Prevalence and genotypic characteristics of Shigatoxin-producing *Escherichia coli* (STEC) along the foodchain and beyond

Background:
Shiga toxin (Stx)-producing *Escherichia coli* (STEC) are foodborne pathogens that cause gastrointestinal illnesses including non-bloody or bloody diarrhea, haemorrhagic colitis (HC), and the life-threatening haemolytic uremic syndrome (HUS). There exist to date two main Stx types, encoded by *stx* genes and designated Stx1 and Stx2, with different subtypes.

WP1: Farm animals as a potential reservoir of Shigatoxin-producing *Escherichia coli*

Feacal shedding of *Escherichia coli* O157, *Salmonella* spp. and *Campylobacter* spp. in Swiss cattle at slaughter
https://pubmed.ncbi.nlm.nih.gov/15083718/

Insights: The percentage of the 2,930 samples that tested positive for E. coli O157 by PCR was 1.6%. Of the six sorbitol-negative strains, five tested positive for stx2 genes (two times for stx2c and three times for stx2), and one strain tested positive for stx1 and stx2c genes. All sorbitol-negative strains belonged to the serotypes O157:H7- and O157:H7 and harbored the eae type gamma 1 and ehxA genes. The 32 sorbitol-positive strains tested negative for stx genes and belonged to the serotypes O157:H2, O157:H7, O157:H8, O157:H12, O157:H19, O157:H25, O157:H27, O157:H38, O157:H43, O157:H45, and O157:H-.

Application of a real-time PCR-based system for monitoring of O26, O103, O111, O145, and O157 Shiga toxin-producing *Escherichia coli* in cattle at slaughter
https://pubmed.ncbi.nlm.nih.gov/22348425/

Insights: Faecal samples were collected from 573 slaughtered cattle aged between three and 24 months in seven abattoirs. 74.1% tested positive for stx genes. Amongst them, the serogroups O145, O103, O26, O157 and O111 were detected in 41.9%, 25.9%, 23.9%, 7.8% and 0.8%, respectively. Shiga toxin genes and the top-five STEC serogroups were frequently found in young Swiss cattle at slaughter, but success rates for strain isolation were low and only few strains showed a virulence pattern of human pathogenic STEC.

Detection of the emerging Shiga Toxin-producing *Escherichia coli* O26:H11/H- ST29 in human patients and healthy cattle in Switzerland
https://pubmed.ncbi.nlm.nih.gov/23811503/

Insights: Shiga toxin-producing Escherichia coli O26:H11/H(-) strains showing the characteristics of the emerging human-pathogenic ST29 clone (stx2a(+) only, eae(+), plasmid gene profile hlyA(+) etpD(+)) were detected from human patients and healthy cattle, indicating a possible reservoir.

Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella* spp. and *Campylobacter* spp. isolated from slaughtered sheep in Switzerland
https://pubmed.ncbi.nlm.nih.gov/15033267/

Insights: Caecum samples collected from 653 slaughtered sheep from two Swiss abattoirs were examined. The percentage of samples testing positive for STEC by a polymerase chain reaction was 29.9%.

Escherichia coli O157 and non-O157 Shiga toxin-producing *Escherichia coli* (STEC) in fecal samples of finished pigs at slaughter in Switzerland
https://pubmed.ncbi.nlm.nih.gov/16496563/

Insights: Fecal samples from 630 slaughtered finisher pigs were examined by PCR to assess the shedding of Escherichia coli O157 (rfbE) and Shiga toxin-producing E. coli (STEC, stx). The proportion of positive samples was 7.5% for rfbE and 22% for stx. Among E. coli O157 strains, 30 were sorbitol positive, 30 had an H type other than H7, and none harbored stx genes. The isolated STEC strains (43 sorbitol positive) belonged to 11 O:H serotypes. All but one strain harbored stx2e. The eae and ehxA genes, which are strongly correlated with human disease, were present in only one O103:H2 strain. High prevalence of STEC was found among finisher pigs, but according to the virulence factors the majority of these strains seem to be of low virulence.

WP2: Non livestock animals as possible reservoir of Shigatoxin-producing *Escherichia coli*

Presence of foodborne pathogens, extended-spectrum β-lactamase -producing Enterobacteriaceae, and methicillin-resistant *Staphylococcus aureus* in slaughtered reindeer in northern Finland and Norway

https://pubmed.ncbi.nlm.nih.gov/28049493/

Insights: Shiga toxins genes (stx1 and/or stx2) were detected in 33% of 470 reindeer fecal samples. Stx2 predominated among the Shiga toxin genes (stx2 alone or in combination with stx1 was found in 25% of the samples). With regard to the frequency and distribution of stx1/stx2, striking differences were evident among the 10 different areas of origin. Hence, reindeer could constitute a reservoir for Shiga toxin-producing E. coli (STEC), but strain isolation and characterization is required for verification purposes and to assess the potential human pathogenicity of strains.

