</th>
<th>Strain used</th>
<th>CFU/ PCR reaction</th>
<th>CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPS1</td>
<td>Serotype 1</td>
<td>6</td>
<td>1.6 X 10^3</td>
</tr>
<tr>
<td>LPS1-9-11</td>
<td>Serotype 9</td>
<td>0.6</td>
<td>1.4 X 10^2</td>
</tr>
<tr>
<td></td>
<td>Serotype 11</td>
<td>0.7</td>
<td>1.8 X 10^2</td>
</tr>
</tbody>
</table>
### Specificity: App field strains

232 strains tested

Isolated from 1990 to 2013 in Canada, USA, France, Brazil, Argentina and Mexico

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number of strains tested</th>
<th>positive strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LPS1-9-11</td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>NT</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>
Atypical strains

- Strains isolated from herds seropositive for App serotype 7, but negative for App serotype 1.
- Tested with different mAb recognizing CPS and LPS of the serotypes 1 and 7.

<table>
<thead>
<tr>
<th>Strains</th>
<th>CPS</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serotype 1</td>
<td>Serotype 7</td>
</tr>
<tr>
<td>Amy91-781f-26b</td>
<td>+</td>
<td>Neg</td>
</tr>
<tr>
<td>A05-1768-1</td>
<td>+</td>
<td>Neg</td>
</tr>
<tr>
<td>A04-1499</td>
<td>+</td>
<td>Neg</td>
</tr>
<tr>
<td>A08-013</td>
<td>+</td>
<td>Neg</td>
</tr>
<tr>
<td>A05-0339-1</td>
<td>+</td>
<td>Neg</td>
</tr>
</tbody>
</table>

Strains K1:O7 (CPS 1 and LPS 7)
BRIEF COMMUNICATIONS

Atypical Actinobacillus pleuropneumoniae isolates that share antigenic determinants with both serotypes 1 and 7

M. Gottschalk, A. Lebrun, S. Lacouture, J. Harel, C. Forget, K. R. Mittal

Abstract. In the present study, the characterization of 3 atypical isolates of Actinobacillus pleuropneumoniae is presented. Two isolates (1B and 27E) showed positive reactions in coagglutination, immunodiffusion, and indirect hemagglutination tests for serotypes 1 and 7, whereas the third isolate (26B) reacted with antisera to serotypes 1, 4, and 7. These atypical isolates of A. pleuropneumoniae possessed a capsular polysaccharide (CPS) antigenically related to serotype 1 as well as an O-chain lipopolysaccharide antigenically related to serotype 7 or to serotypes 4 and 7, as shown by the use of monoclonal antibodies. Results of toxin profile and virulence assays for mice and pigs showed them to be more related to A. pleuropneumoniae serotype 7 field isolates. All 3 isolates induced antibodies mainly against serotype 7/4 O-long-chain lipopolysaccharide (LC-LPS) and, to a lesser extent, to the CPS of serotype 1, in experimentally infected pigs. Diagnostic laboratories that use a LC-LPS-based enzyme-linked immunosorbent assay (ELISA) for serodiagnosis of A. pleuropneumoniae infection in swine would probably diagnose herds infected with these atypical isolates as being infected by A. pleuropneumoniae serotypes 7 or 4, whereas those that use a CPS-based ELISA would probably consider them as infected by A. pleuropneumoniae serotype 1.
Sensitivity in tonsils

- DNA was extracted from suspensions of negative tonsil spiked with $10^7$ to $10^3$ CFU of serotype 1/ml.

<table>
<thead>
<tr>
<th>PCR</th>
<th>CFU/ PCR reaction</th>
<th>CFU/0.1 g tonsil</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPS1</td>
<td>64</td>
<td>1 X $10^4$</td>
</tr>
<tr>
<td>LPS1-9-11</td>
<td>6</td>
<td>1 X $10^3$</td>
</tr>
</tbody>
</table>
Validation in tonsils

- **Negative:** herd serologically negative to all serotypes of App.

  All the tonsils tested so far are negative

- **Positive:** herd with clinical signs of pleuropneumonia,
  - serotype 1 isolated in lungs

  10 tonsils have been tested so far
  
  CPS1: 6 positive tonsils
  LPS1-9-11: 8 positive tonsils

  $2 \times 10^5$ to $2 \times 10^8$ CFU/g of tonsil
Conclusion

• CPS1 PCR specific for serotype 1
• LPS1-9-11 PCR specific for serotypes 1, 9 and 11
• Both PCR have good sensitivity
• Both PCR can be used to detect quantitatively App serotype 1 in tonsils
• Used together and in combinaison with mAb, good tool for atypical strains.
• When validated, PCR LPS1-9-11, highly useful to be used in Europe or to test animals to be imported to Canada
  – Serotype 1 almost absent in Europe
  – Serotypes 9 and 11 absent in NA
Future work

• To complete the validation in tonsils
• To test LPS1-9-11 PCR with more strains of serotypes 9 and 11 (collaboration with AFSSA Ploufragan)
• To test both PCR with nasal and tonsil swabs as well as tonsil biopsies (samples from live animals) (collaboration with CReSA, Barcelona)
Acknowledgements

• Dr. Corinne Marois (Ploufragan, France)
• Dr. Marina Sibila and Dr. Quim Segalés (CRESA, Barcelona, Spain)
• Dr. Réal Boutin (in the selection of the herd)
• Dr. Martin Choinière, Dr. Étienne Tessier, Marie-Audrey Lévesque and Jean Gauthier (tonsils)
• All the students and technicians of the laboratory