Abstract Feecal samples from 630 slaughtered healthy pigs were examined with the aim: (i) to monitor the shedding of non-O157 STEC and \textit{E. coli} O157; (ii) to further characterize isolated strains; and (iii) to discuss the results obtained with their relevance to food safety. The percentage of the 630 samples testing positive for STEC by polymerase chain reaction was 22.2%. The 32 isolated \textit{stx} positive strains (31 sorbitol-positive) belonged to non-O157 STEC and comprised ten serotypes. \textit{Stx1}, \textit{stx2}, and both toxin genes were detected in 3\%, 97\%, and 0\% of strains. Among \textit{stx2}-positive strains, 29 were positive for \textit{stx2e}, one for \textit{stx2/stx2e}, and one for \textit{stx2c/stx2e}. The percentage of the 630 samples testing positive for \textit{E. coli} O157 by polymerase chain reaction was 7.5\%. The 31 isolated \textit{rfbE} PCR positive strains tested negative for \textit{stx} genes. However, four strains harbored \textit{eae} and were classified as EPEC.

Introduction The importance of the Shiga toxin (Stx) -producing \textit{Escherichia coli} (STEC) group has increased since a food-borne infection caused by STEC has first been reported in 1983 (Riley \textit{et al}, 1983). Pathogenicity of STEC in humans is associated with diverse virulence factors. The main factors are the ability to form cytotoxic exotoxins (shiga toxins), which can be subdivided into a shiga toxin 1 group (\textit{Stx1} group) and a shiga toxin 2 group (\textit{Stx2} group), as well as the property of producing attachment-effacement lesions and the presence of an enterohaemolysin gene (EHEC-\textit{hlyA}) (Boerlin \textit{et al}, 1999).

The source of STEC food-borne infection was often found to be foods of bovine origin or other fecally cross-contaminated foods. Cattle are currently considered the main reservoir of STEC pathogenic to humans. The results of a Swiss study of faeces of healthy beef cattle at slaughter showed a STEC carriage rate ranging from 2.3\% to 23.7\% (Stephan \textit{et al}, 2000).

In addition, there are some reports on the isolation of \textit{Stx} positive \textit{E. coli} from pigs, but often strains originating from pigs with diarrhoea or oedema disease were tested (Moon \textit{et al}, 1999; Osek 1999). Although \textit{E. coli} of the serogroups O138, O139 and O141 producing the \textit{Stx2e} variant can cause oedema disease in pigs, they seem not to be able to colonized the human intestinal tract. However, since strains harbouring \textit{stx2e} have also been isolated from patients with diarrhea and HUS (Piérard \textit{et al}, 1991, Muniesa \textit{et al}, 2000) and a high degree of genetic relatedness between O101 strains harbouring \textit{stx2e} genes of human and porcine origin was demonstrated (Franke \textit{et al}, 1995), the role of pigs as asymptomatic carriers of STEC in the epidemiology of human disease needs further research. Rios \textit{et al} (1999) found 69\% of healthy pigs positive for STEC and isolated enterohemorrhagic STEC serogroups O26 and O111 from pig intestinal contents. The strains harboured virulence genes with profiles indicative of potential human pathogens, suggesting that pigs may be an animal reservoir for these organisms. This hypothesis is supported by the results of a study conducted in the United States as part of the National Animal Health Monitoring System’s Swine 2000 study that found a STEC prevalence of 70\%. Of the isolated strains 13.2\%, 6.4\% and 80.4\% were positive for \textit{stx1}, \textit{stx2} and \textit{stx2e} genes (Fratamico \textit{et al}, 2004). Additionally, O157:H7 has been isolated from slaughtered pigs during investigations carried out in the Netherlands (Heuvelink \textit{et al}, 1999), in Japan (Nakazawa \textit{et al}, 1999), in UK (Paiba \textit{et al}, 2000) and in the United States (Feder \textit{et al}, 2003) with a prevalence of positive fecal samples of 0.7\%, 1.4\%, 0.3\% and 2.0\%, respectively. The aim of this study was to investigate the prevalence of STEC in healthy pigs at slaughter in Switzerland and to further characterize such strains by subtyping the toxins and by determination of additional virulence factors.

Materials and Methods This study was based on investigations that were carried out within half a year (October 2004–April 2005) in an EU-approved slaughterhouse in Switzerland. In total, 630 pig fecal samples of animals at slaughter were collected during 22 sampling days.
For the *E. coli* O157 assay, 1 ml of the enrichment sample was examined by immunomagnetic separation technique (IMS) using Dynabeads® anti-*E. coli* O157 (Dynal Biotech ASA, Oslo, Norway). The immunomagnetic-separated material (bacteria beads complex) was streaked onto sheep blood agar (Difco Laboratories, Becton Dickinson; 5% sheep blood, Oxoid Ltd., Hampshire, UK), and after incubation at 37°C for another 24 h, colonies were washed off with 2 ml of 0.85% saline solution. Of each plate eluate, 2 μl were then evaluated by a polymerase chain reaction (PCR) with primers based on sequences targeting the rfbE gene (Abdulmawjood et al., 2002).