Shiga toxin-producing *Escherichia coli* (STEC) isolated from fecal samples of African dromedary camels

https://pubmed.ncbi.nlm.nih.gov/30911597/

Insights: To investigate the occurrence of STEC among grazing dromedaries from Kenya, *E. coli* isolated from fecal matter collected from 163 dromedaries on a large ranch were screened for the presence of *stx1* and *stx2*. STEC was isolated from 20 (12.3%) of the fecal samples. Thereof, nine (45%) isolates were STEC O156:H25, three (15%) isolates typed STEC O43:H2. The remaining isolates occurred as single serotypes or were O non-typeable. Eleven (55%) of the isolates harboured *stx2a*, nine (45%) *eae*, and 14 (70%) *ehx*, respectively. Overall, the results indicate that dromedary camels in Kenya may be reservoirs of STEC, including serotypes possessing virulence markers associated to disease in humans, such as STEC O156:H25.

Animal petting zoos as sources of Shiga toxin-producing *Escherichia coli*, *Salmonella* and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae

https://pubmed.ncbi.nlm.nih.gov/33382208/

Insights: The aim of this study was to assess the occurrence of Shiga toxin-producing Escherichia coli (STEC), Salmonella, extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae and methicillin-resistant Staphylococcus aureus (MRSA) in animal faeces from six animal petting zoos and one farm fair in Switzerland. Furthermore, hygiene facilities on the venues were evaluated. Of 163 faecal samples, 75 contained stx1, stx2 or stx1/stx2 genes, indicating the presence of STEC. Samples included faeces from sika deer (100%), sheep (92%), goats (88%), mouflons (80%), camels (62%), llamas (50%), yaks (50%), pigs (29%) and donkeys (6%), whereas no stx genes were isolated from faeces of calves, guinea pigs, hens, ostriches, ponies, zebras or zebus. This study provides data that underscore the importance of hygiene measures to minimize the risk of transmission of zoonotic pathogens to visitors of animal petting venues.

WP3: Shigatoxin-producing *Escherichia coli* along the food and feed chain

Prevalence and characteristics of shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland

https://pubmed.ncbi.nlm.nih.gov/11759763/

Insights: A total of 400 minced meat samples from 240 small butcheries in Switzerland were collected and analysed for the presence of Shigatoxin-producing E. coli (STEC). The samples comprised 211 samples of minced beef and 189 samples of minced pork. Shigatoxin-producing E. coli was isolated from 7/400 (1.75%) samples. In particular, 5/211 (2.3%) minced beef samples and 2/189 (1%) minced pork samples were contaminated. Serotyping of the seven strains yielded five different serotypes. but none of the strains belonged to O157:H7. Two STEC strains harboured stx1 and stx2 and five strains harboured stx2c genes. Furthermore, four strains harboured one or more additional virulence factors. However, none of the strains was positive for eae.

Characteristics of Shiga toxin-producing *Escherichia coli* isolated from Swiss raw milk cheese within a 3-year monitoring program

https://pubmed.ncbi.nlm.nih.gov/20051209/

Insights: 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 stx(1) was only found in 2 strains, whereas 27 strains carried genes encoding for the Stx(2) group. 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.

Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* in Swiss raw milk cheeses collected at producer level

https://pubmed.ncbi.nlm.nih.gov/18565913/

Insights: Raw milk cheese samples (soft cheese, n = 52; semihard and hard cheese, n = 744; all produced from Swiss cows', goats', and sheep's milk) collected at the producer level throughout Switzerland within the national sampling plan during the period of March 2006 to December 2007 were analyzed. Of the 432 cheese samples obtained in the year 2006 and the 364 samples obtained in the year 2007, 16 (3.7%) and 23 (6.3%), respectively, were found to be stx positive. By colony dot-blot hybridization, non-O157 STEC strains were isolated from 16 samples. Of the 16 strains, 11 were typed into 7 E. coli O groups (O2, O15, O22, O91, O109, O113, O174), whereas 5 strains were nontypeable (ONT). Among the 16 STEC strains analyzed, stx(1) and stx(2) variants were detected in 1 and 15 strains, respectively. Out of the 15 strains with genes encoding for the Stx2 group, 4 strains were positive for stx(2), 6 strains for stx(2d2), 2 strains for stx(2-O118), 1 strain for stx(2-06), 1 strain for stx(2g), 1 strain for stx(2) and stx(2d2), and 1 strain for stx(2) and stx(2g). Furthermore, 3 STEC strains harbored E-hlyA as a further putative virulence factor. None of the strains tested positive for eae (intimin). Results obtained in this work reinforce the suggestion that semihard raw milk cheese may be a potential vehicle for transmission of pathogenic STEC to humans.

Fate of Shiga toxin-producing and generic *Escherichia coli* during ripening of semi-hard raw milk cheese

https://pubmed.ncbi.nlm.nih.gov/23245958/

Insights: The fate of 5 different Escherichia coli strains, including 3 Shiga toxin-producing E. coli (STEC) strains, was analyzed during the production and ripening of semihard raw milk cheese. The strains, which were previously isolated from raw milk cheese, were spiked into raw milk before cheese production at 2 different levels (approximately 10(1) and 10(3) cfu/mL, respectively). Two cheese types were produced, which differed in cooking temperatures (40 and 46°C). The cheeses were sampled during manufacture and the 16-wk ripening period. An increase in E. coli counts of approximately 3.5 log(10) cfu/g occurred from raw milk to fresh cheese at d 1, which was attributed to a concentration effect during cheese production and growth of the strains. During ripening over 16 wk, a slow, continuous decrease was observed for all strains. However, significant differences were found between the E. coli strains at the applied spiking levels, whereas the inactivation was similar in the 2 different cheese types. The 2 generic E. coli strains survived at higher counts than did the 3 STEC strains. Nevertheless, only 1 of the 3 STEC strains showed significantly weaker survival at both spiking levels and in both cheese types. Six of 16 cheeses made from raw milk at a low spiking level contained more than 10 cfu/g of STEC at the end of the 16-wk ripening process. After enrichment, STEC were detected in almost all cheeses at both spiking levels. Particularly because of the low infectious dose of highly pathogenic STEC, even low colony counts in raw milk cheese are a matter of concern.

