Molecular Characterization of Escherichia coli O157 Contamination Routes in a Cattle Slaughterhouse

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ABSTRACT

In a cattle slaughterhouse, sampling was performed over a 1-week period to examine the prevalence and possible contamination routes of Escherichia coli O157. Each sampling day, swab samples were collected from the slaughterhouse environment before onset of slaughter, from the slaughterline, and from 20 successively slaughtered animals. Isolation of E. coli O157 consisted of a 6-hour enrichment followed by immunomagnetic separation and selective plating. From the 394 samples taken, 84 (21%) were positive for E. coli O157. Pulsed-field gel electrophoresis (PFGE) of collected isolates produced 26 different profiles, from which 5 PFGE profiles carried two or more Stx genes. The combination of PFGE profiles and Stx types resulted in 32 different E. coli O157 types. E. coli O157 was found in the slaughterhouse environment before the onset of slaughter. The first two sampling days, feces and carcasses were found negative. On the third sampling day, five fecal samples and four carcasses from animals negative in the feces were positive. Hide of the anal region and the shoulder were found positive every sampling day. The shoulder hide was more than twice as contaminated as the anal region hide. Typing of different isolates from a sample showed that frequently different E. coli O157 types were present. On sampling days 1 and 2, types present in the environment and on the hides of the slaughtered animals differed. On the third sampling day, two dominant types were found in the environment (even before the onset of slaughter), as well as on the hides, feces, and carcasses. Although examined animals originated from different farms, one (two on day 3) dominant E. coli O157 type was present on their hides each sampling day. These data indicated that (i) the progress of contamination can differ from day to day within a slaughterhouse and (ii) contact between animals after the departure from the farm can have a large effect on the spread of E. coli O157 hide contamination.

Since the first outbreak of Escherichia coli O157 in 1982, it is known that healthy ruminants, especially cattle, transiently harbor human pathogenic enterohemorrhagic E. coli (EHEC) O157 in their gastrointestinal tract. EHEC are shiga toxin–producing E. coli with enhanced virulence, allowing them to cause bloody diarrhea in humans and more serious complications such as hemorrhagic colitis and hemolytic uremic syndrome (11). It becomes increasingly clear that direct actions are necessary to improve the microbiological status of cattle going for slaughter, with the aim to reduce the risk of human pathogens entering the food chain (10). Transportation is a potential stressor for farm animals and has been associated with the shedding of pathogenic bacteria such as E. coli O157 and Salmonella spp. The hides and feces of animals presented for slaughter have been shown to be the major sources of pathogens in processing plants (4, 7). During slaughter, microorganisms derived from feces, hides, and the environment can contaminate carcasses (3, 7). It can be expected that at least adherent fecal matter on the hides of animals originating from contaminated farms contain E. coli O157 because this pathogen can survive for several weeks in feces (22). The degree of visible contamination has proved to be correlated with the degree of subsequent contamination of the carcass (15). However, visibly clean hides can have an E. coli O157 prevalence of 10.7% (7), even up to 28.8% (18). On average, 7.2% of the surfaces in the lairages of three cattle slaughterhouses were contaminated with E. coli O157 (20). Nearly 40% of the lairage floor samples were contaminated and consequently can lead to a contamination of the brisket if animals lie in the holding pen.

A recent study (21) revealed that 6.3% of the freshly slaughtered cattle in Belgium carried E. coli O157 in their feces. A national E. coli O157 monitoring program (14) indicated that 1 to 2% of the fresh slaughtered beef carcasses are contaminated with this pathogen. Because of contradictory data in the literature about the origin of such carcass contamination, the present study was initiated to (i) determine the prevalence of E. coli O157 by animals on carcasses and in the slaughter environment and (ii) characterize the isolated E. coli O157 strains by pulsed-field gel electrophoresis (PFGE) technique and shiga-toxin gene typing in order to detect contamination routes.

