Prevalence of virulence genes and haemolytic activity in *Escherichia coli* associated with diarrhoea in grower pigs

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**Introduction**

Enteric diseases are the most common reason for antibiotic treatment in nursery pigs in Denmark. *Escherichia coli* (*E. coli*) harbouring F4 or F18 adhesins are among the most important causes of post weaning diarrhoea in week one and week two after weaning [1]. *E. coli* is also found as cause of diarrhoea outbreaks in grower pigs three to ten weeks after weaning which accounts for the majority of antibiotic treatments [2-3].

**Objectives**

The objective of the current study was to investigate the prevalence of virulence genes in *E. coli* from outbreaks of diarrhoea in growers and assess the value of using haemolytic activity as a predictor of virulence.

**Conclusions**

- The only adhesion factor found in this study was F18.
- Virulence genes were present in 89% of the F18+ isolates.
- Virulent *E. coli* F18+ were isolated from approximately ¼ of the diarrhoeic pigs examined in this study.
- Haemolytic activity was a useful marker for isolates containing both adhesions and toxin genes.
- The positive predictive value for haemolytic activity as virulence marker was 74%, which indicates that false positive or presence of other virulence genes than those examined should be considered when using haemolytic activity of *E. coli* as indicator of virulence.

**Table 1: Prevalence of virulence factor genes**

<table>
<thead>
<tr>
<th>Adhesion factor</th>
<th># Isolates</th>
<th>LT</th>
<th>Sta</th>
<th>Stb</th>
<th>VT2e</th>
<th>Sta,Stb</th>
<th>Stb,LT</th>
<th>Sta,Stb,VT2e</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>F18+</td>
<td>46</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>9</td>
<td>22</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>none</td>
<td>162</td>
<td>1</td>
<td>6</td>
<td>23</td>
<td>4</td>
<td>39</td>
<td>5</td>
<td>1</td>
<td>83</td>
</tr>
<tr>
<td>Total</td>
<td>208</td>
<td>1</td>
<td>6</td>
<td>33</td>
<td>4</td>
<td>48</td>
<td>27</td>
<td>1</td>
<td>88</td>
</tr>
</tbody>
</table>

A total of 208 *E. coli* isolates from the faeces of 86 pigs were included in this study. F18 occurred in 46 isolates (22%) and was the only adhesion factor found. A total of 41 (89%) of the F18+ isolates were positive for toxins and thereby classified as virulent. The combination of toxins found in the 41 F18+ isolates was 22 (53%) STb+LT, 10 (24%) STb and 9 (22%) STA+STb. Virulent F18+ isolates were cultured from the faeces of 22 (26%) of the 86 diarrhoeic pigs.

**Materials and Methods**

Faecal samples from non-treated diarrhoeic pigs two to four week post weaning were collected from three commercial nursery facilities in the eastern part of Denmark. The samples were cultured on blood agar and *E. coli* isolates were analysed for haemolytic activity and by RT-PCR for adhesion factor; (F4, F5, F6, F18, F41), and toxin genes; (VT2e, STA, STb and LT).

**Table 2: Association between haemolytic activity and virulence factor genes**

<table>
<thead>
<tr>
<th>Virulence factors*</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolytic activity</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
<td>153</td>
<td>154</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>167</td>
<td>208</td>
</tr>
</tbody>
</table>

* = Isolates harbouring both adhesion and toxin genes were assigned +, all other were assigned -

<sup>a</sup> - Colonies surrounded by a zone of lysis after overnight growth at 37°C on blood agar.

54 (26%) isolates showed haemolytic activity. By using haemolytic activity as marker for F18+ isolates with virulence genes a sensitivity of 97.6% (CL95% = 87.1%-99.9%), specificity of 91.6% (CL95% = 86.3%-95.3%), positive predictive value of 74% (CL95% = 60.3%-85.04%) and a negative predictive value of 99% (CL95% = 96.44%-99.98%) were obtained.

**References**

Lawsonia intracellularis is an important enteric pathogen in nursery pigs worldwide. High excretion level of Lawsonia intracellularis (>10^6 bacteria/g faeces) is correlated to histopathological lesions of proliferative enteropathy and decrease of daily weight gain [1]. In Denmark doxycycline and tylosine are the most commonly used antimicrobial oral treatments for Lawsonia intracellularis in nursery pigs with a typical treatment period of 5 days[2].

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**Objectives**

The objective of this study was to compare the effect of doxycycline and tylosine treatment on faecal excretion of Lawsonia intracellularis two days after treatment.

**Conclusions**

Oral treatment with 12.5 mg per kilo bodyweight doxycycline was more efficient than treatment with 7.5 mg per kilo bodyweight tylosine.

In pens treated with doxycycline:

a) Fewer pens were positive for Lawsonia intracellularis 2 days after treatment

b) Lower excretion levels of Lawsonia intracellularis 2 days after treatment were observed

c) Fewer pens were positive for high level of Lawsonia intracellularis 2 days after treatment that correlates to gross lesions of proliferative enteropathy and decrease of daily weight gain

Furthermore reduction of Lawsonia intracellularis excretion was only achieved in 50 % of pens treated with tylosine.