An overview of molecular stress response mechanisms in *Escherichia coli* contributing to survival of STEC during the raw milk cheese production

https://pubmed.ncbi.nlm.nih.gov/21549061/

Insights: The ability of foodborne pathogens to survive in certain foods mainly depends on stress response mechanisms. Insight into molecular properties enabling pathogenic bacteria to survive in food is valuable for improvement of the control of pathogens during food processing. Raw milk cheeses are a potential source for human infections with Shiga toxin-producing Escherichia coli (STEC). In this review, we focused on the stress response mechanisms important for allowing STEC to survive raw milk cheese production processes. The major components and regulation pathways for general, acid, osmotic, and heat shock stress responses in E. coli and the implications of these responses for the survival of STEC in raw milk cheeses are discussed.

Occurrence of *Salmonella*, *L. monocytogenes*, Shigatoxin-producing *E. coli* and ESBL-producing Enterobacteriaceae in sprout samples collected from the Swiss market

https://link.springer.com/content/pdf/10.1007/s00003-015-1003-3.pdf?pdf=button

This study evaluated the presence of foodborne pathogens andextended spectrum b-lactamases (ESBL)-producing bacteria in 102 sprout samples collected from the Swiss market. Salmonella, Shigatoxin-producing E. coli and Listeria monocytogenes were not detected but 3 samples were positive for ESBL-producing Enterobacteriaceae (Klebsiella variicola, Enterobacter cloacae, E. coli). The seeds and sprouts of those 3 positive samples originated from Switzerland. The Klebsiella variicola and Escherichia coli isolate harbored the clinically important blaCTX-M-14 gene, whereas blaCTX-M-3 was present in the Enterobacter cloacae isolate. Although these results assert an overall favorable situation in terms of the occurrence of foodborne pathogens in sprouting seeds in Switzerland, these products constitute a possible route for the spread of ESBL-producing Enterobacteriaceae, which co

Microbiological quality and presence of foodborne pathogens in raw milk cheeses and raw meat products marketed at farm level in Switzerland

https://pubmed.ncbi.nlm.nih.gov/30046563/

Insights: This study investigated the microbiological quality and presence of bacterial foodborne pathogens in 51 raw milk cheeses (mainly semihard and hard cheese) and 53 raw meat products (cured meat products and sausages) marketed at farm level. STEC were found in two products. The two STEC isolates harbored *stx*1a (cheese) or *stx*2e (sausage), but both lacked *eae* and did not belong to the top five-serogroups.

Detection, isolation and characterization of Shiga toxin-producing *Escherichia coli* (STEC) in flour
https://pubmed.ncbi.nlm.nih.gov/30707053/

Insights: Wheat flour has recently been described as a novel vehicle for transmission of Shiga toxin-producing Escherichia coli (STEC). Very recently, an outbreak of STEC O121 and STEC O26 infections was linked to flour in the United States. The aim of the present study was to generate baseline data for the occurrence of STEC in flour samples from different retailers in Switzerland. In total, 70 flour samples were analyzed. After enrichment, the samples were screened for stx_1 and stx_2 by the Assurance GDS MPX ID assay. STEC strains were isolated and serotyped by the E. coli SeroGenoTyping AS-1 kit. The determination of stx subtypes was performed with conventional PCR amplification. Screening for eae, aggR, elt, and estIa/Ib was performed by real-time PCR. Nine (12.9%) of the flour samples tested positive for stx by PCR. STEC was recovered from eight (88.9%) of the positive samples. Two isolates were STEC O11:H48 harboring stx_{1c}/ stx_{1d}, two were O146:H28 containing stx_{2b}, one was O103:H2 containing stx_{1a} and eae, and three were O nontypeable: Ont:H12 (stx_{2a}), Ont:H14 (stx_{2a}/ stx_{2g}), and Ont:H31 (stx_{1c}/ stx_{1d}). STEC O103 belongs to the "top five" serogroups of human pathogenic STEC in the European Union, and STEC O146 is frequently isolated from diseased humans in Switzerland. Our results show that flour may be contaminated with a variety of STEC serogroups. Consumption of raw or undercooked flour may constitute a risk for STEC infection.