Moreover, the 630 samples were analyzed by PCR for stx genes. The enrichment samples (BGB) were streaked onto sheep blood agar, and after incubation at 37°C for another 24 h, colonies were washed off with 2 ml of 0.85% saline solution. For the STEC assay, 2 μl of each plate eluate was then evaluated by PCR with primers based on sequences targeting a region conserved between stx1 and stx2 genes (Burnens et al., 1995) and PCR conditions described previously (Zweifel et al., 2004).

Strains were isolated by colony hybridization and one isolate per sample was further characterized. Determination of O and H antigens was performed by the method described by Guinée et al. (1981) with all available O (O1 to O181) and H (H1 to H56) antisera. Antisera were obtained and absorbed with corresponding cross-reaction antigens to remove nonspecific agglutinins. O antisera were produced in the Laboratorio de Referencia E. coli (LREC), and H antisera were obtained from the Statens Seruminstitut (Copenhagen, Denmark). For the genotypic characterization, all isolated strains were examined by PCR for the presence of stx1 and stx2 genes under the conditions described by Rüssmann et al. (1995) and Piérard et al. (1998). stx2e under the conditions of Rüssmann et al. (1995), stx1 encoding for intimin under the conditions of Schmidt et al. (1994), and ehxA encoding for enterohemolysin under the conditions of Schmidt et al. (1995).

**Results and Discussion**

The proportion of positive samples was 22.2% for stx and 7.5% for rfbE. The 32 isolated stx-positive strains (31 sorbitol-positive) belonged to non-O157 STEC and comprised ten serotypes (O8:H9; O9:H-; O65:H-; O100:H-; O103:H2; O141:H17; O159:H-; O176:H1; O179:H10; O181:H19), three of them (O9:H-; O100:H-; O176:H-; O179:H10) accounting for 69% of strains. Stx1, stx2, and both toxins were detected in 3%, 97%, and 0% of strains. Among stx2 positive strains, 29 were positive for stx2e, one for stx2/stx2e, and one for stx2c/stx2e. One strain of serotype O141 harboring stx2e contained a virulence pattern typically associated with diarrhea or oedemic disease in pigs. Among the Stx2e producers no O101 serotype could be found.

Moreover, among the 31 isolated *E. coli* O157 strains, 30 were positive for sorbitol fermentation, all were negative for stx, and four strains were positive for eae.

Most outbreaks and sporadic cases of hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) have been attributed to strains of the enterohemorrhagic serotype O157:H7 STEC, but especially in continental Europe, the importance of non-O157 STEC strains as e.g. O26:H11/H, O103:H2, O111:H1, O113:H21, and O145:H- as causes of HC, HUS and other gastrointestinal diseases is being increasingly recognized. Pathogenicity of STEC in humans is associated with diverse virulence factors. The main factors are the ability to form exotoxins (Shiga toxins), which can be subdivided into a Shiga toxin 1 group (Stx1) and a Shiga toxin 2 group (Stx2). Apart from the capability to produce Shiga toxins, these pathogroups may possess accessory virulence factors associated with the capacity to colonize the gut, such as intimin and a 90 kbp virulence-associated plasmid. Intimin mediates the intimate bacterial attachment to the host cell surface of EPEC and STEC, and is required for the formation of the characteristic A/E lesions. O157 STEC and non-O157 STEC strains isolated from patients with severe symptoms frequently show a typical virulence spectrum, with such strains tending to be stx2- and eae-positive (Boerlin et al., 1999).

In our examinations, none of the isolated strains showed these typical virulence patterns.

The finding of a high number of sorbitol-positive stx negative O157 strains with other H type than H7 was striking. However, these data agree with the results in one of our recent studies, where in minced meat samples (minced pork) taken throughout Switzerland we found O157:H38, O157:Hru, O157:H2 and O157:Hnt strains, and none of these strains was positive for stx genes, too (Fantelli et al., 2001). Interestingly, four strains harbouredeae, and therefore seem to be enteropathogenic *E. coli* (EPEC) strains. Whereas for typical EPEC, humans are the only reservoir; for atypical EPEC, both animals and humans can be reservoirs. Atypical EPEC are more closely related to STEC, and like STEC these strains appear to be emerging pathogens. To our knowledge, the present study is the first, that document the detection of the eae gene in porcine O157 E. coli strains.
Conclusions Based on the results of this study the following situation in Switzerland can be noted: STEC were found in healthy pigs with a remarkable prevalence; most of the isolates harvested stx2e. Moreover, the fact is emphasized that E. coli with the O157 antigen are not always STEC, but can also be EPEC. The fecal carriage of foodborne pathogens among livestock animals at slaughter is strongly correlated with the hazard of carcasses contamination. In order to reduce the risk represented by STEC and EPEC, the maintenance of slaughter hygiene is consequently of central importance in meat production.

References