Zeitschrift: Mitteilungen aus Lebensmitteluntersuchungen und Hygiene = Travaux de chimie alimentaire et d'hygiène

Herausgeber: Bundesamt für Gesundheit

Band: 97 (2006)

Heft: 1

Artikel: Prevalence and characteristics of shigatoxin-producing E. coli (STEC) in fecal samples from farm animals at slaughter in Switzerland - a review

Autor: Stephan, R.

DOI: https://doi.org/10.5169/seals-982017

Prevalence and characteristics of Shigatoxin-producing *E. coli* (STEC) in fecal samples from farm animals at slaughter in Switzerland – a review*

R. Stephan, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, CH-8057 Zurich, Switzerland

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are among the most important causes of food-borne diseases. STEC are responsible for a number of human gastrointestinal diseases, including watery or bloody diarrhea, and hemorrhagic colitis (HC). In a proportion of individuals, commonly children, these symptoms may be complicated by neurological and renal sequelae, including hemolytic-uremic syndrome (HUS) (1). The majority of human infections is correlated with the consumption of fecally contaminated food, particularly undercooked ground beef and unpasteurized cow's milk, but transmission by animal to person and person to person contact has also been reported (2).

Most outbreaks and sporadic cases of HC and HUS have been attributed to strains of serotype O157:H7 STEC. However, especially in continental Europe, the importance of non-O157 STEC, e.g. O26:H11/H-, O91:H21/H-, O103:H2, O111:H-, O113:H21, O121:H19, O128:H2/H-, and O145:H28/H-, as causes of HUS, HC, and other gastrointestinal diseases is being increasingly recognized (3). Non-O157 STEC-associated diseases have probably been underreported, as historically many laboratories have screened only for serogroup O157.

The common feature and main virulence factor of STEC is the production of Shiga toxin 1 (Stx1) and/or Shiga toxin 2 (Stx2) or its variants. Pathogenic STEC strains usually contain other virulence factors such as the outer membrane protein intimin, a protein essential for the intimate attachment and the formation of attaching and effacing (A/E) lesions on gastrointestinal epithelial cells and/or enterohemolysin (1). However, it is not completely clear what combination of virulence

*Lecture presented at the 38th Symposium of the Swiss Society of Food Hygiene, Zurich, 16. September 2005

attributes of STEC is linked to pathogenicity in humans (4). STEC have been isolated from a variety of domestic animals including pigs, poultry, cats, dogs and horses, and wild animals including deer, raccoons, flies and birds (5, 6, 7). However, the bulk of data suggests that prevalence of STEC is greater in ruminants than in other animals and domestic ruminants, especially cattle, represent one of the largest reservoirs of STEC pathogenic to humans.

This review provides data on the prevalence of O157 and non-O157 STEC in cattle, sheep and pigs at slaughter in Switzerland. Moreover, characterization results for isolated O157 and non-O157 strains are summarized.

Prevalence and characteristics of O157 and non-O157 STEC in fecal samples from cattle, sheep and finishing pigs at slaughter

2.1 Cattle

2.1.1 O157 STEC

In a recent study 1.4 % of 2930 fecal samples taken from cattle at slaughter were found to be *E. coli* O157 (*rfb*E)-positive (8). Thirty-seven strains from different animals agglutinating with Wellcolex *E. coli* O157 were isolated. Thirty-one strains tested positive and six strains tested negative for sorbitol fermentation, respectively. All sorbitol negative strains, three strains isolated from calves, two strains isolated from cattle and one strain isolated from a cow, harboured *stx* genes and belonged to the serotypes O157:H- and O157:H7. Of these strains, 5 tested positive for only the *stx*2 genes and one strain for both the *stx*1 and *stx*2 genes. Further characterization of the *stx*2 variants showed three *stx*2-positive strains, and three *stx*2c-positive strains. All strains harboured *eae* type gamma1 and *ehxA* genes.

The isolated sorbitol-positive strains tested negative for *stx* genes and belonged to the serotypes O157:H2, O157:H7, O157:H12, O157:H19, O157:H25, O157:H27, O157:H38, O157:H43, O157:H45 and O157:H-. Serotypes O157:H38 and O157:H45 were most frequently found (20 of 31 strains).

This finding agrees with the assumed low prevalence of O157 STEC in cattle in other European countries, e.g. Germany, Norway and Poland. However, in Europe depending on the country, there are great ranges of O157 STEC prevalence in cattle from 0.2 % to 17.0 %. In United Kingdom, Italy, Spain and the Netherlands prevalence >10 % were found (9, 10, 11). A strong effect of age for high shedding prevalence as well as a seasonality as described in other surveys (12), could not be found in the study done by *Al Saigh et al.* (8).

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 of another study, where in minced beef meat samples 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 (13).

2.1.2 non-O157 STEC

In Switzerland, examinations of cattle (n=544) at slaughter showed a non-O157 STEC prevalence of 14% (14). An age influence on prevalence was evident with younger animals more likely to be STEC positive. Recently, *Kuhnert et al.* (15) detected with a RT-PCR system STEC in 58% of fecal samples taken from organically and conventionally farmed dairy cattle.

In a further study, a total of 42 Shiga toxin-producing (STEC) strains from slaughtered healthy cattle in Switzerland were characterized by phenotypic and genotypic traits (16). The 42 sorbitol-positive, non-O157 STEC strains belonged to 26 O:H serotypes (including eight new serotypes) with four serotypes (O103:H2, O113:H4, O116:H-, ONT:H-) accounting for 38.1% of strains. Out of 16 serotypes previously found in human STEC (71% of strains), nine serotypes (38% of strains) were serotypes that have been associated with hemolytic-uremic syndrome (HUS). Polymerase chain reaction analysis showed that 18 (43%) strains carried the *stx*1 gene, 20 strains (48%) had the *stx*2 gene, and four (9%) strains had both *stx*1 and *stx*2 genes. Of strains encoding for *stx*2 variants, 63% were positive for *stx*2 subtype. Enterohemolysin *(ehxA)* and intimin *(eae)* were detected in 17% and 21%, respectively. Amongst the seven intimin-positive strains, one possessed intimin type beta1 (O5:H-), one intimin gamma1 (O145:H), one intimin gamma2, (O111:H21), and four intimin epsilon (O103:H2). The strains belonged to 29 serovirotypes (association between serotypes and virulence factors). O103:H2 *stx*1 *eae*-epsilon *ehxA*, O116:H- *stx*2, and ONT:H- *stx*2c were the most common accounting for 29% of the strains.

Although six of the seven *eae*-positive non-O157 STEC strains belonged to serotypes previously reported in association with HUS, considering the typical virulence spectrum of pathogen strains *(stx*2- and *eae*-positive), only one strain (2.4%) of serotype O145:H- harboring simultaneously *stx*1, *stx*2, *eae*-gamma1, and *ehxA* showed a pattern characteristic of potentially virulent strain.

Although cattle may harbor many different STEC serotypes in their gastrointestinal tracts, only a restricted number of serotypes (O20:H19; O22:H8; O26:H11; O45:H-; O77:H41; O82:H8; O103:H2/H-; O105:H18, O113:H4/H21; O116:H21; O153:H25; O156:H; O157:H7; O171:H2; O172:H21; O174:H21/H2/H-, O177:H11/H-) have been most commonly found (17). The predominant bovine STEC serotype in the majority of surveys realized in Europe was O113:H21, in America and Australia O26:H11, and in Japan O45:H8/H- and O145:H-. In our study, the serotypes O103:H2, O113:H4, O116:H-, and ONT:H- dominated.

2.2 Sheep

2.2.1 O157 STEC

From October 2004 to June 2005, fecal samples from 630 slaughtered sheep were examined by immunomagnetic separation technique and PCR to assess the preva-

lence of *E. coli* O157 (*rfb*E) (18). Seven samples (1.1 %), distributed throughout the whole examination period, were found to be positive. To assess the potential pathogenicity for humans, *E. coli* O157 strains were isolated by colony hybridization and further characterized. The isolated strains fermented sorbitol, showed four different H types (H7, H12, H38, H48), and were all negative for *stx*. One O157:H7 strain harbored the gene for intimin (*eae*) in combination with *ehxA*. In consequence, the potential health hazard from sheep meat related to O157 STEC seems actually not to be of particular importance in Switzerland.

