VTEC in raw cow's milk in Denmark

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Publication date: 2012

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
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 hazard, 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 to 220 ml of tryptic soy broth supplemented with 1% (v/v) TSB (Tryptone Soy Broth) for non-specific enrichment. DNA was isolated from 1 ml of the enrichment culture using the MagNA Pure LC (Roche Diagnostics, Germany) system (Promega Corporation, USA). The purified DNA was analyzed for genes specific for vt1, vt2, eae and E. coli O157 (3), 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 AP-RT inhibitors in the purified DNA samples. Samples that were real-time PCR positive were further analyzed for genes specific for E. coli O157 following the biological speciation protocol by ISO (3). Real-time PCR was performed on a Rotor-Gene 3000 thermo cycler (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 (2). E. coli O157 strains were performed using E. coli O157 antibody coated magnetic beads (Dynabeads® anti-E. coli O157, Invitrogen) and a BioMérieux instrument (BioRex). Standard Escherichia coli O157 was used as the secondary isolation control.

VTEC was isolated from real-time PCR positive samples by sending the primary enrichment culture to the Escherichia coli O157 laboratory at Statens Seruminstitut (SSI). 1 ml of enrichment culture was investigated in one plate. The positive colonies were verified using conventional methods, where external and internal eae and vtx isolates were also included. The remaining culture was stored at -20°C for subsequent investigation.

#### Genomic System (Promega Corporation, USA).

The purified DNA was analyzed for genes specific for vtx1 and vtx2. The isolated genes were sequenced using the MagNA Pure LC (Roche Diagnostics, Germany) system (Promega Corporation, USA). The purified DNA was analyzed for genes specific for vt1, vt2, eae and E. coli O157 following the biological speciation protocol by ISO (3). Real-time PCR was performed on a Rotor-Gene 3000 thermo cycler (Corbett Research, Australia).

Results and discussion

### Generic E. coli:

The level of generic 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 10–50 CFU/ml. Few samples had a low (~5 CFU) or high (~50 CFU) levels. The 30 spiked samples were made by inoculating 15 samples with 50 CFU of the target organism (high level inoculum). Each strain was used to inoculate 2 x 3 milk samples at high and low levels, respectively.

#### VTEC isolation:

VTEC was isolated from 6.4% of samples but 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 vrt positive enrichment broth. A minimum of 10 and up to 50 colonies were tested for vrt. The approach yielded VTEC isolates from two samples; these were vrt2 positive, eae negative and of serotype O116:H- and O126:H20.

#### Spiking samples:

The vrt positive samples were analyzed 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.

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### 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.

### Acknowledgments:

Thanks to Annie Kaalby, Arla Foods Amba and Sven Erik Sørensen, Eurofins Denmark for providing the milk samples.

### References: