Research Note

Characteristics of Shiga Toxin–Producing *Escherichia coli* Isolated from Swiss Raw Milk Cheese within a 3-Year Monitoring Program


1Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Switzerland Winterthurerstrasse 272, CH-8057 Zurich, Switzerland; 2National Reference Laboratory for *Escherichia coli*, Centre for Infectiology and Pathogen Characterization, Federal Institute for Risk Assessment (BfR), Diersdorfer Weg 1, 12277 Berlin, Germany; and 3Federal Veterinary Office, Schwarzenburgstrasse 155, 3003 Bern, Switzerland

MS 09-351; Received 18 August 2009/Accepted 25 September 2009

ABSTRACT

Food is an important vehicle for transmission of Shiga toxin–producing *Escherichia coli* (STEC). To assess the potential public health impact of STEC in Swiss raw milk cheese produced from cow’s, goat’s, and ewe’s milk, 1,422 samples from semihard or hard cheese and 80 samples from soft cheese were examined for STEC, and isolated strains were further characterized. By PCR, STEC was detected after enrichment in 5.7% of the 1,502 raw milk cheese samples collected at the producer level. STEC-positive samples comprised 76 semihard, 8 soft, and 1 hard cheese. By colony hybridization, 29 STEC strains were isolated from 24 semihard and 5 soft cheeses. Thirteen of the 24 strains typeable with O antisera belonged to the serogroups O2, O22, and O91. More than half (58.6%) of the 29 strains belonged to O:H serotypes previously isolated from humans, and STEC O22:H8, O91:H10, O91:H21, and O174:H21 have also been identified as agents of hemolytic uremic syndrome. Typing of Shiga toxin genes showed that stx2 was only found in 2 strains, whereas 27 strains carried genes encoding for the Stx2 group, mainly stx2 and stx2vh-ab. Production of Stx2 and Stx2vh-ab subtypes might be an indicator for a severe outcome in patients. Nine strains harbored hlyA (enterohemorrhagic *E. coli* hemolysin), whereas none tested positive for eae ( intimin). Consequently, semihard and hard raw milk cheese may be a potential source of STEC, and a notable proportion of the isolated non-O157 STEC strains belonged to serotypes or harbored Shiga toxin gene variants associated with human infections.

Shiga toxin–producing *Escherichia coli* (STEC) is associated with human diseases ranging from uncomplicated diarrhea to hemorrhagic colitis (HC) and life-threatening complications, such as hemolytic uremic syndrome (HUS), which is the main cause of acute renal failure in children (25). Most cases of HC and HUS have been associated with *E. coli* O157:H7, but the importance of non-O157 STEC is increasingly recognized, especially in continental Europe (13). STEC is characterized by the production of one or more Shiga toxins (Stx1, Stx2, and variants). Pathogenic STEC tends to feature the Shiga toxin 2 variant, the outer membrane protein intimin mediating attaching and effacing lesions on intestinal epithelial cells, and/or the enterohemorrhagic *E. coli* (EHEC) plasmid-encoded hemolysin (2, 19).

Worldwide, food is an important vehicle for transmission of human STEC infections. Unpasteurized milk and dairy products including cheese (7–9, 12) have also been linked to STEC outbreaks. Under poor hygienic practices, STEC can gain access to raw milk by fecal contamination during the milking process, since ruminants represent important natural reservoirs of STEC. In bulk-tank milk collected throughout Switzerland, STEC was recently detected in 2.5, 12.7, and 16.3% of the samples from cow’s, goat’s, and ewe’s milk, respectively (18, 29).

According to the Food and Agriculture Organization of the United Nations, world cheese production is estimated to be more than 18 million tons annually (http://faostat.fao.org). Many cheese varieties throughout Europe, including Swiss semihard and hard cheese, are typically made from unpasteurized milk with the natural enzymes and microflora responsible for enhancing desirable flavor characteristics. STEC tends to decline during cheese ripening, but the ability of STEC to survive ripening periods of more than 90 days has repeatedly been demonstrated (6, 16, 23, 29). Even though raw milk cheese has the potential to carry STEC and cheese is marketed worldwide, only limited comprehensive characterization data are available for STEC isolated from cheese (10, 20, 21, 26). Recently, we examined the occurrence of STEC in Swiss raw milk cheese and detected it in 4.9% of the samples (24). The aim of the present study was to update the prevalence data of STEC in soft, semihard, and hard raw milk cheese at the producer level and to provide further characterization data of isolated STEC strains to assess the virulence and potential public health impact of such strains.