This finding agrees with the assumed low prevalence of O157 STEC in sheep in other European countries, e.g. Spain, Norway and United Kingdom. There are ranges of O157 STEC (30) prevalence in sheep from 0.4 % (Spain) to 6.5 % (United Kingdom) (19, 20, 21).

2.2.2 non-O157 STEC

In Switzerland, examinations of sheep at slaughter and sheep carcasses showed a non-O157 STEC prevalence of 29.9 % and 36.6 %, respectively (22, 23). These results are consistent with the increased prevalence of STEC of small ruminants compared with those of cattle (24, 25).

Sixty ovine STEC strains were examined with the aim (i) to serotype the strains, (ii) to characterize virulence factors, and (iii) to discuss possible associations between virulence factors and to assess the potential pathogenicity of these strains for humans (26). The 60 sorbitol-positive, non-O157 STEC strains belonged to 19 O:H serotypes, whereas 68 % were of five serotypes (O87:H16, O91:H-, O103:H2, O128:H2, O176:H4). 52 % belonged to serotypes reported in association with HUS. Five serotypes were not previously reported in sheep strains. Of the 47 strains encoding for *stx*1 variants, 57 % were *stx*1c- and of the 45 encoding for *stx*2 variants, 80 % were *stx*2d-positive. 82 % of the strains showed further virulence factors: 13 % were *eae*- and 60 % *ehxA*. Four strains of serotype O103:H2 and O121:H10 harboring *stx*2, *eae* and *ehxA* showed virulence factors typical for strains associated with severe human disease. However, according to the virulence factors, the majority of the ovine non-O157 STEC strains are assumed low-virulence variants.

2.3 Finishing pigs

2.3.1 O157 STEC

In a recent study fecal samples from 630 slaughtered finisher pigs were examined by polymerase chain reaction to assess the shedding of *E. coli* O157 (*rfb*E) (27). The examinations yielded 7.5 % *E. coli* O157-positive animals.

The *E. coli* O157 prevalence in healthy pigs at slaughter ranged from 0.3 % to 11 % in different countries (28, 29, 30, 31). Our results for pigs shedding *E. coli* O157 at slaughter (7.5 %) are comparable with data from the US (5.9 %) and from Belgium (11 %) (29).

Among *E. coli* O157 strains isolated, the great majority was sorbitol positive (30/31), a high number with other H type than H7 was found, and none harbored *stx* genes. These data agree with the results of a recent study, where in minced pork samples 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 (13). Furthermore, in the present study, genes for intimin (3/31) and enterohemolysin (1/31) were only found infrequently. Non-Shiga toxin-producing *E. coli* O157:non-H7 isolates from pigs that were often lacking other virulence factors as *eae* or *ehxA* have been also reported in other studies, and such strains were therefore considered as an unlikely source of human disease (31).

In contrast to our results, O157 STEC have been isolated from slaughtered healthy pigs in investigations carried out in different countries and the detection rate ranged from 0.1% to 2.0% (19, 30). Interestingly, in a Swedish study, the same strains were present in pigs and ruminants indicating that keeping pigs and ruminants together on farms appears to be a risk factor for establishing O157 STEC (30). Overall, only few healthy pigs appear to harbor potentially pathogenic O157 STEC.

2.3.2 non-O157 STEC

In the above mentioned study, the fecal samples of the 630 slaughtered finisher pigs were also examined by polymerase chain reaction to assess the shedding of Shiga toxin-producing *E. coli* (STEC, *stx)* (27). 22% of the samples were found to be positive for *stx* genes.

Depending on country, author and method applied for detection, STEC shedding rate in pigs at slaughter varies widely and ranges from 2.1% to 70% (29, 32). Our results (22%) are comparable with data from Belgium with 24% STEC-positive colon contents at the slaughterhouse and 32% STEC-positive rectal swabs at the farm (29).

Of the 45 isolated STEC strains (27), 43 were positive for sorbitol fermentation. Thirty-two strains were typed into ten *E. coli* O groups (O2, O8, O9, O26, O65, O100, O103, O141, O159, O180), whereas 13 strains were non-typeable (ONT). Seventeen strains were typed into eight different H types (H2, H4, H9, H10, H17, H19, H21, H32), whereas 28 were non-motile (H-). Among the 32 typeable strains, 11 different O:H serotypes were found: O2:H32, O8:H4, O8:H9, O9:H-, O26:H-, O65:H-, O100:H-, O103:H2, O141:H17, O159:H-, O180:H21. Three of them (O8:H9; O9:H-, O100:H-) accounted for 69% of typeable and 49% of all STEC strains.

Serotypes O8:H9, O9:H-, and O100:H- have also been frequently identified in STEC from pigs in other studies (32). Interestingly, none of the strains isolated in our study belonged to serogroup O101, where a high degree of genetic relatedness among human and porcine strains was demonstrated (33). In literature, a considerable number of STEC serogroups have been isolated from healthy pigs, including O2, O5, O7, O8, O9, O11, O15, O20, O26, O55, O57, O65, O68, O69, O78, O86,

O91, O96, O100, O101, O111, O114, O119, O120, O121, O126, O127, O128, O142, O152, O157, O158, O159, O160, and O163, whereas a second class of STEC mainly belonging to O138, O139, O141, and O149 and producing *stx*2e appears to be predominantly a pathogen of pigs (32). Although fecal samples tested in the study of *Kaufmann et al.* (27) were obtained from apparently healthy pigs, one O141 strain positive for *stx*2e and showing α-hemolysis contained a virulence pattern typically associated with postweaning diarrhea or edema disease in pigs.

In this study, a large proportion of swine STEC harbored *stx*2e, a variant not detected in strains from other animal hosts. The majority of these *stx*2e-positive strains were lacking further virulence factors or only the *astA* gene (heat stable enterotoxin) was found. The *Stx*2e variant was previously often isolated from healthy pigs, as well as from pigs suffering from postweaning diarrhea and edema disease (32). Although some authors have reported the presence of *stx*2e in STEC strains from patients on a few occasions, swine STEC harboring *stx*2e are more likely to cause disease in pigs. In agreement to the results of *Kaufmann et al.* (2005), *stx*2e-positive STEC, in which *eae* or *ehxA* were lacking or only present in low proportion, were also detected in other studies (29).

In consequence, in the study of *Kaufmann et al.* (27) only one (2 %) O103:H2 STEC strain carried a combination of virulence factors *(stx*1, *eae-e, ehxA)* indicative of potential human pathogenic characteristics, although four serotypes were previously described as human STEC (including three serotypes reported in association with HUS). However, even this strain was lacking the *stx*2 (subtype) that is frequently found in STEC causing severe human disease. Contrary to most other studies, (i) STEC strains isolated from pigs in Chile often harbored *stx*1and/or *stx*2, *eae* and *ehxA* suggesting pigs to be an important reservoir for potentially pathogenic strains (34), and (ii) a considerable percentage of STEC isolated from pigs in the US harbored *stx*1 (13.2 %) or *stx*2 (6.4 %) other than *stx*2e (35).

Conclusions

Based on these results the following situation for Switzerland can be noted: (i) Shigatoxin-producing *E. coli* O157 are isolated in low prevalence from livestock animals (only cattle), (ii) non-O157 STEC, however, were found in cattle, sheep and pigs in a remarkable prevalence.

To assess the pathogenicity of STEC for humans, evaluation of serotypes and associated virulence factors is required. O157 and non-O157 STEC isolated from patients with severe symptoms such as bloody diarrhea, HC and HUS frequently show a typical spectrum of virulence factors: numerous authors have underlined the potential key role of the *stx*2 subtype and eae in the severity of disease. The impact of *ehxA* is controversially discussed (36).