High occurrence of Shiga toxin-producing *Escherichia coli* in raw meat-based diets for companion animals – a public health issue
https://www.mdpi.com/2076-2607/9/8/1556

Insights: The aim of this study was to evaluate commercially available RMBDs with regard to the occurrence of STEC. Of 59 RMBD samples, 59% tested positive by real-time PCR for the presence of Shiga toxin genes stx1 and/or stx2. STECs were recovered from 41% of the 59 samples, and strains were subjected to serotyping and virulence gene profiling. Of 28 strains, 29% carried stx2a or stx2d, which are linked to STEC with high pathogenic potential. Twenty different serotypes were identified, including STEC O26:H11, O91:H10, O91:H14, O145:H28, O146:H21, and O146:H28, which are within the most common non-O157 serogroups associated with human STEC-related illnesses worldwide. Considering the low infectious dose and potential severity of disease manifestations, the high occurrence of STEC in RMBDs poses an important health risk for persons handling raw pet food and persons with close contact to pets fed on RMBDs, and is of concern in the field of public health.

WP4: Faecal carriage of Shigatoxin-producing *Escherichia coli* in the community

Virulence factors and phenotypical traits of verotoxin-producing Escherichia coli strains isolated from asymptomatic human carriers
https://pubmed.ncbi.nlm.nih.gov/10203524/

Insights: A total of 5590 stool samples from healthy employees in the meat industry were screened by PCR for verotoxin-producing Escherichia coli (VTEC). The PCR product of VT-encoding genes was detected in 3. 5% of the samples. Phenotypic and genotypic traits of 47 VTEC strains isolated from asymptomatic carriers were characterized. A variety of serotypes was found; one strain belonged to the serotype O157:H7. The majority of the isolates proved to be VT2-positive. Fifty-seven percent of the verotoxin-producing strains harboured the genes for one or several additional virulence associated factors, including intimin (eae, 8.5%), the 60 MDa plasmid (42.5%), enterohaemolysin (EHEC-hlyA, 38.3%), the heat-stable enterotoxin (astA, 6.4%), a serin protease (espP, 6.4%), colicin production (col D157, 12.8%) and a secretion system II (etpD, 10.6%). None of the strains was positive for a specific enzyme with catalase-peroxidase activity (katP).

WP5: Unusual clinical cases

Hemolytic uremic syndrome in a 65 year-old male linked to a very unusual type of *stx2e* and *eae* harboring O51:H49 Shiga-toxin producing *Escherichia coli*
https://pubmed.ncbi.nlm.nih.gov/24501025/

Insights: We report on a 65-year-old male patient with a Shiga toxin-producing Escherichia coli O51:H49 gastrointestinal infection and sepsis associated with hemolytic uremic syndrome (HUS) with a fatal outcome. The strains isolated harbored stx2e and eae, a very unusual and new virulence profile for an HUS-associated enterohemorrhagic E. coli.

Neonatal hemolytic uremic syndrome after mother-to-child transmission of a low virulent stx2b harbouring Shiga-toxin producing *Escherichia coli*
https://pubmed.ncbi.nlm.nih.gov/23042969/

Insights: This case describes evidence for a Shiga toxin-producing Escherichia coli (STEC) O146:H28 infection leading to hemolytic uremic syndrome in a neonate. STEC O146:H28 was linked hitherto with asymptomatic carriage in humans. Based on strain characteristics and genotyping data, the mother is a healthy carrier who transmitted the STEC during delivery. STEC strains belonging to the low-pathogenic STEC group must also be considered in the workup of neonatal hemolytic uremic syndrome.

WP7: Further characteristics of Shigatoxin-producing *Escherichia coli* in a One Health context

Serotypes, intimin variants and other virulence factors of *eae* positive *Escherichia coli* strains isolated from healthy cattle in Switzerland. Identification of a new intimin variant gene (eae-eta2)

https://bmcmicrobiol.biomedcentral.com/articles/10.1186/1471-2180-5-23

Insights: These data confirm that ruminants are an important source of serologically and genetically diverse intimin-harboring *E. coli* strains. Moreover, cattle have not only to be considered as important asymptomatic carriers of O157 STEC but can also be a reservoir of EPEC and *eae* positive non-O157 STEC, which are described in association with human diseases.

Phenotypic and genotypic characteristics of non-O157 Shiga toxin producing *E. coli* from Swiss cattle

https://pubmed.ncbi.nlm.nih.gov/15607082/

Insights:_A total of 42 Shiga toxin-producing (STEC) strains from slaughtered healthy cattle in Switzerland were characterized by phenotypic and genotypic traits. 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 (PCR) analysis showed that 18 (43%) strains carried the stx1 gene, 20 strains (48%) had the stx2 gene, and four (9%) strains had both stx1 and stx2 genes.

Serotypes and virulence genes of ovine non-O157 Shiga toxin-producing *Escherichia coli* in Switzerland

https://pubmed.ncbi.nlm.nih.gov/15240071/

Insights: 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 these factors and to assess the potential pathogenicity of these strains for humans. 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. Of the 47 strains encoding for stx1 variants, 57% were stx1c- and of the 45 encoding for stx2 variants, 80% were stx2d-positive.

