VTEC in raw cow's milk in Denmark

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The objective of this study was to develop and validate fast and reliable real-time PCR based methods for detection of VTEC and *Escherichia coli* O157 in raw milk from cows within 20 hours and to use the methods to obtain information about the occurrence of VTEC and *E. coli* O157 in samples taken from bulk milk tanks on Danish farms. An additional aim was to determine the quantitative levels of *E. coli* in milk samples.

Raw milk from cows may be contaminated with verocytotoxin producing *E. coli* (VTEC) including serogroup O157 (*VTEC O157*). To overcome this kind, milk is usually heat treated before it is used for production of dairy products. Despite the risk of diseases many consumers choose to drink unpasteurized milk and eat dairy products made from minimally heat treated milk, e.g. soft cheeses. A safe production of dairy product made from minimally heat treated milk requires that the milk is free for VTEC.

**Materials and Methods**

Real-time PCR based detection: Twenty four milk samples were inoculated in 229 ml of 0.9 % NaCl supplemented with 15 mg/L 
E. coli

The purified DNA was assayed for genes specific for vtx1, vtx2, and O157 vtx1/2 (SD) respectively, by real-time PCR assays based on dual-labeled probes (1). An internal amplification control (IAC) was included to ensure that no false negative PCR reactions were due to the presence of PCR inhibitors in the purified DNA samples. Samples that were real-time PCR positive were further assayed for genes specific for *E. coli* serum group O157 and O111 following the biochemical specifications laid out in ISO (2). Real-time PCR was performed on a Rotorgene 3000 Thermocycler (Corbett Research, Australia).

**Culture methods for detection of VTEC, *E. coli* O157 and *E. coli* O111**: The milk samples were investigated for *E. coli* O157 using the method described in ISO 16654:2001 (3). The *E. coli* O157 analysis was performed using *E. coli* O157 antibody coated magnetic beads (Dynatech; anti *E. coli* O157, Invitrogen) and a BioRobot instrument (Genepool). Sorbitol MacConkey (SMAC) agar was used as the secondary isolation medium.

**VTEC** was isolated from real-time PCR positive samples by sending the primary enrichment culture to EN (Tropical Bred Agar with Escherichia coli and *Salmonella* broth) and investigated in real-time PCR positive samples were verified by real-time PCR assays using a real-time thermal cycler, with external standard volumes of virus isolates investigated on one test. From the real-time PCR positive isolates were verified by real-time PCR assay using standard geometrical and microsatellite testing and tested for the presence of verocytotoxin producing (vtx1 and vtx2) and was an isolate. The obtained were published by Skibby, Sorensen, and Son by the Genomic System (Promega Corporation, USA). The purified DNA was analyzed for genes specific for vtx1, vtx2, eae, and vtx1 + vtx2 (*O157*) respectively.

**Validation study**: The real-time PCR based methods for detection of VTEC and *E. coli* O157 in raw milk from cows were robust and had specificities and sensitivities that were equal to the standard ISO *E. coli* O157 reference method.

**Conclusions:**

- The real-time PCR based methods for detection of VTEC and *E. coli* O157 in raw milk from cows was isolated from two samples (0.6%).
- *E. coli* O157 was isolated from 6.4% of samples but none of these were VTEC.

**Raw milk survey**: The PCR assays were used to investigate 312 milk samples from dairy farms for the occurrence of the genes vtx1, vtx2, and O157 referred to as percentage of positive samples in the table below:

<table>
<thead>
<tr>
<th>Gene</th>
<th>vtx</th>
<th>vtx1</th>
<th>vtx2</th>
<th>vtx1 + vtx2</th>
<th>eae</th>
<th>vtx + eae</th>
<th>O157</th>
<th>vtx + O157</th>
</tr>
</thead>
<tbody>
<tr>
<td>% positive</td>
<td>19.6</td>
<td>5.6</td>
<td>10.6</td>
<td>3.5</td>
<td>32.7</td>
<td>11.9</td>
<td>8.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Ct mean</td>
<td>27.9</td>
<td>29.8</td>
<td>22.6</td>
<td>22.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>5.2</td>
<td>6.2</td>
<td>4.6</td>
<td>5.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A few of the analyzed non-spiked milk samples generated weak positive PCR signals, all with Ct values of >30.

**Results and discussion**

Generic *E. coli*:

*E. coli* CFU/ml in the 312 analyzed samples of raw milk from bulk tanks on Danish farms is shown in the bar chart. Half of the samples were contaminated at the level of 1-10 CFU/ml.

**References**:


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