MATERIALS AND METHODS

Sampling and STEC detection. At the producer level, raw milk cheese samples were collected within the risk-based national
sampling plan throughout Switzerland. As reported previously, 432 and 364 samples were collected in 2006 and 2007, respectively (24). In 2008, 706 additional samples were tested. During the 3-year STEC monitoring program, 1,502 samples from 80 soft cheeses and 1,422 semihard and hard cheeses were analyzed. These cheeses were produced from cow’s (n = 1,370), goat’s (n = 121), and ewe’s (n = 11) milk.

From each sample, 25 g was enriched in 225 ml of brilliant green bile broth (Becton Dickinson, Sparks, MD) at 42°C for 24 h. The enriched samples were streaked onto sheep blood agar (5% sheep blood, Oxoid Ltd., Hampshire, UK), and, after incubation at 42°C for 24 h, colonies were washed off with 2 ml of 0.85% saline solution. For the STEC assay, 2 μl of each plate eluate were evaluated by PCR with primers targeting a region conserved between stx1 and stx2, complementary to nucleotides 447 to 462 and 943 to 962 of sequence EMBL/GenBank M19473 for stx1 and nucleotides 517 to 538 and 1016 to 1035 of sequence EMBL/GenBank X07865 for stx2. PCR assays were performed according to conditions described previously (31), and E. coli O157:H7 strain 857/03 (stx1 and stx2 positive) was used as a positive control.

STEC isolation and strain characterization. For strain isolation by colony dot-blot hybridization (24), DNA probes were prepared by labeling stx-PCR amplicons from E. coli O157:H7 857/03 with DIG High Prime kit (Roche, Mannheim, Germany). Stx-positive samples were streaked onto sheep blood agar, and, after incubation at 42°C for 24 h, colonies were transferred to a nylon membrane (Roche) and lysed following standard methods. After washing, crosslinking, and prehybridization in DIG-Easy-Hyb buffer (Roche) at 42°C for 60 min, hybridization of membranes with stx probes was performed overnight at 42°C. After washing in primary and secondary wash buffers, the presence of labeled probe was detected with an alkaline phosphatase-conjugated antibody detection kit and nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate, toluidine salt stock solution (Roche). One isolate per sample, corresponding to a colored spot, was picked from the hybridization plate and grown at 42°C overnight on sheep blood agar. Isolated strains were confirmed as STEC by PCR detection of stx as described above.

Serotyping of O (lipopolysaccharide) and H (flagellar) antigens was performed with O (O1 to O181) and H (H1 to H56) monospecific antisera prepared at the National Reference Laboratory for Escherichia coli (Federal Institute for Risk Assessment, Germany). Strains were examined for stx1, stx2, and their variants by stx-specific PCRs and by analysis of restriction fragment length polymorphisms of the PCR products (3). By PCR, strains were tested for eae encoding intimin, and hlyA encoding EHEC hemolysin (2).

RESULTS AND DISCUSSION

In a previous study, examinations of Swiss raw milk cheese samples showed a STEC prevalence of 3.7% in 2006 and 6.3% in 2007 (24). In 2008, 6.5% of 706 additional samples collected at the producer level tested positive for stx. During the 3-year monitoring program (part of the Swiss risk assessment program for raw milk cheese), STEC was detected by PCR after enrichment in 5.7% of the 1,502 raw milk cheese samples, which comprised 8 soft cheeses (half made from cow’s milk and half from goat’s milk), 76 semihard cheeses (66 from cow’s milk, 9 from goat’s milk, and 1 from ewe’s milk), and even 1 hard-ripened cheese made from cow’s milk. Further comprehensive prevalence data of STEC in raw milk cheese are restricted to only a few countries. Studies in France detected STEC in 13.1, 10, and 30.5% of 1,039, 603, and 180 cheese samples, respectively (10, 21, 26). In smaller investigations, the percentage of STEC-positive ewe’s milk cheese ranged from 2.4 to 6.9% in Spain (6, 22). In a study from Peru, E. coli O157 strains were isolated from 7.8% of 102 soft cheese samples (17).