Virulence profiles of Shiga toxin 2e-producing *Escherichia coli* isolated from healthy pig at slaughter

https://www.sciencedirect.com/science/article/pii/S037811350600246X?via%3Dihub

Insights: In the present study, 31 Shiga toxin-producing *E. coli* (STEC) strains harboring *stx*2e, which were previously isolated out of fecal samples from healthy pigs at slaughter were characterized by phenotypic and genotypic traits. Nine of the thirty-one sorbitol-positive non-O157 STEC (*stx*2e) isolated from healthy pigs belonged to serotypes found in STEC isolated from humans, including two serotypes (O9:H−, O26:H−) reported in association with hemolytic-uremic syndrome. Otherwise, the serotypes were different from those isolated from cases of edema disease in pigs. The *eae* (intimin) gene, which is strongly correlated with severe human disease, was not detected. Nine strains tested positive for *astA* (EAST1), one O141:H17 strain for *fedA* (F18 fimbrial adhesin) and one O159:H− strain for *terF* (tellurite resistance). Similar to the Stx2e-producing *E. coli* isolated from humans, which are mainly lacking further virulence factors, genes of an iron uptake system on the high-pathogenicity island (*irp2*, *fyuA*) were detected in three ONT:H10 and ONT:H19 strains from healthy pigs. Consequently, although the isolated strains are unlikely to be associated with severe human diseases, healthy pigs cannot be excluded as a potential source of human infection with Stx2e-producing STEC.

Activatable Shiga toxin 2d (Stx2d) in STEC strains isolated from cattle and sheep at slaughter

https://www.sciencedirect.com/science/article/pii/S0378113508001028?via%3Dihub

Insights: We analyzed 11 STEC strains isolated from healthy cattle and sheep at slaughter that were originally detected to contain the stx_{2c} allele, for the presence of the $stx_{2d\text{-activatable}}$ genotype. Ten of the eleven strains displayed the $stx_{2d\text{-activatable}}$ genotype as determine by *Pst*I restriction fragment length polymorphism (RFLP) of 890-bp fragments of their *stx* genes. Only in 6 of the 10 strains whose *stx* genes were sequenced, the presence of $stx_{2d\text{-activatable}}$ could be confirmed based on the predicted amino acid sequence of their StxA subunits; the remaining four strains contained Stx2c A subunit. Five of the six strains which contained $stx_{2d\text{-activatable}}$ displayed the activatable phenotype on Vero cells.

Phenotypic and genotypic traits of Shiga toxin-negative *E. coli* O157:H7/H- bovine and porcine strains

https://pubmed.ncbi.nlm.nih.gov/19245340/

Insights: Enterohemorrhagic E. coli (EHEC) O157:H7/H(-) (nonmotile) exist as Shiga toxin gene (stx)-positive and stx-negative variants in patients and the environment. We analyzed molecular characteristics, phenotypes, and the phylogenetic background of three stx-negative E. coli O157:H7/H(-) strains isolated from cattle and a pig and compared them with those of human EHEC and stx-negative E. coli O157:H7/H(-). All three animal strains contained fliCH7 and two contained eae. One eae-positive strain (O157:H(-)) was sorbitol-fermenting (SF) and the other (O157:H7) was non-sorbitol-fermenting (nSF). These two strains shared a spectrum of non-stx putative virulence and fitness genes with human nSF and SF EHEC and stx-negative E. coli O157:H7/H(-) and belonged, similar to the vast majority of human isolates, to sequence type (ST) 11 in multilocus sequence typing. In contrast, the eae-negative O157:H7 animal isolate, which was SF, differed in spectrum of virulence genes and also differed phylogenetically (ST717) from the two eae-positive strains and the human EHEC and stx-negative E. coli O157:H7/H(-). In contrast to efforts with human stx-negative E. coli O157:H(-), attempts to transduce the two stx-negative/eae-positive animal O157:H7/H(-) strains with stx(2)-encoding phages from human SF and nSF EHEC O157:H7/H(-) failed, despite the animal strains having intact loci where such phages integrate in human EHEC O157 (wrbA and yecE). The role of animal stx-negative/eae-positive and stx-negative/eae-negative E. coli O157:H7/H(-) in their natural source and in human infections needs further investigation.

Shiga toxin subtypes associated with Shiga toxin-producing *Escherichia coli* strains isolated from red deer, roe deer, chamois and ibex

https://pubmed.ncbi.nlm.nih.gov/22891940/

Insights: A total of 52 Shiga toxin-producing Escherichia coli (STEC) strains, isolated from fecal samples of six ibex, 12 chamois, 15 roe deer, and 19 red deer were further characterized by subtyping the stx genes, examining strains for the top nine serogroups and testing for the presence of eae and ehxA. Eleven of the 52 strains belonged to one of the top nine STEC O groups (O26, O45, O91, O103, O111, O113, O121, O145, and O157). Eight STEC strains were of serogroup O145, two strains of serogroup O113, and one strain of serogroup O157. None of the strains harbored stx2a, stx2e, or stx2f. Stx2b (24 strains) and stx1c (21 strains) were the most frequently detected stx subtypes, occurring alone or in combination with another stx subtype. Eight strains harbored stx2g, five strains stx2d, three strains stx1a, two strains stx2c, and one strain stx1d. Stx2g and stx1d were detected in strains not harboring any other stx subtype.