MATERIALS AND METHODS

Specifications of the cattle processing plant. From the slaughterhouses participating in a previous study (21), one slaughterhouse with a European Union (EU) licence was selected for a more extensive investigation of the occurrence and distribution of E. coli O157 over a 1-week period in May 2002. In the slaughterhouse, a holding pen for 85 animals was available. In the plant,
about 700 animals were slaughtered each week at a maximum slaughter capacity of 30 animals/h. Normal slaughter activity took place from 6:30 a.m. until 2:00 p.m. from Monday until Friday. Conventional slaughtering and processing techniques were used and are described briefly below. The animals were transported to the slaughterhouse on large and small lorries. On arrival at the processing plant, cattle were kept in a holding pen and provided with water. The animals were individually tied. The holding pen had a short race to the stunning box. Stunned animals were hoisted onto the killing line by one hind leg with a shackel for bleeding. After the rump of the animal was rinsed with cold potable water, removal of the hide started by incision of the hide near and around the anus. During the manual pre-dehiding of the hind legs, hooks replaced the shackles. Also at this stage the rectum was released and immediately sealed in a plastic bag. Then the carcasses were further prepared for mechanical dehiding. During this time, process measures, such as the placement of a pair of crocodile clips joined by a plastic cord on the dehided skin of the brisket, were taken to prevent the roll-back of the hide. The hide was pulled downward mechanically. After skinning, the carcasses were eviscerated and split. Before chilling, all carcasses were manually sprayed with potable water (40 to 50°C) for cleaning and cut up (removal of superficial fat tissue) before entering the chilling room.

Each slaughter man had on the work station the possibility to clean and to disinfect (water at 82°C) the knives. At the end of each workday, the holding pen was cleaned by a high-pressure spraying pistol. The equipment on the slaughterline was cleaned daily and disinfected, whereas the work floor was cleaned daily and disinfected every Friday.

**Sample collection.** The first samples were taken on Tuesday after a period of 3 d with no activities in the slaughterhouse because of a prolonged weekend, on Friday of the same week, and a last time on Tuesday of the next week. With the exception of the tonsils (whole organ) and the feces (±100-g portion), all sites were sampled using the swab sampling technique, in which a sterile cotton swab of 0.8–1 g was moistened in 0.1% peptone water, rubbed to an undefined surface of about 400 cm², and placed in a stomacher bag. From large sampling sites (indicated with an asterisk below), four surfaces close to each other were swabbed and pooled. Before the onset of the slaughter activities, the following sites were sampled: five aprons* at five different places on the slaughterline, the stunning box* (one inside and one outside), two shackles, three hooks, the work floor,* three elevators,* and a tray for the bowls.* In the antemortem area of the slaughterhouse, three sites from the race to the stunning box* and five from the holding pen* were swabbed. At the time of sampling, animals ready for slaughtering were presented in this area. Samples from knife blades and a bar, where the manual dehiding started and that came in contact with the hide of the animals, were taken during the first break at 8 a.m. On each sampling day, 20 successive animals were also sampled for the presence of *E. coli O157 at the following sites: the anal region* (skin around the anus, including the anus), skin of the shoulder,* the tonsils, feces, and shoulder* of the carcass ready for chilling.

**Examination of the collected samples.** The collected tonsil samples were examined by adding a volume of nine times (wt/vol) modified tryptone soya broth (Oxoid, Basingstoke, UK) supplemented with 2% novobiocin (mTSBn, Sigma, Bornem, Belgium) (6). A 25-g portion of each feces sample was weighed in a stomacher bag, and 225 ml mTSBn was added. The swab samples were examined by adding 225 or 100 ml mTSBn to bags containing four cotton swabs (1,600 cm²) or one cotton swab (400 cm²), respectively. After addition of the enrichment broth, all bags were homogenized for 1 min in a stomacher blender at normal speed (200 rpm) and incubated for 6 h in warm water (42°C).

Isolation and identification were performed as previously described (21). Briefly, *E. coli O157 was separated by the immunomagnetic separation technique using Dynabeads anti-*E. coli O157 (Dynal, Oslo, Norway) following the manufacturer’s instructions. The final 100-µl volume was streaked onto one sorbitol MacConkey agar (Oxoid) supplemented with 0.05 ml/liter cefixime (Dynal) and 2.5 ml/liter tellurite (Dynal). Plates were incubated for 20 to 22 h at 42°C. A maximum of four sorbitol-negative colonies per agar-cefixime-tellurite plate were selected, purified on plate count agar (Oxoid), and tested for agglutination with a commercial latex antibody test for O157 antigen (Oxoid). Those strains that agglutinated were tested biochemically using an API 20E test strip (bioMérieux, Lyon, France). Each strain was also tested for beta-glucuronidase with a chromogenic diagnostic tablet (Rosco, Taastrup, Denmark).

