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Boel, Jeppe; Jensen, Annette Nygaard

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VTEC in raw cow's milk in Denmark

Jeppe Boel & Annette Nygaard Jensen

Division of Food Microbiology, National Food Institute, DTU, Copenhagen Denmark

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, milk is usually heat treated before it is used for production of dairy products. Despite the risk of diseases many consumers choose to drink unpasteurised 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-eight milk samples were incubated at 320 °C for 5 hours in addition to non-specific enrichment. DNA was isolated from 1 ml of enrichment culture using a MagneSil® KF, Genetic System (Promega Corporation, USA). The purified DNA was analysed for genes specific for vtx1, vtx2, eae and E. coli O157 (vtx1, respectively, by Real-time PCR assays based on duplex 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 analysed for genes specific for vtx1, vtx2 and O157 following the technical specification issued by CEN (2). Real-time PCR was performed on a Roche-Darwin 3500 XL thermocycler (Cocktail Research, Australia).

Culture methods for detection of E. coli and O157 in milk: The milk samples were inoculated for E. coli O157 using the method described in ISO 16654:2001 (3). The E. coli O157 strains were performed using vtx1 and vtx2 antibodies specific for the corresponding genes vtx1 and vtx2 (Promega Corporation, USA). The isolated strains were serotyped by Statens Serum Institut, Copenhagen, Denmark.

VTEC was isolated from real-time PCR positive samples by sending the primary enrichment cultures on TSA (Tryptone Bile Agar with X-vtx) with a 0.45 μm filter obtained from the same dairy farm. The milk samples were inoculated using a one-ml volume which was then incubated at 37ºC for 24 hours before being analysed by the reference culture methods and the real-time PCR based methods.

Validation study: The results of the validation studies are summarized in the Table. There were full agreement between the results of the E. coli O157 real-time based method and the ISO 16654:2001 method; the 30 spiked samples were positive and the 30 non-spiked samples were negative. Similarly, the vtx1 and vtx2 real-time PCR assays gave the expected results. The observed Ct values in the spiked samples were consistent and only marginal differences were observed between the Ct values in the samples that were spiked with high and low levels of VTEC O157. The amplifications of the IAC control indicated that the analysed DNA preparations were free of PCR inhibition. These data indicate that the real-time PCR based methods are robust, and that the performance of the methods are satisfactory and equal to the performance of the reference culture method for detection of E. coli O157.

Conclusions:

- 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.
- The real-time PCR based prevalence of VTEC in raw cow’s milk from bulk tanks was 19.6%.
- VTEC was isolated from two samples (0.6%).
- E. coli O157 was isolated from 6.4% of samples but none of these were VTEC.

Results and discussion

The vpx positive samples were used to investigate 312 milk samples from dairy farms for the occurrence of the genes vtx1, vtx2, and O157; given 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>O157 ISO culture method</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>

The vtx positive samples were analysed for O26 and O111 specific genes; three samples were O26 positive whereas none of the samples were O111 positive. The obtained Ct values for naturally infected samples were generally higher than the Ct values generated in the validation study.

The VTEC isolation protocol was applied on each of the 61 vtx positive enrichment broths. A minimum of 10 and up to 50 colonies were tested for vtx1. The approach yielded VTEC isolates from two samples; these were vtx2 positive, eae negative and of serotype O116:H- and O126:H20.

Twenty five (8.5%) of the samples were positive for E. coli O157 with the real-time PCR based method. Strains of E. coli O157 were isolated from 20 (6.4%) of the samples by investigating subcultures on blood agar. It was not possible to verify the culture remaining five samples when the enrichment broths were investigated in accordance with the ISO protocol for E. coli O157 isolation. The PCR and culture positive samples generated an average Ct value of 20.3 (SD = 3.1), whereas the PCR positive but culture negative samples all had Ct values above 31. The 20 isolated E. coli O157 strains all tested negative for verocytotoxin encoding genes by PCR analysis. The high prevalence of verocytotoxin negative E. coli O157 emphasizes the need for isolation and further characterization of E. coli O157 if the purpose of the analysis is to ensure that the samples are free of human pathogenic VTEC O157.

This study shows that real-time PCR assays are efficient for screening of raw cow’s milk for VTEC, but also highlights the difficulties in obtaining isolates from PCR screening positive samples. The results indicate that the prevalence of VTEC, including serogroup O157 is low in Danish milk, but genes encoding verocytotoxin and other VTEC associated genes are frequently found. The study also shows that non-pathogenic E. coli O157 prevails in raw milk in Denmark.

References:

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