Using colony hybridization, 29 STEC strains were isolated in the current study from 22 cow’s milk semihard cheeses, 2 cow’s milk soft cheeses, 2 goat’s milk semihard cheeses, and 3 goat’s milk soft cheeses. Twenty-four strains were typeable with O antisera, and they belonged to 12 O serogroups (Table 1). Thirteen strains belonged to the serogroups O2, O22, and O91, and these strains were typed into five different O:H serotypes. STEC strains belonging to the serogroups O8, O15, O22, O91, O109, O113, and O174 were also isolated from raw milk cheese in other studies, though other serogroups like O5 and O6 predominated (20, 26). The majority of the serotypes found in the present study have previously been isolated from cattle (Laboratorio de Referencia de E. coli [LREC], Lugo, Spain; www.lugo.usc.es/ecoli). More than half (58.3%) of the 24 STEC strains isolated from cow’s milk cheese belonged to serotypes previously isolated from Swiss cattle, namely O2:H7, O15:H16, O22:H8, O91:H21, O113:H4, O148:H8, and O174:H21 (30).

None of the STEC strains isolated in the present study belonged to classical EHEC types such as O26:H11, O103:H2, O111:H8, O145:H7, or O157:H7. On the other hand, the serotypes of 58.6% of the 29 strains have previously been identified in human STEC strains (Table 1). STEC of serotypes O22:H8, O91:H10, O91:H21, and O174:H21 have additionally been identified as agents of HUS (LREC). E. coli O91 strains are the most common human pathogenic eae-negative STEC, especially in adult patients (1, 28). In a recent German study, serogroup O91 was the second (after O8) and fourth (after O157, O103, and O26) most commonly identified O group of STEC strains isolated from food and patients, respectively (28). Interestingly, Bielaszewska et al. (4) reported that STEC O91:H21, which often expresses mucus and elastase activatable Stx2d, is more frequently associated with HUS than O91 strains with other H types.

Among the 29 STEC strains isolated from Swiss raw milk cheese, genes for toxins of the Stx2 group dominated (Table 1). Of the 27 strains with genes encoding for the Stx2 group, stx2 (11 strains) and stx2v2h-a/b (10 strains) were most frequently detected, and 4 strains harbored a combination of two genes (stx2/stx2g, stx2/stx2v2h-b, and stx2v2h/stx2v2h-b). Furthermore, almost one-third of the isolated STEC harbored hlyA encoding EHEC hemolysin, whereas none tested positive for eae encoding the adhesion factor intimin, which is a virulence trait of classical EHEC (Table 1). In other studies, STEC strains isolated from French raw milk cheese were characterized by the predominance of strains that produced Stx1 or both Stx1 and Stx2 (20, 26). In the study of Pradel et al. (20), stx2v2h-b was the most frequent stx2 variant found in STEC isolated from cheese, whereas stx2d dominated in the study by Vernozy-Rozand et al. (26). Similar to the stx2 subtyping results obtained for STEC
isolated from Swiss raw milk cheese, stx2 and stx2c (stx2vh-a/b) variants have frequently been found in cattle isolates (5, 30).

Overall, three-quarters of the isolated STEC strains, including those of serotypes O22:H8, O91:H10, O91:H21, and O174:H21, harbored Shiga toxin variants associated with STEC strains that have caused hemolytic diarrhea and HUS (Table 1). The production of Stx2 and Stx2c (Stx2vh-a/b) in particular has a high association with severe outcomes in infected patients (2, 19). Furthermore, four STEC strains isolated from two semihard cow’s milk cheeses, a semihard goat’s milk cheese, and a soft goat’s milk cheese harbored stx2g, alone or in combination with stx2. Stx2g is described as an infrequently occurring variant in STEC isolated from cattle (14, 15, 27), and it has also been identified in isolates from cattle wastewater (11). To our knowledge, this is the first report of the isolation of stx2g harboring STEC associated with a species other than cattle. The role of Stx2g as an agent for human disease is not yet clear, but Leung et al. (15) showed that stx2g has high nucleic acid homology with stx sequences associated with human disease, and Stx2g cytotoxicity for HeLa and Vero cells is comparable to that of Stx2-EDL933.