**Results**

The was no difference in positive pens or excretion levels of Lawsonia intracellularis in pens selected for doxycycline or tylosine treatment at the first day of treatment (Table 1).

**Materials and Methods**

A total of 65 pens from batches of nursery pigs in three herds 14 to 28 days post weaning was randomly assigned and treated for 5 days with 12.5 mg per kilo bodyweight doxycycline or 5 days with 7.5 mg per kilo bodyweight tylosine via water through. Treatments was initiated randomly at day 14, 21 or 28 post weaning independently of diarrhoea status of the pigs. Pooled faecal pen samples was collected at the day of initiation of treatment and two days after last day of treatment and analysed by qPCR for Lawsonia intracellularis with a lower detection limit of 2x10^5 bacteria/g faeces.

**References**

Infection dynamics of intestinal pathogens in batches of non-medicated diarrheic nursery pigs

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Introduction

Pooled faecal pen floor samples tested by quantitative PCR (qPCR) can be used as diagnostic tool to assess the average excretion level in groups of pigs. In Denmark a commercial qPCR test for four important intestinal pathogens in nursery pigs E. coli F4 (F4) and F18 (F18), Lawsonia intracellularis (LI) and Brachyspira pilosicoli (PILO) has been developed [1].

Objectives

The objective of this study was to investigate the infection dynamics of the four mentioned pathogens by qPCR testing and assess the diarrhoea prevalence in batches of non-medicated nursery pigs 14-35 days after weaning.

Materials and Methods

A longitudinal study was performed in three production farms in Denmark. Pen floor samples were collected weekly from pens containing nursery pigs (day 14, 21, 28 and 35 post weaning). The samples were examined for F4, F18, LI and PILO by qPCR testing. Furthermore, faecal samples obtained from pigs was clinically assessed as diarrhoeic or not and the diarrhoea prevalence at pen level was calculated. Pens subjected to antibiotic batch medication during the study were excluded.

Results

A total of 78 pens were included in the start of the study at day 14. Due to antibiotic batch medication pens were excluded. For the following observation day: 21, 28 and 35 after weaning, 58, 31, and 13 pens were un-medicated and included for analysis.

Conclusions

- F18 and LI was the most frequently detected pathogens
- F18 was the dominant pathogen in the beginning, LI was dominating in the end of the study period
- Mean pathogen excretion level of positive samples and diarrhoea prevalence increased over time

Agreement of antimicrobial resistance from pooled faecal samples at pen level and diarrhoeic pigs

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Introduction

Antimicrobial resistance testing of enterotoxic Escherichia coli (ETEC) is used as a diagnostic decision tool for selecting classes of antibiotics for treatment in pigs. Resistance testing is often performed on isolates from faecal samples collected from diseased case pigs with the advantage of high certainty of analysing E. coli strains that has caused disease in the individual pig.

Objectives

The objective of this current study was to investigate pen-level agreement of antimicrobial resistance testing of E. coli isolates sampled from diarrhoeic pigs and from pooled faecal pen floor samples.

Conclusions

- In 10 of 31 pens virulent E. coli were isolated in pen floor and pig samples
- Complete agreement between pen resistance and pig resistance classification were observed in 5 of 6 antimicrobial classes
- Antimicrobial resistance testing can be performed on pooled faecal pen floor samples
- Confirmation in larger study needed

Materials and Methods

Pigs were sampled two to four weeks after weaning in three commercial nursery facilities in the eastern part of Denmark.

Results

It was possible to compare pen-level results from pig faecal samples and pen floor samples in 10 pens (Table 1).

Table 1: Pig level

<table>
<thead>
<tr>
<th>Pen level</th>
<th>+ Virulent</th>
<th>- Virulent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Virulent</td>
<td>10</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>- Virulent</td>
<td>2</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>19</td>
<td>31</td>
</tr>
</tbody>
</table>

A total of 89 virulent E. coli isolates were cultured from 22 (26%) of 86 sampled diarrhoeic pigs and from in 13 (41%) of 31 pooled faecal samples from the pen floor. The overall prevalence of antimicrobial resistance for TET, AMP, SUL, TMP, STREP and SPEC and agreement of resistance classification are shown in Table 2.

Table 2: Resistance in pen and pig samples

<table>
<thead>
<tr>
<th>Antimicrobial class</th>
<th>% resistant</th>
<th>Pens with agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphamethoxazole</td>
<td>69.7</td>
<td>10/10</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>69.7</td>
<td>10/10</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>60.7</td>
<td>10/10</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>47.2</td>
<td>10/10</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>34.8</td>
<td>10/10</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>18.0</td>
<td>8/10</td>
</tr>
</tbody>
</table>

Complete agreement was obtained for all antibiotic classes except for SPEC where agreement was found in 8 of 10 pens.

Acknowledgement

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