Further characterization of virulence factors of isolated cattle, sheep and pig strains in Switzerland showed that the majority of STEC strains lacked combinations correlated with severe human disease. Nevertheless, as long as the contribu-

tion and interaction of virulence factors in especially milder disease remains unclear, the possibility that certain non-O157 STEC may represent a potential source of human infection cannot be neglected.

STEC carriers represent a source of carcass contamination as the fecal carriage of food-borne pathogens among livestock animals is strongly correlated with the hazard of carcasses contamination at slaughter. In order to reduce the risk represented by zoonotic agents to the consumer health, it is essential to reduce contamination of carcasses during the slaughtering processes. Therefore, the maintenance of slaughter hygiene, which can be measured in daily practice by "in-process-controls" and regular microbiological monitoring of carcasses, is consequently of central importance in meat production. Moreover, preventive measures, such as implementation of codes of good manufacturing practices, increased care during hiding and evisceration as well as alterations of the veterinary meat inspection practices, should be encouraged.

Summary

The importance of latent zoonoses has increased in recent years in view of food borne diseases: (i) the "healthy" animal represents a reservoir for specific pathogens; (ii) no pathological-anatomical changes in the carcass and its organs show the presence of these pathogens; and (iii) these pathogens may enter the food chain via weak points in the slaughtering or milking process.

Shiga toxin-producing *Escherichia coli* (STEC) are among the most important causes of food-borne diseases. The STEC group represents one of at least six different categories of diarrheagenic *E. coli* recognized at present. STEC are characterized by their ability to elaborate potent phage-encoded cytotoxins called Shiga toxins. Pathogenic STEC often possess other putative virulence factors such as intimin *(eae)* and enterohemolysin *(ehxA)*.

STEC are responsible for a number of human gastrointestinal diseases, including diarrhea, bloody diarrhea and hemorrhagic colitis (HC). In a proportion of individuals, particularly in children, these conditions may be complicated by neurological and renal sequelae, including hemolytic-uremic syndrome (HUS).

STEC have been isolated from a variety of animals. However, the bulk of data suggests that prevalence of STEC is greater in ruminants than in other animals and domestic ruminants, especially cattle, represent one of the largest reservoirs of STEC pathogenic to humans. This review provides data on the prevalence of O157 and non-O157 STEC in cattle, sheep and pigs at slaughter in Switzerland. Moreover, characterization results for isolated O157 and non-O157 strains are summarized.

Zusammenfassung

Shigatoxin-bildende *Escherichia coli* (STEC) gehören zur Gruppe der latenten Zoonose-Erreger, wobei «gesunde» Nutztiere das Reservoir darstellen. Im Rahmen der traditionellen Fleischkontrolle werden diese Tiere nicht erkannt, da keine klinischen Symptome und auch keine pathologisch-anatomischen Veränderungen am

Schlachttierkörper und den Organen vorliegen. Zudem besteht bei der Fleisch- und Milchgewinnung an verschiedensten Prozessstufen die Gefahr einer Kontamination des Fleisches und der Milch.

STEC gehören heute weltweit zu den drei bedeutendsten bakteriellen foodborne pathogens und sind durch ihre Fähigkeit phagencodierte Shigatoxine zu bilden charakterisiert. Zudem besitzen pathogene Stämme weitere Virulenzfaktoren wie z.B. das Intimin *(eae)* und das Enterohämolysin *(ehxA)*.

Das Spektrum klinischer Erscheinungen bei STEC-Infektionen ist sehr breit. STEC verursachen beim Menschen in erster Linie Durchfallserkrankungen, die einen milden wässrigen bis schweren hämorrhagischen Verlauf nehmen können. Bei Kindern kann sich nach Sistieren des Durchfalls als schwere Komplikation ein hämolytisch-urämisches Syndrom (HUS) entwickeln.

Der Verdacht, dass vor allem Rinder und Schafe den Erreger ausscheiden können und daher das wichtigste Reservoir darstellen, bestätigte sich durch weltweite Untersuchungen. Dieser Review zeigt die aktuelle schweizerische Situation hinsichtlich der Prävalenz von O157 und non-O157 STEC bei Schlachtrindern, -schafen und -schweinen auf. Zudem werden Charakterisierungsdaten isolierter Stämme gegenübergestellt und damit ein Beitrag zum risk assessment geschaffen.