In conclusion, semihard and hard raw milk cheese may be a potential source for transmission of pathogenic STEC to humans. Due to the low minimum infectious dose of STEC required to trigger serious disease and the ability of STEC to survive cheese production and ripening, the role of raw milk cheese consumption in human STEC infection must not be neglected. Although none of the STEC strains isolated from Swiss raw milk cheese belonged to classical EHEC serotypes or possessed intimin, a notable proportion belonged to serotypes or harbored Shiga toxin genes associated with human infections (Table 1), and hence such strains might pose a threat to consumer health. To inactivate STEC and reduce this threat, the efficacy of, e.g., subpasteurization heat treatments of raw milk (recently evaluated for milk used for the production of Cheddar cheese (23)) needs to be determined. Furthermore, the occurrence of STEC strains with common characteristics in cattle and cheese samples underlines the importance of reducing the contamination of raw milk during the milking process. On the other hand, note that certain STEC strains might possess special properties, such as enhanced acid and salt tolerance, enabling them to survive the cheese ripening process; therefore, underlying stress response mechanisms need to be further evaluated.

**ACKNOWLEDGMENTS**

The authors thank the different cantonal laboratories for the collection of the samples.

---

**TABLE 1.** Characterization results of 29 Shiga toxin-producing Escherichia coli strains isolated from 29 raw milk cheeses at the producer level in Switzerland

<table>
<thead>
<tr>
<th>No. of strains</th>
<th>Serotype</th>
<th>stx1</th>
<th>stx2</th>
<th>hlyA</th>
<th>eae</th>
<th>Cheese type</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>O2:H27h,c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>3</td>
<td>O2:H27h,c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O2:H27h,c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Soft</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O8:H20</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O9:H21b</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O15:H16c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O15:H16c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O22:H8h,c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O22:H8h,c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O22:HNHb,c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O86:H21</td>
<td>stx1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O91:H10h,c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>2</td>
<td>O91:H12b,c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O109:H16</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O113:H4c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O116:H28</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Soft</td>
<td>Goat’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O148:H8c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>O174:H21b,c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>2</td>
<td>O174:H21b,c</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>ONT:H9</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Soft</td>
<td>Goat’s milk</td>
</tr>
<tr>
<td>1</td>
<td>OR:H45</td>
<td>stx1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
<tr>
<td>1</td>
<td>OR:HNH</td>
<td>-</td>
<td>stx2</td>
<td>-</td>
<td>-</td>
<td>Semihard</td>
<td>Cow’s milk</td>
</tr>
</tbody>
</table>

---

a. -, negative (gene(s) not found); +, positive (gene(s) detected in strain); HNM, H nonmotile; ONT, O not typeable (O1 to O181 negative); Or, O rough (spontaneously agglutinating); HNT, H not typeable.

b. Serotype previously isolated from humans.

c. Serotype previously isolated from cattle.

d. Toxin type associated with STEC strains that caused hemolytic diarrhea and HUS.

---

**References**

1. O2:H27h,c, O2:H27h,c, O2:H27h,c, O2:H27h,c
2. O8:H20, O9:H21b, O15:H16c, O15:H16c, O22:H8h,c
3. O22:H8h,c, O22:H8h,c, O22:H8h,c, O22:H8h,c
4. O22:HNHb,c, O22:HNHb,c, O22:HNHb,c, O22:HNHb,c
5. O86:H21, O86:H21, O86:H21, O86:H21
6. O91:H10h,c, O91:H10h,c, O91:H10h,c, O91:H10h,c
7. O91:H12b,c, O91:H12b,c, O91:H12b,c, O91:H12b,c
9. O113:H4c, O113:H4c, O113:H4c, O113:H4c
12. O174:H21b,c, O174:H21b,c, O174:H21b,c, O174:H21b,c
13. O174:H21b,c, O174:H21b,c, O174:H21b,c, O174:H21b,c
15. OR:H45, OR:H45, OR:H45, OR:H45
16. OR:HNH, OR:HNH, OR:HNH, OR:HNH
17. ONT:HNH, ONT:HNH, ONT:HNH, ONT:HNH

---

**Downloaded from** http://meridian.allenpress.com/jfp/article-pdf/73/1/88/1678552/0362-028x-73_1_88.pdf by guest on 06 September 2020
REFERENCES

1. Bettelheim, K. A. 2007. The non-O157 shiga-toxicigenic (verocyto-