**Characterization of EHEC virulence factors.** *E. coli O157 isolates were further tested for stx genes by PCR using consensus primer pair-amplifying Stx₁, Stx₂, and its variants (13). The isolates were then further characterized by the primers for Stx₁, stx₂, intimin (eaeA), and the plasmid-encoded enterohemolysin (E- Hly) in separate reactions in order to make sure that competition did not result in false negative reactions for some of the genes (16). For each stx₂-positive isolate, the Stx₂ subunit genes were further subtyped using a polymerase chain reaction–restriction fragment length polymorphism scheme described previously (17).

**Pulsed-field gel electrophoresis.** A loopful of the isolated strains was grown overnight on agar-cefixime-tellurite at 42°C. A single colony was inoculated in 5 ml nutrient broth (Oxoid) and grown overnight aerobically at 37°C, shaking until the optical density at 610 nm was near 1. Optical densities of the cells were adjusted with nutrient broth until 0.8. Centrifuged cells (4,000 rpm, 15 min) were washed in SE buffer (75 mM NaCl, 25 mM EDTA adjusted to pH 7.5) kept for 4 min at 50°C. Inert agarose (Bio-Rad Laboratories, Hercules, Calif.) was prepared in SE buffer to a final concentration of 1.2% and maintained at 50°C. plugs were formed by mixing 0.2 ml of bacterial suspension with 0.2 ml inert agarose, and this mixture was pipetted into plug molds (Bio-Rad). After the plugs solidified, they were transferred to 3 ml of lysis buffer (1 mg of proteinase K per ml of 50 mM Tris, 50 mM EDTA adjusted to pH 8.0) for 16–20 h of incubation in a 50°C water bath. The lysis buffer was removed, and the plugs were washed six times with 3 ml TE buffer (10 mM Tris, 1 mM EDTA adjusted to pH 8.0) for 30 min while shaking. After the final wash, plugs were kept at 4°C in 3 ml TE buffer until restriction. Two 1-mm-thick slices of the plugs were preincubated with 140 µl of 1× XbaI buffer (Boehringer Mannheim Corporation, Indianapolis, Ind.) for 30 min at 37°C. The buffer was then removed and replaced by a fresh mixture containing 40 U of XbaI restriction enzyme (Boehringer Mannheim) in 100 µl of 1× restriction buffer and incubated for 4 h at 37°C. A lambda ladder (New England BioLabs, Hitchin, England) was used as a molecular size marker. Electrophoresis of the prepared samples was performed on the CHEF-mapper (Bio-Rad) system by using pulsed-field–certified agarose (Bio-Rad) with 2 liters of standard 0.5× TBE (44 mM Tris, 20 mM EDTA, 44 mM boric acid) running buffer. The electrophoresis conditions were as follows: auto algorithm; molecular weight, low 200 kb to high 2,000 kb; calibration factor, 1.00; 0.5× TBE; 14°C; 1.0% “PFGE agarose”; gradient, 6 V/cm; run time, 24 h; included angle, 120°; initial switch time, 3 s; final switching time, 50 s; ramping factor,
TABLE 1. Isolation of E. coli O157 in the antemortem area and from the slaughter equipment

<table>
<thead>
<tr>
<th>Date of visit</th>
<th>Antemortem area</th>
<th>Slaughter line</th>
<th>After 1:30 h work (8:00 a.m. break)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corridor</td>
<td>Holding pen</td>
<td>Stunning box</td>
</tr>
<tr>
<td>Tuesday 21 May 2002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3/3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1/5</td>
<td>1/2</td>
</tr>
<tr>
<td>Friday 24 May 2002</td>
<td>1/3</td>
<td>1/5</td>
<td>0/2</td>
</tr>
<tr>
<td>Tuesday 28 May 2002</td>
<td>3/3</td>
<td>4/5</td>
<td>1/2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Two shackles, three hooks, the work floor, three elevators, a tray for viscera.
<sup>b</sup> After prolonged weekend because of a bank holiday.
<sup>c</sup> Number of positive samples/number of examined samples.

The pump speed was set on 70. After electrophoresis, the gels were stained for 15 min in 1 liter of sterile distilled water containing 100 μL of ethidium bromide (10 mg/ml) and destained for 30 min in sterile distilled water. The DNA profiles were visualized on UV transillumination and photographed. Band position differences, but not band intensity differences, were used to allocate PFGE profiles and were numbered.

