**Identification of virulotypes and serotypes of enteropathogenic (EPEC) and Shigatoxigenic (STEC) Escherichia coli from healthy cattle at slaughterhouses in Wallonia.**

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**MATERIALS and METHODS**

**INTRODUCTION**

*Escherichia coli* producing the attachment-effacement (AE) lesion (EPEC) and/or Shiga toxins (STEC) cause enteritis and (bloody) diarrhoea in young calves and in humans, and are also present in the intestines of healthy cattle. Besides the O57:H7 serotype, which has been the main serotype causing STEC outbreaks in the world, EPEC and STEC can belong to dozens of O serogroups. Of them, 9 have been frequently identified worldwide: O5, O26, O103, O111, O118, O121, O145 and O165.

**AIM**

The aim of this study is to identify the virulotypes and serotypes of EPEC and STEC isolated from healthy cattle at slaughterhouses in Wallonia by DNA-DNA colony hybridization and multiplex PCRs.

**RESULTS: COLONY HYBRIDIZATION**

*Faecal samples from 216 <1-year-old bulls, 25 cows and 4 heifers collected between April and June 2014 in 2 slaughterhouses in Wallonia were grown overnight at 37°C in Lauryl sulfate Enterobacteriaceae selective broth. The enrichment broths were assayed with an stx1, stx2 (Shiga toxins) and eae (AE lesion) triplex PCR. Positive broths were inoculated onto 4 plates: McConkey’s agar, Chromagar ES, Chromagar ES with tellurite and Chromagar STEC. Of the 2542 coliform isolates were subcultured and tested by the colony hybridization assay with gene probes targeting the stx1, stx2 and eae genes. The triplex PCR was again performed on all probe-positive isolates. The PCR-positive E. coli were subsequently assayed with two pentaplex PCR targeting the specific genes coding for the ten O serogroups listed above.*

**RESULTS: TRIPLEX PCR**

- 744 out of the 2542 coliform isolates were positive with at least one gene probe: stx1, stx2 and/or eae;
- these 744 probe-positive isolates originated from 69 out of the 245 animals sampled.

**RESULTS: PENTAPLEX PCR**

<table>
<thead>
<tr>
<th>eae</th>
<th>stx1</th>
<th>stx2</th>
<th>eae, stx1</th>
<th>eae, stx2</th>
<th>stx1, stx2</th>
<th>eae, stx1, stx2</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC (n=690)</td>
<td>52</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>ES (n=680)</td>
<td>56</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>ES tell (n=483)</td>
<td>98</td>
<td>5</td>
<td>32</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>204</td>
</tr>
<tr>
<td>STEC (n=689)</td>
<td>229</td>
<td>18</td>
<td>49</td>
<td>13</td>
<td>23</td>
<td>6</td>
<td>397</td>
</tr>
<tr>
<td>TOTAL (n=2542)</td>
<td>435</td>
<td>29</td>
<td>112</td>
<td>94</td>
<td>16</td>
<td>50</td>
<td>8</td>
</tr>
</tbody>
</table>

**DISCUSSION and CONCLUSION**

These results confirm that EPEC and STEC, which could represent a public health hazard, are observed in healthy cattle at slaughterhouse in Wallonia. The colony hybridization and the triplex PCR results show over 80% of concordance.

The colony hybridization is useful as a first step assay in large-scale studies and can improve the field surveillance and/or monitoring programs in combination. It can also help to predict the prevalence of EPEC, STEC, and AE-STEC in healthy cattle and humans and to trace the source of an infection along with the different multiplex PCR assays.

Further studies are necessary to compare EPEC and STEC from young calves, healthy cattle and humans in order to identify host- and/or age-specific properties.

**REFERENCES**