Résumé

L'importance des zoonoses latentes dans le cadre des maladies d'origine alimentaire a augmenté ces dernières années: (i) les animaux «sains» représentent un réservoir pour les germes pathogènes spécifiques; (ii) les carcasses et les organes ne subissent aucune altérations pathologique-anatomique permettant de distinguer la présence de ces germes pathogènes; (iii) ces germes pathogènes sont capables de pénétrer la chaîne alimentaire par les points faibles de l'abattage et de la traite.

Les *Escherichia coli* produisant des Shiga-toxines (STEC) comptent parmi les causes les plus fréquentes de maladies d'origine alimentaire. Le groupe des STEC est l'un des six différents groupes des *E. coli* responsables de diarrhée connus à ce jour. Les STEC sont caractérisés par leur capacité à produire des cytotoxines puissantes, codées génétiquement par un phage et appelées Shiga toxine. Les STEC pathogènes possèdent souvent d'autres facteurs de virulence, telles l'intimine *(eae)* et l'entérohémolysine *(ehxA)*.

Les STEC sont responsables de nombreux troubles gastro-intestinaux, dont la diarrhée, la diarrhée hémorragique, et la colite hémorragique (HC). Chez certains individus, en particulier les enfants, ces troubles peuvent être suivis de complications neurologiques et de séquelles rénales, en particulier le syndrome hémolytique-urologique (HUS).

Les STEC ont été isolés chez une grande variété d'animaux. Toutefois, la majorité des données suppose que la prévalence des STEC est plus importante chez les ruminants que chez les autres animaux. Les ruminants domestiques, en particulier le bétail bovin, représentent pour l'homme un des réservoirs les plus importants de

STEC pathogènes. Cet article présente des données sur la prévalence du STEC O157 et non-O157 chez les bovins, les moutons et les chèvres dans les abattoirs de Suisse. De plus, les résultats de la caractérisation des souches isolées O157 et non-O157 sont résumés.

Key words

STEC, prevalence, strain characterization, cattle, sheep, finisher pigs

References

1 *Paton J.C. and Paton A.W.:* Pathogenesis and diagnosis of Shiga toxin-producing *E. coli* infections. Clin. Microbiol. Rev. **11**, 450–479 (1998)

2 *Griffin P.M.:* Epidemiology of Shiga toxin-producing *Escherichia coli* infections in humans in the United States, p. 15–22. In J.B. Kaper, and A.D. O'Brien (eds.), *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. ASM Press, Washington, D.C. (1998)

3 *Blanco J.E., Blanco M., Alonso M.P., Mora A., Dahbi G., Coira M.A. and Blanco J.:* Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin-) producing *Escherichia coli* isolates from human patients: Prevalence in Lugo, Spain, from 1992 through 1999. J. Clin. Microbiol. **42**, 311–319 (2004)

4 *Beutin L., Krause G., Zimmermann S., Kaulfuss S. and Gleier K.:* Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. J. Clin. Microbiol. **42**, 1099–1108 (2004)

5 *Beutin L., Geier D., Steinrück H., Zimmermann S. and Scheutz F.:* Prevalence and some properties of Verotoxin (Shiga-like toxin) producing *Escherichia coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. **31**, 2483–2488 (1993)

6 *Wallace J.S., Cheasty T. and Jones K.:* Isolation of Vero cytotoxin-producing *Escherichia coli* O157 from wild birds. J. Appl. Microbiol. **82**, 399–404 (1997)

7 *Sargeant J.M., Hafer D.J., Gillespie J.R., Oberst R.D. and Flood S.J.:* Prevalence of *Escherichia coli* O157:H7 in white-tailed deer sharing rangeland with cattle. J. Am. Vet. Med. Assoc. **215**, 792–794 (1999)

8 *Al-Saigh H., Zweifel C., Blanco J., Blanco J.E., Blanco M., Usera M.A. and Stephan R.:* Fecal shedding of *Escherichia coli* O157, *Samonella* and *Campylobacter* in Swiss cattle at slaughter. J. Food Prot. **67**, 679–684 (2004)