RESULTS

The isolation of EHEC O157 from the collected samples is reported in Tables 1 and 2.

All isolated strains were positive for the presence of eaeA and the EHEC virulence plasmid. Three (1.5%) of all E. coli O157 strains were Stx negative, one (0.5%) possessed Stx<sub>1</sub> + Stx<sub>2</sub>, 64 (32%) possessed Stx<sub>1</sub> + Stx<sub>2</sub> + Stx<sub>2vh-a</sub>, 8 (4%) possessed Stx<sub>1</sub> + Stx<sub>2vh-a</sub>, 23 (12%) possessed only Stx<sub>2</sub>, 48 (24%) carried Stx<sub>2</sub> + Stx<sub>2vh-a</sub>, and 50 (25%) strains carried Stx<sub>2vh-a</sub>.

PFGE typing of the isolated strains resulted in 26 different PFGE profiles (profiles 1 to 26) (Table 3). The PFGE profiles from 10 strains isolated from samples taken on sampling day 1 are shown in Figure 1. Strains belonging to PFGE profile 1, 9, 21, 23, and 24 carried two to three different Stx genes. Different Stx genes within the same PFGE profile are indicated with a letter in Table 3. The combination of PFGE profiles and Stx types resulted in 32 different E. coli O157 types isolated during the present study.

From the 128 samples taken on the first sampling day, 24 samples (19%) were positive, resulting in 45 E. coli O157 isolates. Although the first sampling day took place after a period of 3 d without slaughter activity, E. coli O157 could be isolated from two aprons and the stunning box before the onset of slaughtering. Also, from the antemortem area, E. coli O157 could be isolated. None of the collected feces, carcasses, or tonsil samples were positive, whereas E. coli O157 was isolated from 4 skin samples taken at the anal region and 12 from the shoulder. Typing of the isolates yielded 14 different PFGE profiles, from which 2 possessed 2 different Stx genes, resulting in 16 different E. coli O157 types. From the environment before, as well as during, slaughtering, nine different types were isolated. Type 12 was found in the stunning box, the race, and the holding pen, whereas type 11 was present on an apron and in the race. None of these types were found on the hides of the animals. From the hide samples, seven types were identified. Type 1c was the predominant type and was found in 3 of the 4 positive samples from the anal region and 10 of the 12 positive samples from the shoulder.

From the 134 samples collected the second sampling day, 13 samples (9.7%) were positive and 39 strains were isolated, resulting in seven different PFGE profiles from which two possessed two different Stx genes. Again, none of the feces, carcass, and tonsil samples were positive for E. coli O157. Only one sample taken at the anal region and eight samples taken from the shoulder were positive for E. coli O157. From the environmental samples, one sample of the holding pen, the race, the knives, and the bar were positive. With the exception of two strains of type 1c isolated from two shoulder samples, all isolate types differed from those found on the first sampling day. PFGE profile 18 and 20 were the predominant types present in positive shoulder samples. As on sampling day 1, types on hide samples were different from those found in other samples.

On the third sampling day, 48 (36%) of the 134 samples were positive, resulting in 113 strains. The environmental samples were highly contaminated with E. coli O157. Four (20%) carcasses, 10 (50%) samples from the anal region, 14 (70%) shoulder samples, 5 (25%) fecal samples, and no (0%) tonsils were contaminated. Only one of four positive carcasses originated from animals previously

TABLE 2. Isolation of E. coli O157–positive samples from cattle on the slaughter line

<table>
<thead>
<tr>
<th>Date of visit</th>
<th>No. of cattle examined</th>
<th>Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anus</td>
</tr>
<tr>
<td>21 May 2002</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>24 May 2002</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>28 May 2002</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>
found positive on the hide, and none of them were found E. coli O157–positive in the feces. Typing of the isolates yielded nine different PFGE profiles from which three possessed 2 different Stx genes, resulting in 12 different E. coli O157 types. Environmental samples, animals, and carcasses contained 4, 11, and 3 types, respectively. Type 22 was the predominant E. coli O157 type present in animal samples and carcasses. This type could also be isolated from all other sampling sites, except the bar where manual dehiding started. Type 24b was isolated from animal samples, carcasses, the antemortem area, and the stunning box. Type 1b was isolated at a low rate from the animals and the environment, but not from carcasses.