Prevalence of Subtilase cytotoxin-encoding subAB variants among Shiga toxin-producing *Escherichia coli* strains isolated from wild ruminants and sheep differs from that of cattle and pigs and is predominated by the new allelic variant subAB2-2

https://pubmed.ncbi.nlm.nih.gov/25488108/

Insights: Subtilase cytotoxin (SubAB) is an AB5 toxin produced by Shiga toxin (Stx)-producing Escherichia coli (STEC) strains usually lacking the eae gene product intimin. Three allelic variants of SubAB encoding genes have been described: subAB1, located on a plasmid, subAB2-1, located on the pathogenicity island SE-PAI and subAB2-2 located in an outer membrane efflux protein (OEP) region. SubAB is becoming increasingly recognized as a toxin potentially involved in human pathogenesis. Ruminants and cattle have been identified as reservoirs of subAB-positive STEC. The presence of the three subAB allelic variants was investigated by PCR for 152 STEC strains originating from chamois, ibex, red deer, roe deer, cattle, sheep and pigs. Overall, subAB genes were detected in 45.5% of the strains. Prevalence was highest for STEC originating from ibex (100%), chamois (92%) and sheep (65%). None of the STEC of bovine or of porcine origin tested positive for subAB. None of the strains tested positive for subAB1. The allelic variant subAB2-2 was detected the most commonly, with 51.4% possessing subAb2-1 together with subAB2-2. STEC of ovine origin, serotypes O91:H- and O128:H2, the saa gene, which encodes for the autoagglutinating adhesin and stx2b were significantly associated with subAB-positive STEC. Our results suggest that subAB2-1 and subAB2-2 is widespread among STEC from wild ruminants and sheep and may be important as virulence markers in STEC pathogenic to humans.

Characteristics of Shiga toxin-producing *E. coli* O157 in slaughtered reindeer from northern Finland

https://pubmed.ncbi.nlm.nih.gov/28207302/

Insights: Fecal samples collected from 470 slaughtered reindeer 6 to 7 months of age were screened by real-time PCR (after enrichment) for Shiga toxin genes (stx) and then for Escherichia coli serogroup O157. Shiga toxin genes were found frequently (>30% of samples), and serogroup O157 was detected in 20% of the stx-positive samples. From these samples, a total of 25 E. coli O157:H^- isolates (nonmotile but PCR positive for $fliC_{H7}$) were obtained. Twenty-four of these E. coli O157:H^- isolates did not ferment sorbitol and originated from one geographic area. These 24 isolates belonged to the multilocus sequence type 11, typical for Shiga toxin-producing E. coli (STEC) O157:H7 and O157:H^-, and harbored genes stx_{1a}, stx_{2c}, eae, and hlyA; the stx_{2c} subtype has been associated with high virulence. In contrast, one E. coli O157:H^- isolate (multilocus sequence type 11) did ferment sorbitol, lacked Shiga toxin genes, but was positive for eae, hlyA, and sfpA. This isolate closely resembled an STEC that has lost its Shiga toxin genes. Additional examination revealed that reindeer can be colonized by various other STEC isolates; 21 non-O157 STEC isolates belonged to four multilocus sequence types, harbored stx_{1a} (8 isolates) or stx_{2b} (13 isolates), and in the stx_{2b}-positive isolates the recently described new allelic variants (subAB2-2 and subAB2-3) for subtilase cytotoxin were identified. Hence, slaughtered semidomesticated Finnish reindeer might constitute a little known reservoir for STEC O157:H7/H^- and other serogroups, and the risk of direct or indirect transmission of these pathogens from reindeer to humans and domestic livestock must not be overlooked.

Human infections with non-O157 Shiga toxin–producing *Escherichia coli*, Switzerland, 2000–2009
https://wwwnc.cdc.gov/eid/article/17/2/10-0909_article

Insights: We characterized 97 non-O157 Shiga toxin (stx)–producing *Escherichia coli* strains isolated from human patients during 2000–2009 from the national reference laboratory in Switzerland. These strains belonged to 40 O:H serotypes; 4 serotypes (O26:H11/H$^-$, O103:H2, O121:H19, and O145:H28/H$^-$) accounted for 46.4% of the strains. Nonbloody diarrhea was reported by 23.2% of the patients, bloody diarrhea by 56.8%. Hemolytic uremic syndrome developed in 40.0% of patients; serotype O26:H11/H$^-$ was most often associated with this syndrome. Forty-five (46.4%) strains carried *stx*2 genes only, 36 strains (37.1%) carried *stx*1, and 16 (16.5%) strains carried *stx*1 and *stx*2. Genes encoding enterohemolysin and intimin were detected in 75.3% and 70.1% of the strains, respectively. Resistance to >1 antimicrobial agent was present in 25 isolates. High genetic diversity within strains indicates that non-O157 stx–producing *E. coli* infections in Switzerland most often occurred as single cases.

Shiga toxin-producing *Escherichia coli* O157 associated with human infections in Switzerland: 2000-2009
https://pubmed.ncbi.nlm.nih.gov/20875198/

Insights: A total of 44 O157 strains isolated from different patients from 2000 through 2009 in Switzerland were further characterized and linked to medical history data. Non-bloody diarrhoea was experienced by 15.9%, BD by 61.4% of the patients, and 29.5% developed HUS. All strains belonged to MLST type 11, were positive for stx2 variants (stx2 and/or stx2c), eae and ehxA, and only two strains showed antibiotic resistance. Of the 44 strains, nine phage types (PTs) were detected the most frequent being PT32 (43.2%) and PT8 (18.2%). By PFGE, 39 different patterns were found. This high genetic diversity within the strains leads to the conclusion that STEC O157 infections in Switzerland most often occur as sporadic cases.

Human infections with Shiga toxin-producing *Escherichia coli*, Switzerland, 2010-2014
https://www.frontiersin.org/articles/10.3389/fmicb.2017.01471/full

Insights: The five most common serogroups were O157, O145, O26, O103, and O146. Of the 95 strains, 35 (36.8%) carried *stx1* genes only, 43 strains (45.2%) carried *stx2* and 17 (17.9%) harbored combinations of *stx1* and *stx2* genes. *Stx1a* (42 strains) and *stx2a* (32 strains) were the most frequently detected *stx* subtypes. Genes for intimin (*eae*), hemolysin (*hly*), iron-regulated adhesion (*iha*), and the subtilase cytotoxin subtypes *subAB1, subAB2-1, subAB2-2*, or *subAB2-3* were detected in 70.5, 83.2, 74.7, and 20% of the strains, respectively. Multilocus sequence typing assigned the majority (58.9%) of the isolates to five different clonal complexes (CC), 11, 32, 29, 20, and 165, respectively. CC11 included all O157:[H7] and O55:[H7] isolates. CC32 comprised O145:[H28] isolates, and O145:[H25] belonged to sequence type (ST) 342. CC29 contained isolates of the O26:[H11], O111:[H8] and O118:[Hnt] serogroups, and CC20 encompassed isolates of O51:H49/[Hnt] and O103:[H2]. CC165 included isolates typed O80:[H2]-ST301, all harboring *stx2d, eae*-ξ, *hly*, and 66.7% additionally harboring *iha*. All O80:[H2]-ST301 strains harbored at least 7 genes carried by pS88, a plasmid associated with extraintestinal virulence. Compared to data from Switzerland from the years 2000–2009, an increase of the proportion of non-O157 STEC infections was observed as well as an increase of infections due to STEC O146. By contrast, the prevalence of the highly virulent German clone STEC O26:[H11]-ST29 decreased from 11.3% during 2000–2009 to 1.1% for the time span 2010–2014. The detection of O80:[H2]-ST301 harboring *stx2d, eae*-ξ, *hly, iha*, and pS88 related genes suggests an ongoing emergence in Switzerland of an unusual, highly pathogenic STEC serotype.

Serotypes and virulence profiles of Shiga toxin-producing *Escherichia coli* strains isolated during 2017 from human infections in Switzerland
https://pubmed.ncbi.nlm.nih.gov/30042042/

Insights: This study aimed to identify the serotypes and virulence genes of 120 STEC isolated from human clinical stx positive specimens during 2017 in order to estimate any changes in serotype distribution and toxin profiles of STEC compared to the time span 2010-2014. STEC were recovered from 27.5% of the stx positive samples. STEC O157:H7 accounted for 7.5% of all isolates, and STEC O80:H2, O91:H10/H14/H21, O103:H2/H11, and O26:H11 accounted for 36.9% of the non-O157 strains. Forty-five isolates with stx1 variants, 47 with stx2 variants and 28 isolates with both stx1 and stx2 variants were identified. Forty (33.3% of all isolates) carried the subtypes associated with high pathogenic potential, stx2a, stx2c, or stx2d. The eae gene for intimin was detected in 54 strains (45% of all strains). Compared to 2010-2014, our data show that the proportion of the so called "top five" serogroups, STEC O26, O111, O103, and O157 declined from 53.7% to 28.3% in 2017. The proportion of isolates with stx2a, stx2c, or stx2d decreased from 50.5% to 33.3%. We also observed an increase of STEC harbouring the low pathogenic subtypes stx2b and stx2e from 12.6% to 29.2%, and of eae negative STEC from 29.5% in 2010-2014 to 55% in 2017. Simultaneously, there was a sharp increase of the patients' median age from 24 years to 46.5 years. Clinical manifestations in the patients included abdominal pain without diarrhea (22.3%), diarrhea (77.7%), and the haemolytic-uremic syndrome (HUS) (7.4%). Our data show that a greater number and a wider range of STEC serotypes are detected by culture-independent testing, with implications for public health services.

Genetic characterization of Shiga toxin producing *Escherichia coli* belonging to the emerging hybrid pathotype O80:H2 isolated from humans 2010-2017 in Switzerland
https://pubmed.ncbi.nlm.nih.gov/29884331/

Insights: Shiga toxin-producing E. coli (STEC) O80:H2 is an uncommon hybrid pathotype that has recently emerged in France. We analysed 18 STEC O80:H2 isolated from humans in Switzerland during 2010-2017. All isolates carried stx2a or stx2d, the rare eae variant eae-ξ and at least seven virulence genes associated with pS88, a plasmid that is found in extraintestinal pathogenic E. coli (ExPEC). Whole genome sequencing (WGS) identified additional chromosomal extraintestinal virulence genes encoding for type 1 fimbria (fimA, fimC and fimH), aerobactin (iuc/iutA) and afimbrial adhesins (afaA/C/D/E-VIII). Core genome multi-locus sequence typing (cgMLST) detected two closely related but distinct subclusters with different stx2 and iuc/iutA genotypes. All isolates were multidrug resistant (MDR), but susceptible to third generation cephalosporins and azithromycin. STEC/ExPEC hybrid pathotypes such as STEC O80:H2 represent a therapeutical challenge in the event of extraintestinal infection.