9 *Heuvelink A.E., van den Biggelaar F.L., de Boer E., Herbes R.G., Melchers W.J., Huis in't Veld J.H. and Monnens L.A.:* Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and sheep. J. Clin. Microbiol. **36**, 878–882 (1998)

10 *Bonardi S., Maggi E., Pizzin G., Morabito S. and Caprioli A.:* Faecal carriage of Verocytotoxin-producing *Escherichia coli* O157 and carcass contamination in cattle at slaughter in northern Italy. Int. J. Food Microbiol. **66**, 47–53 (2001)

11 *Chapman P.A., Cerdan Malo A.T., Ellin M., Ashton R. and Harkin M.A.: Escherichia coli* O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. Int. J. Food Microbiol. **64**, 139–150 (2001)

12 *Nielsen E.M., Tegtmeier C., Andersen H.J., Gronbaek C. and Andersen J.S.:* Influence of age, sex and herd characteristics on the occurrence of Verocytotoxin-producing *Escherichia coli* O157 in Danish dairy farms. Vet. Microbiol. **88**, 245–257 (2002)

13 *Fantelli K. and Stephan R.:* Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. Int. J. Food Microbiol. **70**, 63–69 (2001)

14 *Stephan R., Ragettli S. and Untermann F.:* Occurrence of verotoxin-producing *Escherichia coli* (VTEC) in fecal swabs from slaughter cattle and sheep – an observation from a meat hygiene view. Schweiz. Arch. Tierheilkd. **142**, 110–114 (2000)
15 *Kuhnert P., Dubosson C.R., Roesch M., Homfeld E., Doherr M. and Blum J.W.:* Prevalence and risk-factor analysis of Shiga toxigenic *Escherichia coli* in faecal samples of organically and conventionally farmed dairy cattle. Vet. Microbiol. **109**, 37–45 (2005)
16 *Zweifel C., Schumacher S., Blanco M., Blanco J.E., Tasara T., Blanco J. and Stephan R.:* Phenotypic and genotypic characteristics of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) from Swiss cattle. Vet. Microbiol. **105**, 37–45 (2005)
17 *Blanco M., Blanco J.E., Mora A., Dahbi G., Alonso M.P., Gonzales E.A., Bernardez M.I. and Blanco J.:* Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin-) producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae ζ). J. Clin. Microbiol. **42**, 645–651 (2004)
18 *Zweifel C., Kaufmann M., Blanco J. und Stephan R.:* Bedeutung von *E. coli* O157 beim Schlachtschaf in der Schweiz. Schweiz. Arch. Tierheilk. submitted (2005)
19 *Johnsen G., Wasteson Y., Heir E., Berget O.I. and Herikstad H.: Escherichia coli* O157:H7 in faeces from cattle, sheep and pigs in the southwest part of Norway during 1998 and 1999. Int. J. Food Microbiol. **65**, 193–200 (2001)
20 *Blanco M., Blanco J.E., Mora A., Rey J., Alonso J.M., Hermoso M., Hermoso J., Alonso M.P., Dahbi G., González E.A., Bernárdez M.I. and Blanco J.:* Serotypes, virulence genes and intimin types of Shiga toxin (Verotoxin)-producing *Escherichia coli* isolates from healthy sheep in Spain. J. Clin. Microbiol. **41**, 1351–1356 (2003)
21 *Ogden I.D., MacRae M. and Strachan N.J.:* Concentration and prevalence of *Escherichia coli* O157 in sheep faeces at pasture in Scotland. J. Appl. Microbiol. **98**, 646–651 (2005)
22 *Zweifel C. and Stephan R.:* Microbiological monitoring of sheep carcass contamination in three Swiss abattoirs. J. Food Prot. **66**, 946–952 (2003)
23 *Zweifel C., Zychowska M.A. and Stephan R.:* Prevalence of Shiga toxin-23. producing *Escherichia coli* (STEC), Salmonella spp. and *Campylobacter* spp. isolated from slaughtered sheep in Switzerland. Int. J. Food Microbiol. **92**, 45–53 (2004a)
24 *Haring V. and Desmarchelier P.:* Verotoxin-producing *E. coli* in sheep. Aust. Vet. J. **75**, 676 (1997)
25 *Urdahl A.M., Beutin L., Skjerve E. and Wasteson Y.:* Serotypes and virulence factors of Shiga toxin-producing *Escherichia coli* isolated from healthy Norwegian sheep. J. Appl. Microbiol. **93**, 1026–1033 (2002)
26 *Zweifel C., Blanco J.E., Blanco M., Blanco J. and Stephan R.:* Serotypes and virulence genes of ovine non-O157 Shiga toxin-producing *Escherichia coli* in Switzerland. Int. J. Food Microbiol. **95**, 19–27 (2004b)
27 *Kaufmann M., Zweifel C., Blanco M., Blanco J.E., Blanco J., Beutin L. and Stephan R.: Escherichia coli* O157 and non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) in Fecal Samples of Finished Pigs at Slaughter in Switzerland. J. Food Prot. in press (2005)
28 *Bonardi S., Brindani F., Pizzin G., Lucidi L., D'Incau M., Liebana E. and Morabito S.:* Detection of *Salmonella* spp., *Yersinia enterocolitica* and verocytotoxin-producing *Escherichia coli* O157 in pigs at slaughter in Italy. Int. J. Food Microbiol. **85**, 101–110 (2003)
29 *Botteldoorn N., Heyndrickx M., Rijpens N. and Herman L.:* Detection and characterization of verotoxigenic *Escherichia coli* by a VTEC/EHEC multiplex PCR in porcine faeces and pig carcass swabs. Res. Microbiol. **154**, 97–104 (2003)
30 *Eriksson E., Nerbrink E., Borch E., Aspan A. and Gunnarsson A.:* Verocytotoxin-producing *Escherichia coli* O157:H7 in the Swedish pig population. Vet. Rec. **152**, 712–717 (2003)
31 *Feder I., Wallace F.M., Gray J.T., Fratamico P., Fedorka-Cray P.J., Pearce R.A., Call J.E., Perrine R. and Luchansky J.B.:* Isolation of *Escherichia coli* O157:H7 from intact colon fecal samples of swine. Emerging Infect. Dis. **9**, 380–383 (2003)