**Table 3.** E. coli O157 types based on PFGE profiles and present Stx genes isolated from samples collected at the slaughterhouse over three sampling days

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Tuesday</th>
<th>Friday</th>
<th>Tuesday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before slaughter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aprons</td>
<td>10a(1)/11(1)</td>
<td>—</td>
<td>1b(2)/22(1)</td>
</tr>
<tr>
<td>Stunning box</td>
<td>12(1)</td>
<td>—</td>
<td>22(1)/24b(1)</td>
</tr>
<tr>
<td>Race to the stunning box</td>
<td>10b(3)/11(1)/12(1)/16(1)</td>
<td>21a(1)</td>
<td>1b(1)/22(2)/24b(1)</td>
</tr>
<tr>
<td>Holding pen</td>
<td>9(1)/12(3)/13(1)</td>
<td>20(1)/21a(1)/22(1)</td>
<td>1a(1)/1b(1)/22(2)/24b(1)</td>
</tr>
<tr>
<td>Animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anus</td>
<td>1c(3)/2(1)</td>
<td>17(1)</td>
<td>1b(2)/22(9)/23b(2)/24b(1)</td>
</tr>
<tr>
<td>Shoulder</td>
<td>1b(1)/1c(10)/3(1)/6(1)/7(1)/8(1)/9(1)</td>
<td>1c(2)/18(5)/19(1)/20(4)</td>
<td>1a(1)/1b(2)/5(1)/21a(1)/21b(1)/22(8)/23b(1)/24b(3)</td>
</tr>
<tr>
<td>Tonsils</td>
<td>—</td>
<td>—</td>
<td>1b(1)/4(1)/21b(1)/22(1)/24b(4)/25(1)/26(1)</td>
</tr>
<tr>
<td>Feces</td>
<td>—</td>
<td>—</td>
<td>22(3)/24a(1)/24b(2)</td>
</tr>
<tr>
<td>Carcass</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>After 1.30 h work (break, 8 a.m.)</td>
<td>10b(1)/14(1)/15(1)</td>
<td>1b(1)</td>
<td>23a(1)</td>
</tr>
<tr>
<td>Bar at first incision</td>
<td>—</td>
<td>1b(1)</td>
<td>22(1)</td>
</tr>
<tr>
<td>Knife used for first incision</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Other knives</td>
<td>—</td>
<td>—</td>
<td>1b(1)/22(2)</td>
</tr>
</tbody>
</table>

*a* Number, PFGE profile; letter, one PFGE profile containing different Stx genes; ( ), number of samples containing that type of E. coli O157.

*b* —, no strains isolated.

**DISCUSSION**

In the present study, the prevalence of E. coli O157 in a cattle slaughterhouse was monitored during 3 d within a 1-week period. Samples were collected from the antemortem area and the slaughterline before the onset of slaughter. During slaughter, samples were taken from the hide of the anal region and the shoulder, tonsils, feces, and carcasses of 20 successively slaughtered animals, as well as from knives. From isolated E. coli O157, Stx genes were typed and PFGE profiling was carried out. This typing should allow the study of contamination routes in the slaughterhouse.

**FIGURE 1.** PFGE patterns of E. coli O157 isolated on the first sampling day. 1 and 2, shoulders of two cattle; 3, bar at first incision; 4 and 5, aprons; 6–10, race to the stunning box; L, lambda ladder. Selected marker sizes (kilobases, Biolabs, New England) are indicated to the right. Lane 1, profile 8; lane 2, profile 9; lanes 3, 5–7, and 10, profile 10; lanes 4 and 9, profile 11; lane 8, profile 16.
Examination of the slaughterhouse environment before the onset of slaughter showed that the slaughterline was free of \textit{E. coli} O157 contamination with the exception of the stunning box and some aprons on two sampling days. This finding indicated that the applied sanitation procedure was inefficient in eliminating \textit{E. coli} O157 contamination from the slaughterline environment and can, as shown in other studies \cite{1, 9}, survive several days on such surfaces. The antemortem area was found to be contaminated at a high level. Contaminated animals presented in the area at sampling time could have been the source of this contamination, as could \textit{E. coli} O157 surviving routine cleaning \cite{9, 20}.