WP8: Methodological aspects

Evaluation of the CHROMagarTM STEC medium for the detection of Shiga toxin-producing *Escherichia coli*

https://www.zora.uzh.ch/id/eprint/122072/

Insights: Fast and reliable isolation of Shiga toxin-producing Escherichia coli (STEC) remains a challenge in routine diagnostics. The present study evaluated the performance of the CHROMagar™ STEC medium by testing the growth capacity of 39 STEC strains from human patients and of 35 non-target strains. The majority (83.3 %) of the 18 STEC strains belonging to the top-five serogroups (O26, O103, O111, O145, O157) did grow on and showed typical mauve colonies (exceptions were one O26 isolate and two sorbitol-fermenting non-motile O157 isolates). However, only five of the 21 STEC strains not belonging to the top-five serogroups showed growth and typical colonies. Of the 28 stx-negative E. coli strains (including 13 O157 isolates) and the seven non E. coli strains, 10 stx-negative/eae-positive E. coli strains of serogroups O2, O26, O113, O128, O145 and O177 did grow and showed typical colonies. Thus, the CHROMagar™ STEC medium can not be recommended as a primary STEC screening method in routine diagnostics. The CHROMagar™ STEC medium should therefore only be used for specific questions or in routine STEC diagnostics in combination with another method.

Evaluation of seven different commercially available real-time PCR assays for detection of Shiga toxin 1 and 2 gene subtypes

https://pubmed.ncbi.nlm.nih.gov/23643131/

Insights: Various real-time PCR-based methods enabling detection of Shiga toxin genes (stx) have been developed and can be used for applications in food microbiology. The present study was conducted to evaluate the reliability of seven commercially available real-time PCR systems for detection of stx1 and stx2 subtypes. For this purpose, pure cultures of 18 STEC strains harboring all known stx1 and/or stx2 subtypes were tested. Only one of the seven real-time PCR systems detected all known stx1 and stx2 subtypes. Six systems failed to detect the stx2f subtype. One system missed stx2 subtypes reported in association with severe human disease. Because the presence of certain stx genes (subtypes) is considered an important indicator of STEC virulence, systems differentiating between the stx1 and stx2 gene groups provide added value. Reliable and fast detection of stx genes is of major importance for both diagnostic laboratories and the food industry.

Evaluation of different buffered peptone water (BPW) based enrichment broths for detection of Gram-negative foodborne pathogens from various food matrices

https://pubmed.ncbi.nlm.nih.gov/26267889/

Insights: This study evaluated the effects of changing the composition of the pre-enrichment medium buffered peptone water (BPW) on the growth of stressed and unstressed Gram-negative foodborne pathogens in a one-broth enrichment strategy. BPW supplemented with an available iron source and sodium pyruvate, along with low levels of 8-hydroxyquinoline and sodium deoxycholate (BPW-S) improved the recovery of desiccated Cronobacter spp. from powdered infant formula. Growth of Salmonella and STEC was comparable in all BPW variants tested for different food matrices. In products with high levels of Gram-negative background flora (e.g. sprouts), the target organisms could not be reliably detected by PCR in any of the BPW variants tested unless the initial level exceeded 10(3) cfu/10 g of sprouts. Based on these results we suggest BPW-S for a one-broth enrichment strategy of stressed Gram-negative foodborne pathogens from dry products. However, a one-broth enrichment strategy based on BPW variants tested in this evaluation is not recommended for produce with a high level of Gram-negative background flora due to very high detection limits.

Effects of different media on the enrichment of low numbers of Shiga toxin-producing *Escherichia coli* in mungo bean sprouts and on the development of the sprout microbiome

https://pubmed.ncbi.nlm.nih.gov/27240217/

Insights: Sprouted seeds have been implicated in a number of serious outbreaks caused by Salmonella and Shiga toxin-producing Escherichia coli. Sprouts pose a very complex challenge to bacterial pathogen enrichment and detection since they naturally contain high levels of background microflora including members of the Enterobacteriaceae. As such, the currently used method cannot ensure reliable detection of STEC in sprouts. In this study, we compared different media for the enrichment of Enterobacteriaceae in their ability to promote the growth of stressed STEC at 37°C and 42°C. Mung bean sprouts were spiked with low levels of STEC and their growth was recorded over time. In addition, the microbiome of mung bean sprouts was analysed before and after enrichment. Our results indicate that the growth of dry-

stressed STEC is comparable in all of the tested enrichment media except for mTSB+Novobiocin and not influenced by the incubation temperature. Low levels of STEC spiked into the sprouts resuspended in media only grew to levels of around 4logcfu/ml during enrichment, which could reduce the probability of detection. Proteobacteria was the dominant phylum detected within the microbiome of non-enriched mung bean sprouts. During enrichment in EE-broth, Proteobacteria remained the most abundant phylum. In contrast, during enrichment in BPW the relative abundance of Proteobacteria decreased whereas Firmicutes increased when compared to the non-enriched mung bean sprout microbiome. The microbiome composition was not significantly influenced by the incubation temperature during enrichment in both BPW and EE-broth. This is the first study to examine the microbiome on sprouted mung bean seeds during BPW and EE enrichment and relates the bacterial community composition changes to the enrichment of pathogens.