32 *DesRosiers A., Fairbrother J.M., Johnson R.P., Desautels C., Letellier A. and Quessy S.:* Phenotypic and genotypic characterization of *Escherichia coli* Verotoxin-producing isolates from humans and pigs. J. Food Prot. **64**, 1904–1911 (2001)
33 *Franke S., Harmsen D., Caprioli A., Piérard D., Wieler L.H. and Karch H.:* Clonal relatedness of shiga-like toxin-producing *Escherichia coli* O101 strains of human and porcine origin. J. Clin. Microbiol. **33**, 3174–3178 (1995)
34 *Rios M., Prado V., Trucksis M., Arellano C., Borie C., Alexandre M., Fica A. and Levine M.M.:* Clonal diversity of Chilean isolates of enterohemorrhagic *Escherichia coli* from patients with hemolytic-uremic syndrome, asymptomatic subjects, animal reservoirs, and food products. J. Clin. Microbiol. **37**, 778–781 (1999)
35 *Fratamico P.M., Bagi L.K., Bush E.J. and Solow B.T.:* Prevalence and characterization of Shiga toxin-producing *Escherichia coli* in swine feces recovered in the National Animal Health Monitoring System's Swine 2000 study. Appl. Environ. Microbiol. **70**, 7173–7178 (2004)
36 *Friedrich A.W., Bielaszewska M., Zhang W.L., Pulz M., Kuczius T., Ammon A. and Karch H.:* *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J. Infect. Dis. **185**, 74–84 (2002)

Corresponding address: Institute of Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Winterthurerstrasse 272, CH-8057 Zurich, Switzerland, Phone: +41-44-635-8651, Fax: +41-44-635-8908, e-mail: stephanr@fsafety.unizh.ch