Only feces from five slaughtered animals on sampling day 3 were found positive. In contrast, each sampling day, hides were found positive and the contamination rate was higher on the shoulder than the anal region. The present shoulder contamination is much higher than those previously reported for the brisket hide area \cite{22.2\% \cite{18} and 32.9\% \cite{2}}. However, suggesting that the shoulder hide is more contaminated than the brisket hide is not directly possible since sampling of the brisket hide was not involved in the present study.

Contamination of hides did not always lead to contamination of the resultant carcasses: only on the third sampling day were four carcasses found contaminated. However it can be assumed that the contamination rate of the carcasses could be higher because the results of only one carcass area was available (because of the limited opportunity to sample different areas of the carcasses on the slaughterline). Because the anal area frequently was found to be contaminated, contamination of the inside leg area during manual dehiding can be expected. Such contamination can also occur on all other parts of carcasses that are manually dehided. As a consequence, the applied slaughter technique and hygiene will have a large influence on the transfer of \textit{E. coli} O157 from the hide to the carcass. On the other hand, the quantitative aspect of hide contamination also will influence carcass contamination. However, no data on the latter are available in the literature. Direct or indirect fecal contamination, as mentioned in another study \cite{5}, did not seem to occur in this slaughterhouse since the first contaminated carcass (animal 4) was detected five carcasses before the first animal (animal 9) with a positive fecal culture and the next positive carcasses were found two carcasses following the last animal carrying \textit{E. coli} O157 in the feces.

Typing of strains demonstrated that a large number of types was presented in the slaughterhouse. Each sampling day, most isolates belonged to different \textit{E. coli} O157 types, suggesting that a type was not predominant for a long period in the slaughterhouse environment. Moreover, typing of different strains from the same sample showed that different types of samples contained two and even three different \textit{E. coli} O157 types. These data indicated that an accurate study of the epidemiology or the transmission routes of \textit{E. coli} O157 needs the typing of different isolates from one sample. Typing data of strains isolated on the first two sampling days showed that strains present in the antemortem area differed from those presented on the hides, indicating that the antemortem area is not an important source for contamination of the examined regions of the animal hide. Other studies \cite{2, 20} suggested that floor contamination of this area contributed to contamination of some parts of the animal hide, such as the brisket hide, while the animals are lying down. On each of the 2 d, dominant types (day 1: 1c; day 2: 19 and 21) were found on the hide of different animals. Direct hide-to-hide contamination between the departure from the farm and the slaughter seemed to be the most probable transmission route because (i) none of the examined animals carried \textit{E. coli} O157 in their feces; (ii) the animals originated from different, unlinked farms, which are mostly contaminated with distinguishable PFGE profiles \cite{8, 12, 19}, although indistinguishable profiles are occasionally found \cite{19} if \textit{E. coli} O157 are present; and (iii) the antemortem area was contaminated with different types. Hide-to-hide contamination could be possible since some animals were transported with the same lorry. At the slaughterhouse, further spreading of this contamination was possible because all animals were kept in the same holding pen. However, contact between the animals was reduced because they were bound from the arrival up to the time of slaughter. Nevertheless, multiple contacts between the animals were still possible.

Results for the third sampling day showed that two types (22 and 24b) were predominant. In contrast to the other sampling days, these types could be isolated from nearly all sampling sites, even for the slaughterhouse environment before slaughter started. In the latter case, it is supposed that environment-to-hide contamination also cannot be excluded. On the other hand, typing also revealed that type 24b, which was dominant in the feces, was less abundant on the hides. Even from the five animals positive in the feces, only two and one animal harbored the same type on the anal area and shoulder hide, respectively, as in their feces. This finding indicates that feces-to-hide contamination can be considered a minor transmission route.

Sampling was carried out during a week in May 2002. A previous study \cite{21} showed that, as in other countries, a seasonal variation in the shedding of \textit{E. coli} O157 with a peak in the late summer takes place. Consequently, it can be estimated that the observed contamination rates during the present study will be higher; and a shift in transmission routes is possible if the study was performed in the summer months.

The present study has shown that \textit{E. coli} O157 can be largely present in cattle slaughterhouse, especially on the hide of the animals. Hide-to-hide contamination after departure from the farm seemed to be an major transmission route. However hide contamination did not lead to a high contamination rate of the carcasses. Future research has to confirm the present findings and should included more sampling sites. If possible, quantitative aspects have to be explored to obtain more accurate data about the presence of \textit{E. coli} O157 on different parts of the beef carcasses and about possible transmission routes of contamination in cattle slaughterhouses.
REFERENCES


